Anti-Inflammatory Response in Severe Sepsis and Septic Shock

Doctoral dissertation

To be presented by permission of the Faculty of Medicine of the University of Kuopio for public examination in Auditorium, Päijät-Häme Central Hospital, on Friday 25th April 2008, at 12 noon

Faculty of Medicine
University of Kuopio
Activation of the systemic inflammatory response is an essential part of effective host defence mechanism in sepsis. In certain circumstances the activation of inflammatory pathways can be excessive, and overactive proinflammatory response may trigger pathophysiologic mechanisms, which lead to the development of multiple organ failure (MOF). To ensure that the effects of proinflammatory response do not become destructive, the compensatory anti-inflammatory response (CARS) is also activated in severe sepsis. The release of various anti-inflammatory cytokines and the activation of hypothalamic–pituitary adrenal axis are major components of this response. These anti-inflammatory mechanisms may have an important role in the controlling proinflammatory reactions, but the clinical significance of this response in sepsis is not fully established.

The objective of the present study was to evaluate the clinical significance of the compensatory anti-inflammatory response in severe sepsis and septic shock. The specific objectives were to investigate the role of relative adrenal insufficiency in the development and resolution of multiple organ failure (study I), to study the role of anti-inflammatory cytokine response in the pathogenesis of multiple organ failure (study II), to investigate changes in adrenocortical function in critically ill patients (study III) and to study the hemodynamic and metabolic effects of hydrocortisone therapy in septic shock (study IV).

One-hundred-seventy-three critically ill patients were included in the study. Adrenal insufficiency was detected in 22% of the patients with severe sepsis and 40% of septic shock patients. In severe sepsis, impaired adrenal function was associated with a poor resolution of multiple organ failure. In patients with severe multiple organ failure the IL-6/IL-10 ratio was significantly higher in the early phase of sepsis compared to those patients who did not develop MOF. In the identification of adrenal insufficiency, the current diagnostic methods turned to be unsatisfactory. Especially in septic shock a single ACTH stimulation test could not reveal accurately those patients who had impaired adrenal function, and the results of the two consecutive ACTH tests were poorly reproducible.

This study demonstrated that both adequate adrenal function and IL-10 response seemed to have an important protective function in the pathophysiology of sepsis and MOF. In septic shock the changes in adrenocortical function were very rapid and the single ACTH test was not reliable method in detecting adrenal insufficiency. In the treatment of septic shock, continuous hydrocortisone infusion was more effective in the maintenance of strict normoglycemia than conventional bolus treatment.

National Library of Medicine Classification: QW 568, QZ 140, WC 240, WK 515, WK 765,

Medical Subject Headings: Adrenal Cortex; Adrenal Insufficiency; Adrenocorticotropic Hormone; Anti-Inflammatory Agents; Blood Glucose; Hydrocortisone; Hyperglycemia; Interleukin-10; Interleukin-6; Multiple Organ Failure; Sepsis; Shock, Septic
To Eetu and Elina
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Lahti, March 2008

Pekka Loisa
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AAR</td>
<td>Adequate adrenal response</td>
</tr>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
</tr>
<tr>
<td>AVP</td>
<td>Arginine vasopressin</td>
</tr>
<tr>
<td>ARDS</td>
<td>Acute respiratory distress syndrome</td>
</tr>
<tr>
<td>APACHE</td>
<td>Acute physiologic and chronic health evaluation score</td>
</tr>
<tr>
<td>APC</td>
<td>Activated protein C</td>
</tr>
<tr>
<td>CARS</td>
<td>Compensatory anti-inflammatory response syndrome</td>
</tr>
<tr>
<td>CBG</td>
<td>Cortisol binding globulin</td>
</tr>
<tr>
<td>CRH</td>
<td>Corticotropin-releasing hormone</td>
</tr>
<tr>
<td>DHEA</td>
<td>Dehydroepiandrosterone</td>
</tr>
<tr>
<td>DHEAS</td>
<td>Dehydroepiandrosterone sulfate</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Granulocyte-colony stimulating factor</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Granulocyte-macrophage colony-stimulating factor</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal axis</td>
</tr>
<tr>
<td>HMGB</td>
<td>High mobility group box protein</td>
</tr>
<tr>
<td>IAR</td>
<td>Inadequate adrenal response</td>
</tr>
<tr>
<td>ICU</td>
<td>Intensive care unit</td>
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<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>Interleukin-1 receptor antagonist</td>
</tr>
<tr>
<td>LIF</td>
<td>Leukemia inhibitory factor</td>
</tr>
<tr>
<td>MIF</td>
<td>Macrophage migration inhibitory factor</td>
</tr>
<tr>
<td>MOF</td>
<td>Multiple organ failure</td>
</tr>
<tr>
<td>SAPS</td>
<td>Simplified acute physiology score</td>
</tr>
<tr>
<td>SIRS</td>
<td>Systemic inflammatory response syndrome</td>
</tr>
<tr>
<td>SMR</td>
<td>Standardized mortality ratio</td>
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<tr>
<td>SOFA</td>
<td>Sequential organ failure assessment</td>
</tr>
<tr>
<td>TFPI</td>
<td>Tissue factor pathway inhibitor</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor-α</td>
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</table>
LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals.


