REVIEW ARTICLE

Drugs 2004; 64 (8): 837-859
0012-6667/04/0008-0837/$34.00/0

Advances in Sepsis Therapy

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Abstract

During the past 3 years new insights have been gained into the fundamental elements that underlie the pathogenesis of sepsis, and after years of frustrating failures, progress in the basic understanding of sepsis has translated into successful new therapies. These new treatment strategies have significantly improved the outcome of patients experiencing the puzzling syndrome of severe sepsis. More effective supportive therapies with early, goal-oriented therapy including volume

resuscitation, catecholamine therapy and transfusion improve the chances for survival in septic shock. Novel endocrine management with hydrocortisone replacement therapy for relative adrenal insufficiency in septic shock patients and strict blood glucose control provide a survival advantage in critically ill patients. Administering appropriate antimicrobial therapy, nutritional support and ventilation protocols with low tidal volumes have now been shown to benefit septic patients. Finally, human recombinant activated protein C (drotrecogin alfa), which ameliorates sepsis-induced disseminated intravascular coagulation and exerts several other favourable effects on endothelial cells, has been shown to reduce mortality in patients with severe sepsis.

On the basis of newly discovered pathophysiological mechanisms of sepsis, several other adjuvant therapies for sepsis are in various stages of preclinical and clinical development. Individualised and optimal supportive care with efforts to reverse the precipitating cause of sepsis remains the mainstay of therapy for severe sepsis. How these new and often expensive regimens will fit into the standard treatment approach to sepsis remains to be defined by future clinical investigations.

Sepsis is among the leading causes of death in noncardiac intensive care units (ICUs) in the US, totalling approximately 750 000 sepsis cases and up to 200 000 deaths per year.[1,2] The incidence rate has been estimated to be three in 1000 per year in North America and similar data have been found in other developed countries. In a recently published survey of 28 ICUs from eight European countries, patients with infections in the ICU were considered to have sepsis in 28%, severe sepsis (i.e. sepsis with at least one organ failure) in 24% and septic shock in 30%[3] when standard consensus definitions of sepsis were used.[4]

Over the past 20 years a large number of clinical trials in septic patients have been performed. In these trials, new therapeutic agents for sepsis have been evaluated, based largely on progress in the understanding of the pathophysiology of sepsis. Most of these therapies were tested successfully in animal models of sepsis prior to their first use in humans. The vast majority of these laborious and expensive clinical trials failed to show any beneficial effect of the compounds or procedures under investigation.[5] These frustrating clinical trial results did generate a new understanding of the complexity of sepsis and the pitfalls of clinical trial design with novel, experimental adjuvant treatments for sepsis.

Progress in the molecular mechanisms of sepsis has provided a new level of understanding into the complex clinical course of human sepsis. Appropriate design of clinical trials in patients with sepsis needs to account for genetic and environmental variations found in patients and potential microbial pathogens. It has become evident that patients with sepsis are a very heterogeneous population. Certainly not every patient who meets the clinical criteria for severe sepsis is an ideal candidate for a clinical trial with a new therapeutic agent. It is now clear that there is no 'magic bullet' for sepsis, and it is likely there will never be one because most patients with sepsis have some form of underlying morbidity that predisposes them to develop sepsis. One of the major conclusions derived from the 'first generation' of sepsis trials was the insight that patients with a high risk of dying during their septic episode because of their underlying illness should not have been enrolled into clinical trials. Such patients are unlikely to have any benefit from any therapy. In clinical trials these patients introduce statistical 'noise', making it more difficult to detect a beneficial 'signal' of the compound under investigation, especially if this signal (effect) is weak.[6]

Historically, mediators such as cytokines (e.g. tumour necrosis factor [TNF], interleukin [IL]-1) were considered most important in the pathophysiology of sepsis. This view was derived from experiments indicating that when such mediators were injected into animals or healthy volunteers a syndrome with many features of septic shock developed.[7] However, in the mid 1990s it became evident that this view of the pathophysiological alterations in sepsis was clearly too narrow and in reality there is a very complex interaction between microbial pathogen, immunocompetent cells, their mediators, endothelial cells and the coagulation system.

The application of these new insights into the pathophysiology of sepsis together with optimised patient selection and conduct of clinical trials eventually yielded the long-sought-after progress in sepsis therapy. In this review we highlight the important steps in the understanding of severe sepsis and describe the current status of implementation of this knowledge into therapeutic concepts, clinical trials and daily clinical practice.

1. Pathophysiology of Sepsis

1.1 Endotoxin and Other Microbial Antigens

Pfeiffer[8] in 1892, and independently Centanni[9] in 1894, isolated heat-stable compounds from *Vibrio cholerae* and *Salmonella typhi,* respectively, which were called 'endotoxin'. Endotoxin was known for its lipid solubility, its non-protein nature, and its 'toxic' properties – fever and development of shock – if injected into laboratory animals or volunteers.[8-10] It took almost a century of further research until the precise structure of the macromolecule endotoxin was clarified.[11] The large and complex, polar molecule is now referred to as lipopolysaccharide (LPS). LPS is an essential component of the outer membrane of most Gram-negative bacteria. Its structure is similar in all Gram-negative species and consists of a core oligosaccharide, a serotype-specific sugar side chain and a lipid A structure with usually six asymmetrically bound C12–C14 fatty acids (figure 1). This macromolecule is hydrophilic at its polysaccharide end and lipophilic at its lipid A end. It holds important structural and functional attributes of the outer membrane of Gram-negative bacteria. The tertiary structure of lipid A resembles a truncated cone and this spatial structure seems to determine the pathogenic properties, as lipids structurally similar to lipid A, but with a different three-dimensional shape, are not biologically active.[11] Several investigations have demonstrated elevated levels of LPS in patients with sepsis.[12] The levels of LPS correlate somewhat with severity of disease and mortality. Interestingly enough, elevated LPS levels were found not only in Gram-negative but also in Gram-positive bacterial and even fungal infections. Reservoirs of LPS other than pathogens primarily responsible for the septic episode, such as the enormous amount of bacteria and LPS in the intestine,[13] presumably account for most circulating LPS in septic shock.

Besides LPS as the prototype molecule for an inflammatory reaction in the host, several other microbial structures induce an inflammatory reaction. Among these are peptidoglycan and lipoteichoic acid from Gram-positive bacteria, lipoarabinomannan from mycobacteria, flagellin, some fungal cell wall fragments or even (cytosine-phosphate-guanosine [CPG]-rich) fragments of microbial DNA.[14,15] These other pathogen-associated molecular patterns are, on a weight basis, much less 'potent' than LPS in their ability to induce inflammation. Independent of the actual pathogen, LPS seems to possess the greatest pathophysiological potential in sepsis.

1.2 Extracellular and Intracellular Signalling Pathways for Microbial Antigens

A hallmark in the pathophysiology of sepsis and septic shock is the systemic inflammatory response in reaction to circulating microbial antigens. This response is phylogenetically highly conserved and represents a major part of the innate immune reaction.[16] Key molecules in this process are the LPS-binding serum proteins – LPS-binding protein (LBP) and soluble CD14 (sCD14) – together with endotoxin docking molecules such as mCD14, the CD11/CD18 complex and the Toll-like receptors