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1. INTRODUCTION

Severe sepsis and septic shock are major challenges in intensive care (ICU) units. Despite the development of critical care medicine, the mortality from sepsis has remained considerably high. Severe sepsis is associated with a mortality rate of 25 - 30% and in septic shock the hospital mortality is still 40 - 70% (Bernard 2001, Rivers 2001, Dellinger 2003). Severe sepsis and septic shock are frequent causes of death in intensive care units and in 2001, severe sepsis and septic shock were responsible for approximately 750 000 hospital admissions and 210 000 deaths in United States (Angus 2001). Recent epidemiological population-based studies suggest that sepsis is becoming more common (Martin 2003, Brun-Buisson 2004). In Finland, the incidence of severe sepsis in ICUs is 0.38 / 1000 in the adult population (Karlsson 2007).

In addition to high mortality, patients with sepsis consume a considerable amount of ICU resources and the cost associated with sepsis are substantial (Angus 2001, Weycker 2003, Brun-Buisson 2004). Especially the development of multiple organ failure (MOF) causes significant prolongation of ICU stay, and MOF further worsens patients’ prognosis (Beal 1994, Vincent 1998). A better understanding about the pathophysiology of sepsis has demonstrated that microbes themselves do not cause multiple organ failure, but rather infection initiates underlying host reactions, which cause endothelial damage, increased vascular permeability, activation of intravascular coagulation and apoptosis that ultimately lead to the development of progressive organ dysfunction.

A prolonged and amplified systemic inflammatory response (SIRS) and concomitant release of proinflammatory cytokines has been traditionally considered to be a central pathophysiologic mechanism in the development of multiple organ failure in sepsis (Pinsky 1993). The concept of uncontrolled inflammation behind MOF has led to the numerous clinical trials which aimed at blocking various proinflammatory cascades in the early phase of sepsis. The results of these studies consistently failed to show any benefit of the immunomodulatory therapies. These findings have led to a re-evaluation of the model of sepsis.
Recent studies have suggested that sepsis is a bimodal entity. In addition to the activation of the inflammatory response, numerous anti-inflammatory reactions are launched during sepsis and the production and release of cytokine receptor antagonists, the soluble cytokine receptors and the anti-inflammatory cytokines are enhanced (Opal 2000). Sepsis also causes numerous endocrinological alterations. Especially the activation of the hypothalamopituitary-adrenal-axis has an important role in the regulation of the inflammatory response (Chrousos 1995, Beishuizen 2004). These anti-inflammatory responses control the magnitude of the inflammatory reactions. In clinical sepsis pro- and anti-inflammatory mechanisms are linked and interrelated to each other, forming a complex interactive network of endogenous immunological host reactions.

The anti-inflammatory reactions in sepsis have been named as compensatory anti-inflammatory response syndrome (CARS) by Roger Bone, inventor of the SIRS concept (Bone 1996). In theory, it is possible that anti-inflammatory reactions may have an important role in controlling inflammatory reactions, but the clinical significance of these reactions is so far not fully understood. In some studies anti-inflammatory reactions are considered to be protective (Taniguchi 1999). In other studies magnitude of anti-inflammatory response have been associated with profound immunosuppression and increased mortality (Gogos 2000).

In this study, the aim was to further investigate the clinical significance of anti-inflammatory mechanisms in severe sepsis and septic shock. Special attention was focused on the role of anti-inflammatory cytokines IL-10 and IL-1ra and endogenous cortisol production in the pathogenesis of multiple organ failure and changes in adrenocortical function in severe sepsis and septic shock. The second purpose in this study was to investigate different hydrocortisone treatment modalities and their metabolic and hemodynamic effects in the treatment of septic shock.
2. REVIEW OF LITERATURE

2. 1. Systemic inflammatory response and sepsis

Definition of sepsis

Sepsis is defined as the systemic inflammatory response to infection (American College of Chest Physicians / Society of Critical Care Medicine Consensus Conference 1992). This definition was introduced by the American College of Chest Physicians and the Society of Critical Care Medicine consensus conference in 1991. Before this consensus conference the terms sepsis, bacteremia, septicemia and sepsis syndrome were used interchangeably to characterize patients with severe generalized infection. The need for firm and generally accepted definitions became apparent when studies assessing the effect of high-dose corticosteroid therapy in the treatment of sepsis were published in the 1980s (Sprung 1984, Bone 1987, VASSCS 1987). At that time point, the heterogeneity of the study populations and the absence of uniform definitions of sepsis prevented comparison of the study results.

In the ACCP / SCCM consensus conference, new definitions for sepsis were agreed. According to these guidelines, sepsis was defined as a systemic response to infection and the conference proposed a new term, systemic inflammatory response syndrome (SIRS) to describe inflammatory process that occurs in conjunction with generalized infection (Bone 1992). The systemic inflammatory response syndrome has several clinical manifestations, including abnormalities of body temperature, respiratory rate, heart rate and leukocyte count. In addition to SIRS criteria, the consensus conference set the definitions for severe sepsis, septic shock and multiple organ dysfunction syndrome. These definitions are presented in the Table 1. In 2001 American and European critical care societies re-examined the 1991 ACCP/SCCM consensus conference definitions. The conclusion was that the concepts based on SIRS, although overly sensitive and nonspecific, are still useful in the diagnosis of sepsis and septic shock (Levy 2003).
Table 1. ACCP/SCCM consensus conference criteria for the systemic inflammatory response syndrome, sepsis, severe sepsis and septic shock.

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>SIRS</td>
<td>Systemic inflammatory response syndrome. The systemic inflammatory response is manifested with two or more of the following criteria:</td>
</tr>
<tr>
<td></td>
<td>Fever (body temperature &gt; 38°C) or hypothermia (body temperature &lt; 36°C)</td>
</tr>
<tr>
<td></td>
<td>Tachycardia (heart rate &gt; 90 beats/min)</td>
</tr>
<tr>
<td></td>
<td>Tachypnea (&gt; 20 breaths/min) or PaCO2 &lt; 4.3 kPa</td>
</tr>
<tr>
<td></td>
<td>Leukocytosis or leukopenia (white blood cell count &gt; 12,000 or &lt; 4,000/mm³) or &gt; 10% immature forms</td>
</tr>
<tr>
<td>Sepsis</td>
<td>Presence of SIRS in response to infection. SIRS in manifested by two or more of the criteria mentioned above</td>
</tr>
<tr>
<td>Severe Sepsis</td>
<td>Sepsis associated with organ dysfunction, hypoperfusion or hypotension. Organ dysfunction and hypoperfusion abnormalities may include, but are not limited to lactic acidosis, oliguria, or an alteration in mental status</td>
</tr>
<tr>
<td>Septic shock</td>
<td>Sepsis with hypotension despite adequate fluid resuscitation, along with the presence of perfusion abnormalities. Hypotension is defined as a systolic blood pressure &lt; 90 mmHg or a decrease of systolic blood pressure by 40 mmHg or more from the baseline</td>
</tr>
</tbody>
</table>
Epidemiology of SIRS

SIRS, sepsis, severe sepsis and septic shock represent the continuum of the same systemic response with increasing severity of the disease process. Using the ACCP/SCCM definitions, Rangel-Frausto et al. provided evidence of a clinical progression from SIRS to sepsis and further to severe sepsis and septic shock. In three intensive care units and three general wards 26 per cent of the patients with SIRS developed sepsis, 18% severe sepsis and 4% septic shock (Rangel-Frausto 1995). 44% to 71% of patients in any category demonstrated a disease progression from one state to another. Furthermore, a stepwise increase in mortality was observed as the disease process progressed from SIRS to sepsis, to severe sepsis and to septic shock. The mortality rates were 7% in patients with SIRS, 16% in patients with sepsis, 20% in severe sepsis and 46% in septic shock. A similar progressive increase in mortality from SIRS to sepsis to severe sepsis and to septic shock was observed in the epidemiological study performed in 99 Italian intensive care units (Salvo 1995). The mortality rates in this study were 27%, 36%, 52% and 82%, respectively.