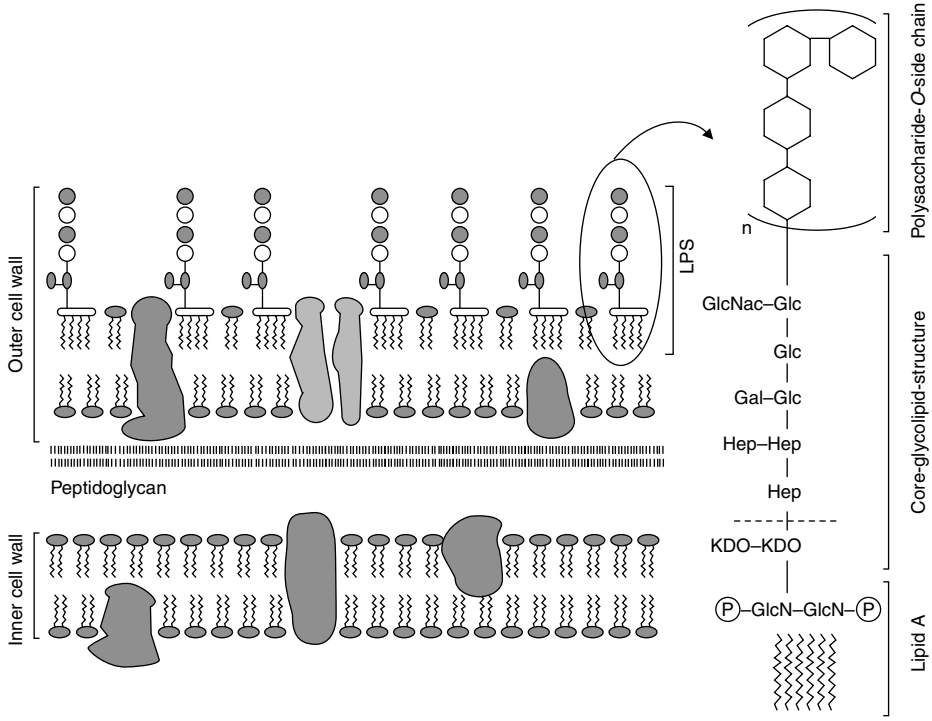

Fig. 1. Structure of the cell wall of Gram-negative bacteria and structure of lipopolysaccharide (LPS) [reproduced from Lynn,[10] with permission]. **Gal** = D-galactose; **Glc** = D-glucose; **GlcNac** = *N*-acetyl-D-glucoasamine; **Hep** = L-glycero-D-manno-heptose; **KDO** = 3-deoxy-D-manno-octulosonic acid.

(TLR) on the monocyte and macrophage surface. According to our current understanding, this process is initiated through binding of fragments of bacterial cell walls or even whole bacteria to LBP. This acute phase serum protein facilitates the transfer of LPS and lipoteichoic acid to mCD14. Alternative pathways for this reaction without LBP involvement may exist; however, these are much less effective.[17] Membrane-bound CD14, originally termed 'endotoxin receptor', binds to endotoxin (LPS), and many other microbial structures as well and it is therefore assigned to the group of pattern recognition receptors.[18-20]

Soon after CD14 had been discovered and its structure clarified, it became evident that this molecule could not activate cells, as it is attached to the cell membrane by a glycosyl-phosphatidyl-inositol (GPI) anchor and does not have an intracellular domain.[21] The receptors that actually transduce the signal after contact with microbial structures intracellularly were long sought. These were eventually discovered in 1998 after a thorough analysis of the genome of LPS-resistant mouse strains (C3H/HeJ and C57BL/10SeCr-mice) as TLR.[22] Ten members of this group of receptors have been identified so far. They are characterised by a number of structural and functional similarities, mainly by a series of leucine-rich repeats in the extracellular domain of the molecule and an intracellular domain that shares considerable homology to the intracellular domain of the IL-1 receptor.[15]

When a macrophage comes into contact with LPS the receptors mCD14, TLR-4 and the adapter-protein MD-2 closely co-localise on the cell surface and form a functional receptor complex ('raft').[23] Various TLRs are involved in the recognition of different types of bacteria. Contact with LPS initiates a homodimerisation or homomultimerisation of TLR4 as the first step in the activation of macrophages. Signalling by lipoteichoic acid is mediated

by heterodimerisation of TLR2 and TLR1, and peptidoglycan or zymosan signalling is by heterodimerisation of TLR2 and TLR6. The signal after contact of a cell with microbial structures is rapidly transduced in the cytoplasm through a series of kinases very similar to the IL-1 cell activation pathway.[24] At the end of this pathway nuclear factor (NF)κB is translocated into the nucleus, which eventually activates the cell by NFκB-induced expression of specific genes in the nucleus.[15,24] The CD14/TLR receptor complex is also involved in the mechanisms of phagocytosis of bacteria and microbial debris[25] (for schematic overview see figure 2[26]).

The role of sCD14 is less clear in this respect. In the presence of sCD14, cells usually not expressing mCD14 and unresponsive to LPS can be activated by LPS and contribute to cytokine production in sepsis (e.g. endothelial cells). Several studies showed an association of higher sCD14 levels and excess mortality.[27] It remains unclear whether this represents a pathophysiological mechanism or just an association with severity of disease, as sCD14 has also been identified as an acute phase protein in other noninfectious inflammatory diseases (e.g. systemic lupus erythematosus). In mice, administration of recombinant sCD14 reduced mortality after LPS-injection, even if the recombinant sCD14 was given (minutes) after the LPS injection.[28] sCD14 may have different effects in low or supraphysiological concentrations.[29]

1.3 Cytokines

After contact with microbial mediators, markedly elevated levels of TNF and IL-1 can be found in the circulation within 30–90 minutes. These are the principal cytokines in the inflammatory reaction in sepsis. If TNF or IL-1 are injected into laboratory animals or volunteers in appropriate concentrations, they induce a syndrome that resembles fulminant septic shock.[30] Administration of anti-TNF or anti-IL-1 therapy before or together with these cytokines was able to prevent the systemic inflammatory response to these mediators and to LPS.[31,32] These

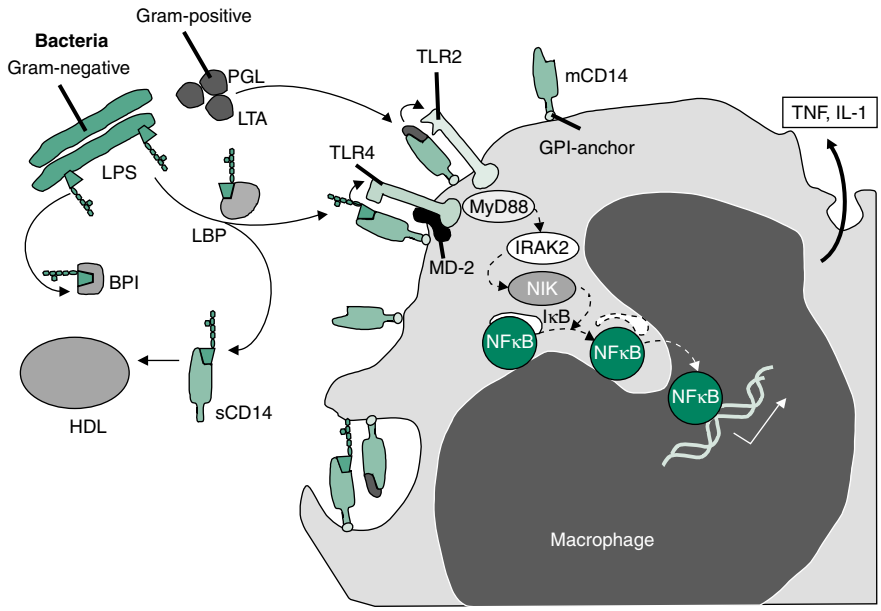

Fig. 2. Interaction between microbial antigens and macrophages (reproduced from Glück et al.,[26] with permission). **BPI** = bactericidal permeability increasing protein; **GPI** = glycosyl-phosphatidyl-inositol chain; **HDL** = high-density lipoprotein; **IL** = interleukin; **IRAK** = IL-1 receptor-associated kinase; **LBP** = lipopolysaccharide-binding protein; **LPS** = lipopolysaccharide; **LTA** = lipoteichoic acid; **m-/sCD14** = membrane-/soluble endotoxin receptor; **MD-2/MyD88** = adapter proteins; **NF** = nuclear factor; **NIK** = NFκB-inducing kinase; **PGL** = peptidoglycan; **TLR** = Toll-like receptor; **TNF** = tumour necrosis factor.