Since the ACCP/SCCM consensus conference the SIRS concept has been implemented into critical care terminology worldwide. Despite the general agreement, however, the concept has raised extensive criticism. Several authors have emphasized that significant limitations exist in the application of sepsis definitions into clinical practice (Salvo 1995, Opal 1998). The definitions of SIRS are broad, and the clinical manifestations of the systemic inflammatory response are sensitive, but at the same time the specificity is very poor. SIRS can be triggered either by an infectious agents but also a numerous noninfectious insults can launch cascades that results to the development of SIRS. The majority of the ICU patients and patients with trauma, recent surgery, myocardial infarction or pulmonary embolism meet SIRS criteria without any evidence of sepsis (Muckart 1997, Pittet 1995, Vincent 1997). Bossink et al. demonstrated that 95% of the febrile medical patients met the two or more clinical criteria for SIRS, but only 44% of the patients developed sepsis (Bossink 1998). Pittet et al. demonstrated similar figures in surgical patients (Pittet 1995). Table 2 summarizes clinical frequencies of SIRS, sepsis, severe sepsis and septic shock. Because of poor specificity it has been suggested
that SIRS criteria in the diagnosis of sepsis can be misleading and potentially even harmful (Vincent 1997). The presence of infection is a fundamental part of the pathophysiology of sepsis, and sepsis should be only diagnosed at the presence of SIRS when infection is confirmed or strongly suspected. However, in 30% of patients the definitive origin of infection cannot be determined (Brun-Buisson 1995).

Table 2. Clinical frequency of SIRS, sepsis, severe sepsis and septic shock.

<table>
<thead>
<tr>
<th>Reference</th>
<th>No patients</th>
<th>SIRS</th>
<th>Sepsis</th>
<th>Severe sepsis</th>
<th>Septic shock</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rangel-Frausto 1995</td>
<td>3 708</td>
<td>68%</td>
<td>18%</td>
<td>13%</td>
<td>3.0%</td>
</tr>
<tr>
<td>Salvo 1995</td>
<td>1 101</td>
<td>58%</td>
<td>16%</td>
<td>5.5%</td>
<td>6.1%</td>
</tr>
<tr>
<td>Pittet 1995</td>
<td>170</td>
<td>74%</td>
<td>19%</td>
<td>12%</td>
<td>5.3%</td>
</tr>
<tr>
<td>Muckart 1997</td>
<td>450</td>
<td>88%</td>
<td>14%</td>
<td>14%</td>
<td>20%</td>
</tr>
</tbody>
</table>
2.2. Pathophysiology of SIRS

Activation of innate and adaptive immunity

Activation of the systemic inflammatory response is needed for effective host defense against infection. Multiple inflammatory pathways are activated in the initial stage of sepsis in order to handle the bacterial invasion. These mechanisms include the release of cytokines, activation of neutrophils, monocytes, macrophages and endothelial cells, and activation of complement, coagulation, fibrinolytic and contact systems (Hack 2000). The release of tissue-damaging proteinases, eicosanoids and oxygen and nitrogen radicals are also enhanced as a part of effective host defense mechanisms (Hack 2000).

Toll-like receptors regulate antimicrobial host defense mechanisms and play a central role in the activation of innate immunity (Kopp 1999). Toll-like receptors are a family of cellular surface protein receptors that recognize molecular components of various micro-organisms. Bacterial components including lipopolysaccharide, lipoteichoic acid, flagellin and other cell wall components interact with Toll-like receptors and different microbial products bind to different receptors. TLR2 and TLR6 has been shown to react with lipoteichoic acid, TLR4 with lipopolysaccharide, and TLR5 with flagellin (Warren 2005). These findings implicate that the innate immune response is tailored in a pathogen specific manner (Kopp 1999).