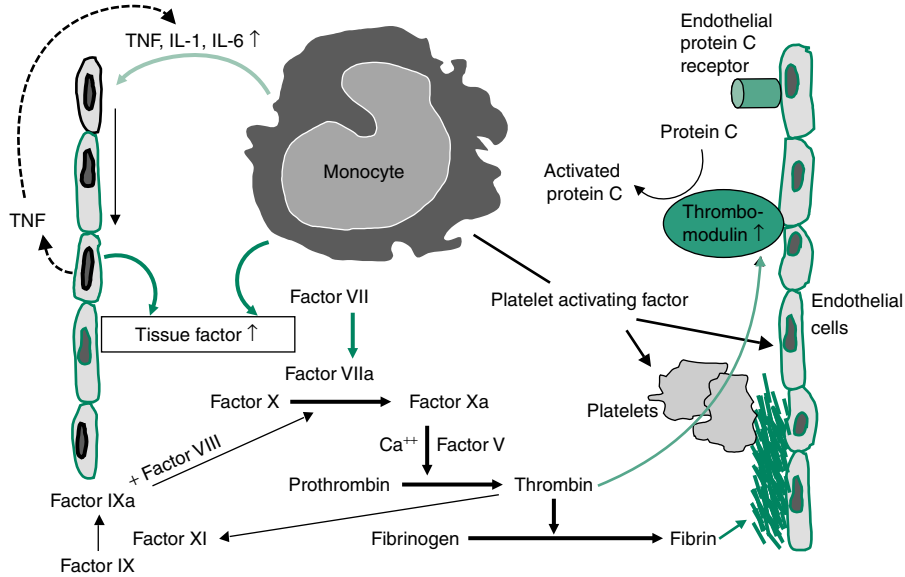

Fig. 3. Schematic overview on the coagulation system in sepsis (reproduced from Glück et al.,[26] with permission). **IL** = interleukin; **TNF** = tumour necrosis factor.

seminal revolutionary experiments provided the rationale for the mediator hypothesis in the pathogenesis of sepsis, which has dominated sepsis research for the past decade.

1.4 Inflammation and Coagulation

When the effects of the innate immune system and systemic inflammation of sepsis were investigated, it became apparent that this system is closely interlinked with other essential defence systems of the body. The finding of a close link between inflammation and the coagulation system, therefore, provided a major step forward in the understanding of the pathophysiology of sepsis.[33,34] One of the most important links between these two systems is the endothelial cell.[35,36] Proinflammatory cytokines can activate endothelial cells and induce the release of tissue factor, which activates factor VII and thus initiates the coagulation cascade (figure 3). Activated macrophages also release tissue factor and other procoagulant mediators, such as platelet-activating factor (PAF). PAF has platelet-activating and platelet-aggregation-inducing properties. PAF is also a potent inducer of proinflammatory cytokines from granulocytes and monocytes/macrophages. Like platelets, these cells have specific surface receptors for PAF. Not only PAF but oxidised phospholipids of similar structure may serve as ligands for the PAF-receptor, and such phospholipids are released in large amounts in inflamed tissues.[37]

Under physiological conditions in a localised process the conjunction of proinflammatory mechanisms and the coagulation system appears to be highly appropriate to seal off an injured, infected area in order to prevent blood loss and further spread of the infection. However, if these processes become systemic under the condition of severe sepsis, they lead to disseminated intravascular coagulation (DIC) with impairment of the microcirculation to vital tissues and multiorgan failure ensues.[38] Procoagulant mechanisms are opposed by physiological anticoagulant and fibrinolytic pathways, and among these the protein C pathway has an essential function. Deposition of thrombin on endothelial cells promotes the generation of thrombomodulin **:** thrombin complexes on the cell surface and this activates protein C. Protein C and its cofactor protein S inhibit factors V and VIII of the coagulation cascade, as well as the plasminogen-activator-inhibitor which facilitates plasmin activation.[39]

Besides its anticoagulant/fibrinolytic effects, protein C exhibits a number of other properties of potential benefit in sepsis, such as an anti-apoptotic effect on endothelial cells and inhibitory effects on cytokine release from macrophages by inhibition of NFκB activation.[40]

Antithrombin is another regulatory molecule with anticoagulant effects via inhibition of factor X, especially when administered with heparin. Antithrombin also shows anti-inflammatory properties that may be of therapeutic utility.[41]

The mechanisms of disseminated intravascular coagulation described here may lead to depletion of the anticoagulant and fibrinolytic pathways. In early stages of severe sepsis, concentrations of protein C, activated protein C and antithrombin are markedly reduced.[42] DIC *per se* and reduced concentrations of the anticoagulant and fibrinolytic factors are associated with higher mortality in sepsis.[43,44]

From these pathophysiological findings within the coagulation system in sepsis, it seems logical to inactivate the excess procoagulant activity and substitute anticoagulant and fibrinolytic factors as an adjuvant therapy for sepsis. Investigations in animal sepsis models confirmed the benefits of this approach and show beneficial effects on mortality both with antithrombin and with (activated) protein C substitution.[41,45]

2. Therapeutic Strategies Derived from Pathophysiological Findings

2.1 Anti-Lipopolysaccharide (LPS) Strategies

2.1.1 Neutralisation of Endotoxin/LPS

LPS is clearly the initiator of the inflammatory and coagulation networks leading to severe sepsis, multiorgan failure, shock and eventually death, at least for Gram-negative infections. It seems reasonable to remove these triggers from the circulation as early as possible after the manifestation of signs and symptoms of sepsis.[46] Several approaches to achieve this goal have been proposed in the past 20 years and were tested in clinical trials.

Anti-Endotoxin Antibodies

Antibodies against LPS may be directed against the polysaccharide portion of the molecule with serotype-specific binding, or may be directed against the core of the lipid-A portion, providing theoretically broad cross-reactivity against LPS of various Gram-negative bacteria. Such antibodies have raised the perspective of 'passive immunotherapy' of sepsis: anti-endotoxin antibodies could prevent further activation of the immune system and the disadvantageous release of cytokines by binding and inactivating excess LPS circulating in the blood.

The anti-endotoxin antibodies first used in clinical trials were polyclonal immunoglobulin preparations from the blood of volunteers who had received a vaccination with heat-inactivated *Escherichia coli* 0111-J5. The first trial with this 'J5 serum' showed lower mortality in patients who had a microbiologically documented Gram-negative infection.[47] Several subsequent investigations using the same serum preparation, however, were unable to confirm these positive results.[48-50]

To overcome the problems associated with immunoglobulin preparation from human blood (e.g. lot-to-lot variability and safety issues), the monoclonal anti-endotoxin antibodies HA-1A (nebacumab; Centocor, Malvern, PA, USA) and E5 (Xoma, Berkeley, CA, USA) were developed. Both were IgM antibodies, E5 a murine and nebacumab a human monoclonal antibody. Both antibodies were tested in large multicentre, placebo-controlled, randomised trials and no overall benefit could be demonstrated. Nebacumab seemed to improve survival in a subgroup of patients with Gram-negative bacteraemia,[51] and E5 in Gram-negative infection not in shock.[52] On the basis of these retrospective subgroup analyses nebacumab (Centoxin®)[1] was approved in Europe for the therapy of Gram-negative sepsis in 1992. However, approval was withdrawn a year later because follow-up studies were unable to confirm the apparently beneficial results of the subgroup analyses in the first studies with these two anti-endotoxin antibodies.[53-56] Patients who did not have Gram-negative bacteraemia showed a non-

1 The use of trade names is for identification purposes only and does not imply endorsement.

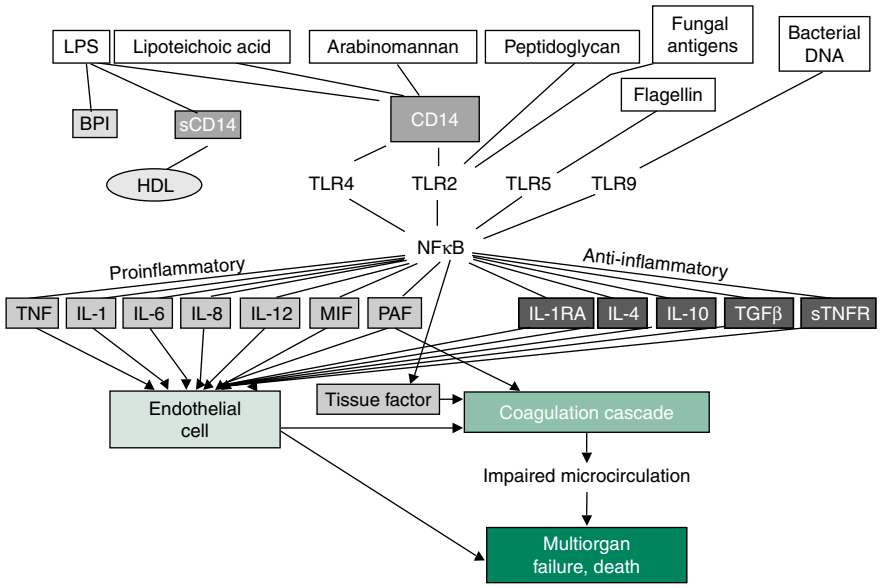

Fig. 4. Schematic overview on key steps in the activation of the innate immune system and the coagulation cascade in sepsis (reproduced from Opal and Glück,[46] with permission). **BPI** = bactericidal permeability increasing protein; **HDL** = high-density lipoprotein; **IL** = interleukin; **LPS** = lipopolysaccharide; **MIF** = macrophage migration inhibitory factor; **NF** = nuclear factor; **PAF** = platelet-activating factor; **sCD14** = soluble endotoxin receptor; **sTNFR** = soluble tumour necrosis factor receptor; **TGF** = transforming growth factor; **TLR** = Toll-like receptor; **TNF** = tumour necrosis factor.

significant trend towards higher mortality with nebacumab. Another antibody developed against a common Enterobacteriaceae antigen (monoclonal antibody T88, Chiron Corporation, Emeryville, CA, USA)[57] also failed to decrease mortality in Gram-negative sepsis.[58]

A thorough review of all anti-LPS antibodies showed that the avidity of these antibodies against LPS was only weak and nonspecific, and also preclinical animal experiments with these compounds had shown little evidence of effective LPS binding.[59,60] Currently, there are no clinical trials with anti-LPS antibodies under way.[61] The concept of passive immunotherapy for sepsis received some revival recently by the observation that patients with high titres of naturally occurring antibodies against the core glycolipid structure of LPS develop fewer postoperative complications than patients without such antibodies.[62]

2.1.2 Modulation of LPS Signal Transduction at the Endotoxin Receptor (CD14)

The endotoxin receptor mCD14 plays a central role in the activation of the immune system by different microbial antigens[14] (figure 2, figure 4). Together with NFκB, CD14 represents one of the bottle-necks in the innate immune response during sepsis and, therefore, represents an attractive target for pharmacological intervention (figure 4). This hypothesis is backed by the observation that CD14-knockout mice are completely resistant against LPS doses which cause 100% mortality in CD14-positive, normal control mice.[63] The CD14-knockout animals are also much more resistant against Gram-negative infections, while their susceptibility to Gram-positive bacteria remains unchanged.[64] Anti-CD14 antibodies were able to reduce the LPS-induced mortality in animal sepsis models and in volunteers it dramatically decreased the inflammatory response to LPS injection.[65,66] Other animal experiments with Gram-negative infections, however, showed that CD14 inactivation by antibodies was associated with increased bacter-

ial dissemination and higher mortality, if the animals did not receive appropriate antimicrobial therapy.[67,68] From this observation a reduced bacterial clearance can be assumed if CD14 and the CD14-dependent pathways are impaired.

Recently a phase Ib/II study with a humanised monoclonal anti-CD14 antibody (IC14) was completed in 40 patients with severe sepsis. IC14 doses of 2–4 mg/kg for 4 days were sufficient to saturate for 7 days more than 90% of the mCD14 receptors of monocytes in the peripheral blood. IC14 antibodies were well tolerated and no increase in secondary infections could be documented.[69] The study was not powered to show differences in any clinical outcome parameter and there were no significant differences in survival; however, a nonsignificant trend for lower organ failure scores was observed in the patients who received IC14 therapy.

2.1.3 Substitution of Bactericidal/Permeability Increasing Protein

Bactericidal/permeability increasing protein (BPI) belongs to a group of cationic antibacterial peptides from granulocytes and is characterised by LPS-neutralising and antibacterial properties.[59] The biologically active N-terminal 21 kDa domain of this physiological antimicrobial peptide appeared useful for neutralising circulating LPS and invasive Gram-negative bacteria. Beneficial effects were confirmed in studies in animal sepsis models,[70] and phase II trials in patients revealed little immunogenicity and good tolerability. The largest controlled clinical trial on recombinant BPI (rBPI) has so far been a phase III study among 393 children with meningococcal sepsis. Children treated with rBPI received fewer blood products, developed fewer long-term neurological sequelae and had fewer amputations than those who received placebo. Overall mortality was 7.5% under rBPI and not significantly different from the 9.9% mortality in the placebo recipients.[71] Children who were treated per protocol for the full 24-hour period, however, showed a more pronounced reduction in mortality (2.2 versus 6.2% with placebo; $p = 0.07$). The trial was not large enough to show the possible benefit associated with rBPI, as the reduction in mortality was only 4–5% and less than anticipated. On the basis of these encouraging results, rBPI will be tested in other clinical sepsis trials in the future.

2.1.4 Phospholipids and Lipoproteins as LPS Inhibitors

sCD14 can transfer circulating LPS into high-density lipoproteins (HDL), which may be an essential physiological role of this plasma protein.[72] Patients with severe disease and especially septic patients quite often have very low levels of circulating HDL. Low HDL levels are associated with a significantly higher mortality in such patients.[73] Experiments in laboratory animals and in human volunteers confirmed that reconstituted HDL may clearly mitigate the effects of subsequently injected LPS.[74,75] Clinical trials with similar lipid preparations are currently in progress.

2.1.5 Absorption of LPS in Columns and Continuous Haemofiltration

Haemofiltration and haemoabsorption for sepsis therapy follow a simple principle: to remove from the circulation any microbial toxins of the infecting organism and/or deleterious host mediators that further activate the immune system. There are several anecdotal reports, clinical trials with small numbers of patients and investigations in laboratory animals which indicate that haemofiltration, haemodialysis or haemoabsorption may partly remove microbial antigens and host mediators from the circulation.[26] However, the main proinflammatory cytokines, TNF and IL-1, will not pass through standard dialysis membranes,[76] and it is not clear whether the removal of these or other factors from the circulation (but usually not from the site of inflammation and infection) will be associated with a clinical benefit for the patient.[77] Other data indicate that continuous haemofiltration or haemodialysis is associated with better survival in sepsis-induced renal failure as compared with intermittent haemodialysis, but it remains unclear if the potential beneficial effects afforded by haemofiltration are related to removal of host-derived or microbial pathogen-derived mediators.[77]

A Japanese group used a column containing polymixin B bound to polystyrol fibres for specific

LPS absorption and reported some success.[78] In Europe LPS absorbers containing albumin-coated plastic beads (MATISSE system) were tested in phase I and II studies.[79] After an interim analysis the phase II study with this device was continued only in patients with severe sepsis and peritonitis. A total of 141 patients were included and a small mortality reduction of 2.3% (relative risk [RR] −8.3%) was observed with an improvement in organ dysfunction (using sequential organ failure assessment [SOFA] score).[80] A follow-up study with the MATISSE system is planned.

Extracorporeal circulation and a large-bore vascular access are essential prerequisites for any of the dialysis and apheresis techniques, and there is need for anticoagulation to keep tubing and filter open. All this may be difficult to accomplish in severe sepsis and septic shock with possible thrombocytopenia due to DIC and is one of the limitations of this approach.

2.1.6 Plasmapheresis

A few centres have performed plasmapheresis in severe sepsis and septic shock, with varying success. This procedure not only removes circulating microbial antigens and host mediators but may also improve DIC by supplementing coagulation factors, and also the immunoglobulins in the pooled plasma preparations used for substitution may have beneficial effects in some forms of sepsis. These aspects make plasmapheresis more than an apheresis technique. The largest controlled trial of plasmapheresis for severe sepsis was only recently presented by a Norwegian-Russian collaboration. One or two plasmaphereses of 30–40 mL/kg bodyweight with substitution of fresh frozen plasma in addition to standard therapy yielded a 28-day mortality reduction in the treatment group (n = 54) from 53.8 to 33.3%, which just achieved significance ($p = 0.05$).[81] There were some imbalances in severity of disease at study entry between the treatment and the control group, which should call for caution in the interpretation of these interesting results.

2.1.7 Lipoproteins and Soluble CD14

One of the pathways for LPS removal from the circulation uses LBP and sCD14 for LPS transfer into lipoproteins, for example HDL particles, making LPS biologically inactive.[72,82] In an animal sepsis model substitution with recombinant sCD14 was found to be beneficial,[28] and in one clinical study, sepsis patients with naturally occurring high sCD14 levels tended to have a better outcome.[83]

Low HDL levels are associated with a dismal prognosis in patients with severe disease,[73] and application of reconstituted HDL particles to human volunteers decreased the inflammatory reaction after LPS exposure.[75] However, these experimental data provide at best indirect evidence that sCD14 or lipoproteins might be of potential benefit in sepsis, and it remains to be proven that such concepts actually improve the outcome of clinical sepsis.