In the initial phase of infection Toll-like receptors active innate immune system and invading pathogens are destroyed by macrophages, natural killer cells and complement system. In the second phase, Toll-like receptors from an important link between innate and adaptive immunity, and these receptors activate adaptive immune system by activating T and B lymphocytes (Modlin 2000). In this process, cytokine production has a fundamental role. Cytokines are endogenous immunomodulating proteins which have important role in the activation and regulation of various inflammatory reactions. Numerous host cells are capable of secreting cytokines upon stimulation. Activated macrophages and monocytes are the primary cells that produce cytokines but
fibroblasts, neutrophils and endothelial cells are also involved in the production of cytokines (Hack 1997).

Cytokines usually influence adjacent cells, but they can also have actions throughout the body or on the secreting cell itself. Cytokine signaling is in most conditions a local process, but once cytokines access to the bloodstream, they can induce a systemic response. Cytokines can be classified into proinflammatory and anti-inflammatory cytokines depending on their principal function, but many cytokines have pleiotropic effects (Hack 2000). As more and more studies are available, it has become evident that the majority of proinflammatory cytokines have also anti-inflammatory properties and vice versa (Opal 2000). The net effect of any cytokine is dependent on the timing of cytokine release, the local milieu in which it acts, the presence of competing or synergistic elements, cytokine receptor density, and tissue responsiveness to a specific cytokine (Opal 2000).

The most extensively studied cytokines in sepsis are TNF-α, IL-1, IL-6, IL-8 IL-10 and IL-1ra, but also large number of other cytokines (IL-4, IL-12, IF-γ, LIF, MIF, G-CSF, GM-CSF, HMGB-1) are involved in the pathogenesis of sepsis (Hack 1997, Yang 2001). The central cytokines and their main actions in the pathophysiology of sepsis are presented in the Table 3.
### Table 3. Principal cytokines and their actions in sepsis.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Nature</th>
<th>Main source</th>
<th>Principal actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>proinflammatory</td>
<td>monocytes, macrophages</td>
<td>- activates release of other proinflammatory cytokines</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- activates coagulation and complement systems</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- activates adhesion molecule synthesis</td>
</tr>
<tr>
<td>IL-1β</td>
<td>proinflammatory</td>
<td>monocytes, macrophages</td>
<td>- physiological actions similar and overlapping with TNFα</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- together with TNFα exerts synergistic effects</td>
</tr>
<tr>
<td>IL-6</td>
<td>proinflammatory anti-inflammatory</td>
<td>monocytes, macrophages, endothelial cells</td>
<td>- regulates B and T lymphocyte differentiation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- stimulates synthesis of acute phase proteins</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- inhibits production of proinflammatory cytokines</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- activates HPA axis</td>
</tr>
<tr>
<td>IL-8</td>
<td>proinflammatory anti-inflammatory</td>
<td>monocytes, macrophages, endothelial cells, epithelial cells</td>
<td>- induction of chemotaxis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- activates neutrophils</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- regulates neutrophil migration</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>anti-inflammatory</td>
<td>monocytes, macrophages</td>
<td>- inhibits activity of IL-1</td>
</tr>
<tr>
<td>IL-10</td>
<td>anti-inflammatory</td>
<td>lymphocytes, monocytes, macrophages</td>
<td>- inhibits production of proinflammatory cytokines</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- stimulates Th2-mediated immunity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- regulates T and B cell proliferation</td>
</tr>
</tbody>
</table>
Proinflammatory cytokines

Tumor necrosis factor alpha (TNF-α) and interleukin-1β (IL-1β) are the principal proinflammatory cytokines that are responsible for the initial activation of the systemic inflammatory response in sepsis (Hack 1997). Although these cytokines bind to different cellular receptors, they have multiple overlapping and synergistic effects in inflammation (Waage 1988). Both of these cytokines have powerful proinflammatory effects. Especially TNF-α is considered to be extremely cytotoxic. TNF-α is produced mainly by monocytes and macrophages. TNF-α induces the production of adhesion molecules in endothelial cells, it activates the production of various other cytokines like IL-6 and IL-8 and it also activates coagulation and complement systems (van der Poll 1990, Dinarello 1997). Administration of TNF-α have resulted in fever, tachycardia, hypotension, leukocytosis or leucopenia, elevated liver enzymes, elevated creatinine levels and coagulopathy, all typical features in septic shock (Tracey 1986, Natanson 1989). In experimental sepsis, the neutralization of TNF-α with monoclonal antibodies has prevented the development of shock and death (Beutler 1985).