Another innovative anti-endotoxin approach is the use of lipid A antagonist.[61] A synthetic lipid A analogue, known as E5564, has been developed. This molecule specifically binds to TLR4 and effectively blocks LPS-mediated events in human endotoxin-challenged volunteers.[84] This LPS inhibitor is now in clinical trials.

2.1.8 Limitations of Anti-LPS Strategies

LPS and other microbial structures initiate the chain of events leading to sepsis, multiorgan failure and death over the course of a severe infection. Therefore, it seems logical to use any anti-LPS strategy only in the early phases of sepsis, as the benefit of such strategies may be lost once the inflammatory networks have been activated and end organ damage has occurred. It must be noted that several of the mechanisms and anti-endotoxin strategies presented herein interfere with physiological mechanisms of clearance of pathogens from the body (e.g. the CD14/TLR receptor complex). Modulation of such mechanisms may be disadvantageous, even if the use of antibiotics in clinical sepsis may partly compensate for the reduced elimination of pathogens after, for example, blockade of the CD14/TLR receptor complex.[68]

Finally, modern concepts of sepsis-associated immunosuppression and immuno-paralysis speculate that, besides the switch from pro- to anti-inflammatory mediators, constant low-level challenge of the innate immune system by LPS may

continue to play a role throughout the course of septic shock evolution.[85,86] If this hypothesis can be confirmed, anti-LPS therapeutic strategies might also be of benefit in later stages of sepsis. Only well designed clinical studies will answer these questions.

2.2 Neutralisation of Cytokines

The observation that injection of proinflammatory mediators may cause most of the symptoms occurring after LPS injection or in fulminant septic shock provided the rationale for therapeutic modulation of these mediators.[30] Anticytokine antibodies were shown to prevent most of these reactions.[32] On the basis of this mediator concept of sepsis pathogenesis, several monoclonal antibodies against TNF were developed,[87-94] as well as hybrids of the p55- or p75-TNF receptors linked to immunoglobulin-Fc fragments (for prolongation of the plasma half-life),[95-97] and the naturally occurring IL-1 receptor antagonist (recombinant IL-1 RA).[98-100] Numerous experiments with anticytokine strategies found beneficial effects on most parameters including survival in laboratory animals using LPS injection as a sepsis model. However, this was the case in only some of the models investigating real infections in the animals; others gave rather unfavourable results.[7,101] Clinical trials with anticytokine strategies for sepsis were unequivocally disappointing.[31] Post hoc analyses seemed to show beneficial effects in subgroups of trials but these analyses lacked statistical power. The expensive and labour-intensive confirmatory trials designed after these subgroup analyses all failed to verify a therapeutic effect.[102] A synopsis of the main characteristics and results of phase II and III clinical trials on anticytokine-strategies is presented in table I.

Thorough re-evaluations of some of these recently completed trials on cytokine-neutralising antibodies revealed that potentially beneficial effects of anticytokine antibodies appear only if analyses included only selected patient populations. If patients with dismal prognosis and those who received inadequate antibiotic and/or surgical therapy are excluded, the results of anticytokine therapies appear more favourable.

A meta-analysis of 11 large multicentre, randomised, controlled, sepsis anti-TNF trials with 8631 patients recently presented in an abstract found a significant but small mortality reduction (2%; RR = 0.88–0.99; p = 0.03).[107] The mortality reduction was greater among patients who had received anti-TNF antibodies (3%) than among patients who had received TNF-receptor hybrids, where no benefit was seen. Patients in shock had a larger benefit (5% mortality reduction) than patients not in shock (no difference).

Such meta-analyses indicate that the concept of TNF neutralisation in patients with sepsis is of potential benefit but the achievable reduction in mortality is relatively small. When the studies were designed, a greater mortality reduction had been assumed, and this may be another reason why no single trial had ever shown convincingly positive results. It is questionable whether the small effect of TNF neutralisation on sepsis mortality according to the results of the meta-analysis is clinically relevant or cost effective.

There is a clear discrepancy between the promising results of the preclinical laboratory investigations in animal sepsis models and the clinical trials. A major difference between these two settings is the problem of establishing adequate animal models for sepsis. If relatively large amounts of LPS are injected abruptly into healthy animals (or human volunteers) this provokes, likewise, an abrupt release of cytokines and a shock-like syndrome. However, such a situation is more representative of an intoxication by LPS rather than an infection, except (perhaps) fulminant meningococcal sepsis in the form of a Waterhouse-Friederichsen syndrome. Less fulminant infections, however, make up the vast majority of sepsis cases, for example peritonitis, pneumonia or wound infections. In addition, a high percentage of septic patients have severe comorbidities that increase the risk for infection and facilitate the development of sepsis. In these situations mechanisms other than excess cytokine release may well be operative. It has not been possible to imitate the very

Table I. Sepsis mortality in clinical trials of anticytokine-therapies

Clinical trial (year)	Therapy	n	Mortality (%)		Benefit
			controls	anticytokine	
Anti-TNF antibodies					
Fisher et al.[87] (1993)	CB0006	80	31.6	44.3	−12.7
Dhainaut et al.[89] (1995)	CDP571	42	60.0	62.5	−2.5
Abraham et al.[88] (1995) [NORACEPT I]	Nerelimomab (Bay 1351)	971	33.1	30.4	+2.7
Cohen & Carlet[90] (1996) [INTERSEPT]		553	39.5	37.3	+2.2
Abraham et al.[92] (1998) [NORASEPT II]		1878	42.8	40.3	+2.5
Reinhart et al.[91] (1996)	Afelimomab (MAK195F)	122	41.4	47,3	−3.9
Reinhart et al.[93] (2001) [RAMSES]		446	57.7	54.0	+3.7
Panacek et al.[94] (2000) [MONARCS]		998	47.6	43.6	+4.0
Soluble TNF receptors					
Fisher et al.[95] (1996)	Lenercept (p75-fusion-protein)	141	30.3	45.4	−15.1
Abraham et al.[96] (1997)		498	38.6	38.0	+0.6
Abraham et al.[97] (2001)		1342	28	27	+1.0
IL-1 receptor antagonist					
Fisher et al.[99] (1994)	IL-1RA (anakinra)	99	44.0	24.3	+19.7
Fisher et al.[99] (1994)		893	33.7	29.9	+3.8
Opal et al.[100] (1997)		696	36.4	33.1	+3.3
PAF inhibitors					
Dhainaut et al.[103] (1994)	PAF-RA (ginkgolide B;	262	51	42	+9
Dhainaut et al.[104] (1998)	BN52021)	609	49	47	+2
Opal[105] (2002)	PAF-AH (epafipase)	124	44.2	21.4	+22.8
Opal[106] (2003)		1261	24	25	−1.0

IL = interleukin; **PAF** = platelet-activating factor; **PAF-AH** = PAF-acetylhydrolase; **RA** = receptor antagonist; **TNF** = tumour necrosis factor.

complex 'real-world sepsis patient' in an animal model, and this may in part explain the discrepancy between the preclinical and clinical data.

Another explanation for the failing anti-TNF and anti-IL-1 strategies might be based in the fact that TNF and IL-1 reach their peak concentrations as early as a few hours after the onset of sepsis symptoms and decline rapidly thereafter. Clinically, by the time a patient receives medical attention for sepsis several downstream mechanisms have already been triggered and act independently of the initiating (and eventually blocked) cytokine-signal. There may be only a narrow window of opportunity for successful initiation of an anti-TNF or anti-IL-1 therapy, and any application of anticytokine strategies after this period may be too late or even disadvantageous. Regrettably, only few patients are diagnosed appropriately in this narrow time frame in the initial phase of sepsis.

Finally, it was long overlooked that TNF and IL-1 pathways are highly redundant in the induction of cytokine release (see figure 4). Moreover, TNF represents a critical factor in the innate immune response to eliminate microbial pathogens. Essential studies in this respect have been performed by Echtenacher et al.,[101] who showed that TNF neutralisation after the induction of an intraperitoneal infection by cecal ligation and puncture was associated with a higher mortality in experimental animals.[101,108]

Interestingly enough, cytokine-neutralising drugs that failed to benefit patients with sepsis yielded

considerable success in chronic inflammatory diseases refractory to other medical therapy, for example in the treatment of active rheumatoid arthritis,[109] refractory Crohn's disease[110] or the spondylarthropathies. TNF inhibitors induce a higher rate of infectious complications in treated patients,[111,112] underlining the special role of TNF in the elimination of microbial pathogens.

In summary, current evidence from experimental investigations and clinical trials indicates that proinflammatory cytokines are a driving force in the pathophysiology of sepsis but cannot be neutralised without potentially compromising the innate defence mechanisms.[113]

Currently there are no further anticytokine strategies for sepsis therapy in clinical trials.

2.3 Anticoagulant and Fibrinolytic Therapeutic Strategies for Sepsis

2.3.1 Antithrombin

Many centres have administered antithrombin replacement in septic patients and some continue to do so. Several smaller studies had indicated a potential benefit for antithrombin replacement; however, there was no formal evidence-based rationale for this approach.[114] The investigators of the KyberSept trial, therefore, examined the efficacy and safety of antithrombin for the reduction of sepsis mortality. This placebo-controlled, randomised, international multicentre study aimed at establishing and maintaining supraphysiological antithrombin concentrations of approximately 200% during the first 4 days in patients with severe sepsis.[115] A total of 2314 patients were enrolled in this study. There was overall no beneficial effect of antithrombin on mortality. Bleeding events were significantly more common if heparin was given with antithrombin (23.8 versus 13.5% without heparin; $p < 0.001$). In the subgroup of 698 patients not receiving heparin, bleeding events were not significantly more frequent and a trend towards decreased 28-day mortality was seen (43.6 versus 37.8%; $p = 0.08$), which became significant after day 90 (52.5 versus 44.9%; $p = 0.03$). However, prespecified subgroup analyses cannot be interpreted as a proof for the efficacy of antithrombin treatment without heparin as an adjuvant therapy for sepsis because the KyberSept trial had not been powered for such an endpoint. The result of the KyberSept trial was unexpected for many experts and generated considerable discussion.[114] After KyberSept, it remains unclear whether substitution of markedly decreased antithrombin levels in severe DIC by replacement doses of antithrombin are beneficial. According to the current evidence, adjuvant therapy of sepsis with antithrombin cannot be recommended.

2.3.2 Activated Protein C

Recombinant activated protein C (rhAPC, drotrecogin alfa [activated]) is currently the largest recombinant protein manufactured on a large scale by genetic engineering. This 405 amino acid protein is produced in a human cell line and requires post-translational modifications and activation by thrombin followed by further purification. Protein C is highly species-specific and human (activated) protein C is biologically inactive in mice and other laboratory animals. Preclinical studies in animal models of sepsis were hampered by this fact and clinical trials were started relatively early in the development of this drug. Plasma half-life of drotrecogin alfa (activated) is only about 13 minutes and requires continuous infusion. In a phase II study, a dose of 24 μg/kg/hour proved to be well tolerated and effective in the reduction of DIC parameters and was used for the subsequent phase III studies.[116] Between 1998 and 2000 drotrecogin alfa (activated) was tested in patients with severe sepsis in a multicentre, multinational, placebo-controlled, double-blind trial (Protein C Worldwide Evaluation of Severe Sepsis [PROWESS] trial). This trial was stopped on the occasion of the second interim analysis when 1690 patients had been enrolled because the predefined stopping criteria had been met by the results. Treatment of patients with severe sepsis with drotrecogin alfa (activated) reduced 28-day mortality from 30.8% in the placebo group to 24.7% in the treatment group. This represents a relative reduction in the risk for mortality of 19.4% ($p = 0.006$; 95% CI 7, 30%).[117] A follow-up study to the PROWESS trial (Lilly protocol EVBI), which

obtained outcome data as far as 30 months after enrolment into PROWESS, confirmed the beneficial effects of drotrecogin alfa (activated) out to day 90 ($p = 0.027$)[118] and showed that mortality after day 30 was generally related to age and comorbidity rather than to sepsis.

The US FDA and the respective European licensing agencies requested extensive subgroup analyses of the PROWESS trial data.[119,120] These analyses showed that drotrecogin alfa (activated) was more effective in patients with more severe disease, assessed by the Acute Physiology and Chronic Health Evaluation (APACHE) II scores ≥25, multiorgan failure and/or DIC. In patients with APACHE II scores ≥25, an absolute reduction in mortality of 13% was observed, representing a relative reduction in the risk for mortality of 29% (95% CI 15, 41%). However, protein C levels at study entry were not able to clearly identify a subgroup of patients likely to benefit from drotrecogin alfa (activated). It appears that: (i) the beneficial effects of the compound are not only related to its anticoagulant and fibrinolytic properties; and (ii) activation of naturally occurring protein C may be severely compromised in severe sepsis by impairment of endothelial cell function.

The most relevant adverse effect of drotrecogin alfa (activated) is haemorrhage. Severe bleeding events were seen during the infusion period in 2.5% of patients receiving drotrecogin alfa (activated) and in 1.0% of placebo recipients ($p = 0.025$) in the PROWESS trial. Analysis of all controlled applications of drotrecogin alfa (activated) [>2500 persons treated by the end of 2002] showed a rate of fatal haemorrhage of 0.4% and a rate of intracerebral haemorrhage of 0.5% during the infusion period.[121] Intracerebral haemorrhage was seen more frequently in patients with meningitis and/or thrombocytopenia, which should call for caution if patients with platelet counts <50 000/μL and meningitis are to be treated with drotrecogin alfa (activated). Despite this, other subgroup analyses of the PROWESS data indicate that just those patients with severe thrombocytopenia of 30 000–50 000/μL due to severe DIC may have a greater benefit from therapy with drotrecogin alfa (activated).[119] It has to be emphasised that great caution is necessary in the interpretation of such subgroup analyses with small patient numbers. There is no experience with drotrecogin alfa (activated) in patients with platelet counts <30 000/μL, as this has been an exclusion or termination criterion in all trials performed thus far.

In November 2001 drotrecogin alfa (activated was approved for therapy of severe sepsis in the US (Xigris®) and in November 2002 approval was given in Europe. The drug is indicated for patients with APACHE II scores ≥25 (in the US), or patients with ≥2 organ dysfunctions despite optimal standard therapy (in Europe). The approved indications for drotrecogin alfa (activated) focus on the subgroup of patients with severe sepsis and multiorgan dysfunction, following the results of the PROWESS subgroup analyses.[122]

There are currently three published pharmacoeconomic evaluations for drotrecogin alfa (activated) from different health systems, all based on the results of the PROWESS-trial. Pharmacoeconomic estimates for therapy with drotrecogin alfa (activated) vary depending on the age of the patient and the health system. Costs per life-year gained were estimated to be $US16 000–30 000 in the Canadian system,[123] $US27 000 in the US system[124] and approximately €15 000 in the German system.[125] Cost-effectiveness values of a similar magnitude have been calculated for other generally accepted medical therapies or procedures.[124]

As efficacy of drotrecogin alfa (activated) has not been established for patients with lower risk of death from sepsis, another large multicentre, multinational, placebo-controlled, double-blind clinical trial with this agent has been initiated. The ADministration of DRotecogin alfa (activated) in Early Severe Sepsis (ADDRESS) trial will enrol more than 11 000 patients in the early stages of severe sepsis and only one sustained organ failure or an APACHE score <25. The results of this truly extensive study will take several years to complete.

2.3.3 Tissue Factor Pathway Inhibitor

Tissue factor plays a critical role in the initiation of the coagulation cascade in sepsis.[126] Its effects

Table II. Synopsis of key clinical trials with significant mortality reduction in sepsis (published 1999 and later)

Clinical trial (year)	No. of trials (patients enrolled)	Intervention	Mortality	p-Value
Kollef et al.[130] (1999), Ibrahim et al.[131] (2000)	2 (1147)	Adequate antimicrobial therapy (critically ill patients)	RR = 0.42 (all infections) RR = 0.45 (bloodstream infections)	<0.001
Rivers et al.[132] (2001)	1 (263)	'Goal-oriented therapy' (severe sepsis)	RR = 0.58	0.009
ARDS-Network[133] (2000)	1 (861)	Protective ventilation (ARDS)	RR = 0.78	0.007
Annane et al.[134] (2002)	1 (299)	Hydrocortisone (septic shock)	Overall: RR = 0.90 insufficiency of adrenal cortex: RR = 0.84	Overall: 0.09 insufficiency of adrenal cortex: 0.04
van den Berghe et al.[135] (2001)	1 (1548)	Strict blood glucose control (surgical patients)	Overall: RR = 0.58 critical care therapy >5 days: RR = 0.52	Overall: 0.04 critical care therapy >5 days: 0.005
Busund et al.[81] (2002)	1 (106)	Plasmapheresis (severe sepsis)	RR = 0.62	0.05
Bernard et al.[117] (2001)	1 (1690)	Drotrecogin alfa (activated) [severe sepsis]	Overall: RR = 0.81 APACHE II >25: RR = 0.71	0.005

APACHE = Acute Physiology and Chronic Health Evaluation; **ARDS** = acute respiratory distress syndrome; **RR** = relative risk.

may be counterbalanced by the naturally occurring tissue factor pathway inhibitor (TFPI). Analogous to protein C and antithrombin, decreased TFPI functional activity is observed in the early stages of sepsis. Therefore, it seemed logical to evaluate substitution therapy with TFPI for adjuvant sepsis therapy.[127] A phase II trial with TFPI in 210 patients with severe sepsis yielded decreased IL-6 release and a trend for mortality reduction with TFPI.[128] However, a large phase III trial was terminated in late 2001 without showing any benefit of TFPI; publication of these results is still pending.[129]

2.3.4 Platelet-Activating Factor

Blockade of the procoagulant and proinflammatory effects of PAF was identified quite early as a potential target for an adjuvant therapy of sepsis. On the basis of experience from animal models, improvement in the endothelial functions, decreased vascular leakage and reversal of disturbances of the microcirculation in sepsis were expected. Two phase III trials with a PAF-receptor antagonist BN52021 (Ginkgolide B) did not find any effect on survival and the further development of this compound was stopped[103,104] (table II).

PAF-acetylhydrolase (PAF-AH; epafipase) is a naturally occurring enzyme that is capable of inactivating PAF and PAF-like oxidised phospholipids. It is produced principally by macrophages and has anti-inflammatory effects *in vitro* and *in vivo*.[37] Results from a phase II trial suggested a reduction in mortality and prevention of acute respiratory distress syndrome (ARDS) in patients with sepsis and trauma by a 5-day treatment with epafipase at a dose of 1 mg/kg/day.[105,136] Epafipase was then tested in patients with severe sepsis. However, after an interim analysis of the first 1261 patients had been performed in December 2002, the study was stopped prematurely because of medical futility, as it was not able to demonstrate any effect of the drug compared with placebo.[106]

2.3.5 Heparin

The role of heparin in the therapy of sepsis is absolutely unclear. Davidson et al.[137] recently published a *post hoc* analysis of the placebo group data from the largest recently completed clinical sepsis trials, PROWESS and KyberSept, in the form of a letter. The results seem to suggest that heparin therapy may have a beneficial effect on sepsis mortality. This is an interesting observation but the data are not suitable for a recommendation, as neither study was planned for these endpoints and they did not control for post-randomisation bias. It may well be that patients with severe disease and a high risk of dying from sepsis and low platelet count due to DIC were

less likely to receive heparin for presumably higher risk of bleeding. Patients without these unfavourable symptoms and a better prognosis are more likely to have received heparin for thrombosis prophylaxis.

Therefore, it is still unclear whether heparin therapy is beneficial for sepsis and, if so, in which dose or application form heparin should be used for sepsis therapy. Despite this lack of evidence, in many centres patients routinely receive low-dose heparin therapy equivalent to 200–600U of unfractionated heparin per hour.

2.4 Modulation of the Endocrine Systems

Several clinical trials have been performed with high doses of corticosteroids for adjuvant sepsis therapy with the rationale of preventing excessive release of cytokines. These trials did not show any beneficial effect of high-dose corticosteroids on sepsis mortality, although corticosteroids may reduce the release of proinflammatory cytokines. Meta-analyses of corticosteroid trials at doses of several 100 to more than 1000mg prednisone or prednisolone per day found a trend towards increased mortality with corticosteroids.[102,138] The use of corticosteroids for sepsis therapy was, therefore, not considered further for some time.

However, in the past few years the use of corticosteroids for sepsis has received renewed interest because it was appreciated that in many patients with sepsis there is an insufficiency in the pituitary gland-adrenal cortex axis.[139] Bollaert et al.[140] and Briegel et al.,[141] who presented promising data from pilot studies with 41 and 40 patients, respectively, first suggested substitution of hydrocortisone at the maximum physiological dose of 200–300 mg/day.

A French multicentre study recently presented data from 299 patients with septic shock who first received an ACTH test for evaluation of the pituitary adrenocortical axis and were then randomised to either hydrocortisone 200mg and fludrocortisone 50μg per day, or placebo.[134] Overall a nonsignificant trend towards reduced mortality was seen with corticosteroid substitution (RR = 0.895; 95% CI 0.39, 1.07; p = 0.09). In the predefined subgroup of patients with relative adrenal insufficiency, a significant reduction of sepsis mortality (RR = 0.84; 95% CI 0.47, 0.95; p = 0.04) was observed. Hydrocortisone substitution was also associated with a decreased need for vasopressor support. In a recently published, multicentre, clinical trial with a crossover design, patients with severe sepsis treated with hydrocortisone 10 mg/hour benefited. Such doses of corticosteroids had anti-inflammatory but not immuno-suppressive effects. Substitution with hydrocortisone should be tapered off and not stopped abruptly, to prevent a rebound.[142] Today many centres use hydrocortisone for therapy of septic shock despite the fact that the efficacy of this approach is not firmly established.

Quite often patients receiving critical care for severe sepsis or other severe disease develop elevated blood glucose levels, even if these patients did not have diabetes mellitus before the episode leading to sepsis. Blood glucose control had not been regarded as critical with respect to outcome until van den Berghe et al.[135] published a fascinating study in surgical, mechanically ventilated, critically ill (but not necessarily septic) patients. Tight control of blood glucose between 80 and 110 mg/dL by administration of insulin doses up to 50 units/hour was associated with a reduction in mortality compared with 'loose' control with blood glucose levels <215 mg/dL. This was most pronounced in patients needing critical care for more than 5 days. Among those, a pronounced reduction in 28-day mortality from 20.2 to 10.5% was achieved with tight blood glucose control (RR = 0.52; p = 0.005).[135] The incidence of secondary infections, as well as duration of ventilation and stay on the ICU, was decreased with tight blood glucose control. These surprising effects need further confirmation in other critically ill patients, for example nonsurgical patients, patients meeting criteria of severe sepsis, etc.

2.5 Supportive Therapy

2.5.1 Protective Ventilation

A high proportion of patients with severe sepsis need mechanical ventilation.[117] The respiratory tract is quite often the primary source of sepsis, and

was in 53.6% of patients enrolled into the PROWESS and 34.5% in the KyberSept trials.[115,117] A detailed discussion of the progress in mechanical ventilation is beyond the scope of this review. It should be noted that the use of protective ventilator settings with low tidal volumes has been shown to decrease mortality in patients requiring mechanical ventilation. In the ARDS-Network study, ventilation with a tidal volume 6 mL/kg was associated with a significantly lower mortality rate of 31.0% as compared with a rate of 39.8% with a tidal volume of 12 mL/kg (RR = 0.78; p = 0.007).[133] The application of this approach to daily ICU clinical practice may contribute to better outcomes for patients with severe sepsis.

The therapeutic value of high positive end expiratory pressure (PEEP) values versus lower PEEP values along with the low tidal volume strategy has recently been studied by the ARDSnet group (presented by Bower R: American Thoracic Society annual meeting, 18 May 2003, Seattle, WA, USA). No apparent survival benefit was observed in the initial analysis of this study with over 500 patients enrolled into the trial. It is hoped that further innovations in lung protection ventilator approaches will improve the supportive management and overall outcome of septic patients in the ICU.

2.5.2 Fluid Resuscitation, Transfusion and Vasopressor Therapy

There is no doubt that patients with severe sepsis need fluid resuscitation to compensate for increased capillary permeability and vasodilation. It is still unclear whether in this situation crystalloid or colloid solutions should be used. The use of albumin in such situations was discouraged by the results of a Cochrane analysis in 1998, but this analysis had several flaws and a second, better selected meta-analysis did not confirm higher mortality rates associated with the use of human albumin for fluid therapy.[143,144] Still, the high costs of human albumin preparations preclude their unrestricted use for fluid therapy in most clinical situations.

Rivers et al.[132] showed in a very elegant study that early timing and magnitude of supportive therapy with fluid and vasopressor therapy and transfusion do have considerable impact on the outcome in patients with sepsis. In this single-centre trial, 263 patients with newly diagnosed sepsis were randomised to receive supportive therapy according to 'early goal-directed therapy guided continuous monitoring of mixed venous oxygen saturation' or standard care. The early goal-directed therapy group (n = 130) was treated in the emergency room for at least 6 hours according to a strict algorithm aimed at achieving as soon as possible a mean arterial pressure ≥65mm Hg, central venous pressure of 8–12mm Hg, mixed venous oxygen saturation ≥70%, a haematocrit ≥30% and urine output ≥0.5 mL/kg/h. The second group (n = 133), after initial stabilisation, was transferred to the ward as soon as a bed became available and received standard care according to the same guidelines except for the monitoring of central venous oxygen saturation, and (presumably) without strict adherence to the algorithm and time frames.

Patients randomised to the early goal-directed therapy group received, in the first 6 hours of their hospital stay, significantly more fluids, more red blood cell transfusions and more frequent vasopressor therapy as compared with the standard therapy group. This difference between the two groups was equalised over the full course of the hospital stay; however, hospital all-cause mortality among the patients with early goal-directed therapy was dramatically reduced compared with that in the standard therapy group (30.5 versus 46.5%; p = 0.009). The 28-day mortality was 33.7 and 49.2% (p = 0.01), and 60-day mortality 44.3 and 56.9% (p = 0.03), respectively. Early intervention with appropriate measures to maintain adequate tissue perfusion and oxygen delivery by prompt institution of fluid therapy, stabilisation of arterial pressure and providing sufficient oxygen transport capacity are highly effective in reducing mortality.

2.5.3 Importance of Adequate Antimicrobial Therapy

It seems obvious that an infection leading to the life-threatening syndrome of (severe) sepsis or septic shock should be treated with antimicrobials. However, adequate antimicrobial therapy may not

always be easily achievable in times of growing resistance among microbial pathogens and expert advice on appropriate choice of antimicrobial therapy may not be available at all times. Several recent publications demonstrate nicely the special importance of this aspect. One study found that 8.5% of patients with infections needing critical care had received inadequate empirical antimicrobial therapy. In most of these patients (79.5%) the infection was nosocomially acquired. Inadequate antimicrobial therapy was in this study associated with a considerably higher mortality (42.0 versus 17.7%; RR = 2.37; $p < 0.001$).[130] The same group investigated patients with bacteraemia and fungaemia. Among these, the rate of inadequate therapy was even higher at 29.9% and, in addition, this investigation found mortality rates approximately twice as high with inappropriate therapy (61.9 versus 28.4%; RR = 2.18; $p < 0.001$).[131] In this study, pathogens isolated most often in patients with inadequate antimicrobial therapy were vancomycin-resistant enterococci, *Candida* spp., methicillin-resistant *Staphylococcus aureus*, coagulase-negative staphylococci and *Pseudomonas aeruginosa*.

There is evidence that besides selecting adequate antimicrobial therapy, the timing of the initiation of such therapy is essential for an optimal outcome. In another study, Kollef and colleagues[145] investigated delays of appropriate empirical antimicrobial therapy for ventilator-associated pneumonia. If the initiation of appropriate therapy was delayed for at least 24 hours after clear symptoms of the nosocomial infections were present, the patients' risk for mortality in this situation increased almost 4-fold.

However, such data should not be interpreted as a reason for empirical broad-spectrum antimicrobial therapy in every patient treated in the hospital or in the ICU. Such an approach often leads to progressive antimicrobial resistance and other possible associated adverse effects. A certain rate of inadequate empirical antimicrobial therapy cannot be avoided in the light of generally increasing antimicrobial resistance and the problematic pathogens associated most often with inadequate antimicrobial therapy. Appropriate selection of empirical antimicrobial therapy has to be considered carefully for every individual patient. Careless choice of inadequate therapy will be associated with higher mortality. Increased risks for special pathogens need to be considered for the individual patient and in many cases the input of infectious diseases specialists will be helpful.

Every effort should be undertaken to minimise the rate of nosocomial infections in the ICU by appropriate infection control programmes. The microbiological laboratory processing the specimens from the ICU should be able to provide regularly updated reviews of resistance patterns for the most important pathogens isolated in the respective ICU. This information should serve, in turn, as a basis for the selection of actual guidelines for empirical therapy in this individual ICU. Such an approach may contribute to minimising inadequate antimicrobial therapy and associated excess mortality in patients with sepsis in the ICU.[146]

3. Conclusion

Over the past 5 years, progress in the understanding of the pathophysiology of sepsis has been translated into several well designed clinical studies. After years of frustration these studies have confirmed the efficacy of a number of therapeutic approaches for severe sepsis and septic shock (for an overview see table II). It cannot be expected that sepsis-related mortality will be reduced to zero. Severe sepsis will continue to have a considerable mortality based on the underlying comorbidity most patients have as the risk factor for developing sepsis.

However, most of the interventions recently found to be beneficial for the outcome of patients with sepsis can be directly implemented in daily practice with minimal financial or logistic effort. Adequate fluid resuscitation, appropriate antimicrobial therapy, maintenance of the endocrine axes and protective ventilation are readily adapted to modern ICU care. Optimising these therapeutic measures should form the basis of appropriate therapy for sepsis and many septic patients will improve rapidly with these simple measures. If a patient is

receiving such optimised therapy and his or her condition still does not improve, new adjuvant therapeutic strategies such as drotrecogin alfa (activated) should be considered.

Drotrecogin alfa (activated) has been shown to improve the outcome of patients with severe sepsis and APACHE II scores of ≥25 or ≥2 organ dysfunctions. When considering the use of drotrecogin alfa (activated), the time frame, contraindications for use, limited experience with various diagnoses and the prognosis of the underlying disease in the individual patient have to be considered. The potential benefits need to be balanced against the potential adverse effects and the costs of this drug in order to use financial resources responsibly, as the limitation of these resources is becoming more and more an issue in many countries. If used appropriately, however, all information available indicates that drotrecogin alfa (activated) is an expensive but highly cost-effective therapy for sepsis.

There are several other adjuvant therapeutic strategies for sepsis in various stages of clinical development; which of these will actually achieve approval for clinical use, like drotrecogin alfa (activated), cannot be predicted.

With the exception of drotrecogin alfa (activated), the development of new therapies for sepsis has almost uniformly followed the pattern: reasonable approach based on pathophysiological considerations → promising phase II trials → expensive and laborious, but disappointing phase III trials. Careful investigation of the reasons for this apparent paradox is likely to influence the fate of many of the therapeutic strategies investigated in future clinical trials.

Finally, it is unlikely that a single therapeutic approach may fit for all patients with sepsis (it may well be that the several different mechanisms by which drotrecogin alfa [activated] works, have provided its success). There is most probably no magic bullet for sepsis. Further development of therapies for sepsis may well depend on the availability of rapid diagnostic methods for determining which patient will have the most benefit from one or another adjuvant therapeutic intervention. Functional genomics and systemic biology research in this field should be encouraged if the full benefits of adjunct therapies for sepsis are to be realised in actual clinical practice.

Acknowledgements

S.M. Opal has received grant support from the Genetics Institute (Cambridge, MA, USA), is an investigator for Tissue Factor Pathway Inhibitor (Chiron, Emeryville, CA, USA) and is also a member of the Data and Safety Monitoring Board for both Eisai (Andover, MA, USA) and Lilly (Indianapolis, IN, USA). T. Glück has received honoraria for lectures from Lilly (Indianapolis, IN, USA) and has participated in clinical trials (Enhance, Address) which were sponsored by Eli Lilly.

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