In experimental sepsis, the peak concentrations of TNF-α are detected very early after the administration of endotoxin, and no detectable concentrations of TNF-α are observed after 10 hours period due to short half-life of TNF-α (Michie 1988, Hack 2000). In clinical sepsis, Waage et al. were first to demonstrate increased circulating TNF-α levels in 30% of patients with severe meningococcal disease (Waage 1987). Furthermore, increased plasma levels of TNF-α correlated with patient outcome (Waage 1987). Increased TNF-α levels generally correlate with the severity of illness, but there are also studies that have failed to confirm any correlation between elevated TNF-α levels and patients’ prognosis (Damas 1992, Pinsky 1993, Casey 1993, Martin 1994). An evident reason of this discrepancy is the very short half-life of TNF-α, which makes the timing of the cytokine samples very crucial.

Together with TNF-α, interleukin-1 (IL-1) is considered to be a central endogenous proinflammatory mediator in sepsis. IL-1 is mainly produced by monocytes and macrophages (Dinarello 1991). IL-1 consists of two structurally related cytokines IL-1α
and IL-1β. IL-1α is very rarely found in circulation, and IL-1α functions mainly as an intracellular messenger (Dinarello 1991). In contrast to IL-1α, IL-1β is released into extracellular space. The biologic actions of IL-1β are similar to TNF-α, and these two cytokines have a synergistic effect (Waage 1988). IL-1β induces the secretion of other cytokines including IL-6, IL-8 and TNF-α, and it is capable to induce hemodynamic changes similar to septic shock (Dinarello 1991). In experimental sepsis circulating IL-1β reach the peak levels after 2-3 h after the endotoxin challenge (Granowitz 1999). The half-life of IL-1β is very short and in clinical sepsis IL-1β levels are often undetectable (Cannon 1990). In most studies, IL-1β levels have correlated very poorly with the severity of the disease (Damas 1992, Pinsky 1993, Casey 1993, Goldie 1995).

**Multimodal cytokines**

Interleukin-6 (IL-6) is the most extensively studied cytokine in sepsis. IL-6 levels are elevated for a longer period of time than TNF-α and IL-1β. In most studies IL-6 levels are significantly elevated in the majority of patients with sepsis. IL-6 is produced mainly by monocytes, macrophages and endothelial cells, but virtually every cell in the body can synthesize IL-6 upon appropriate stimulation (Hack 1997). TNF-α, IL-1 and endotoxin are the main inducers of IL-6 production (Hack 2000).

In several studies, the circulating IL-6 levels correlate well with the severity of sepsis (Hack 1989, Calandra 1991, Damas 1992), and the persistently high levels of IL-6 seem to associate with the development of MOF and poor prognosis (Pinsky 1993). Although elevated IL-6 levels associate with increased mortality in sepsis, the exact role of IL-6 in the pathogenesis of sepsis is not clear (Hack 1997). IL-6 is relatively non-toxic cytokine. It does not activate neutrophils or endothelial cell and it does not induce a septic shock-like state (Preiser 1991). IL-6 regulates the growth of various cells, especially the differentiation of B and T lymphocytes (Hack 1997). IL-6 is an endogenous pyrogen, and fever in patients with sepsis may be induced by IL-6 (Dinarello 1997). IL-6 plays a major role as a mediator of the acute-phase response, and it induces the synthesis of acute-phase proteins in liver. In sepsis, elevated IL-6 levels
reflect the activation of inflammatory response, and IL-6 is considered to be an alarm hormone during inflammation (Hack 1989). IL-6 has also specific anti-inflammatory properties (Xing 1998). IL-6 inhibits the production of other proinflammatory cytokines and an adequate IL-6 response may also have important role in the activation of the hypothalamic-pituitary-adrenal axis in critical illness (Chrousos 1995, Xing 1998).

Interleukin-8 (IL-8) is a prototype of a chemotactic cytokine. The primary function of IL-8 is to activate and chemoattract neutrophils to the sites of inflammation (Hack 1997). Monocytes, macrophages, neutrophils, endothelial and epithelial cells are able to synthesize IL-8 and IL-8 production is also enhanced by other proinflammatory cytokines (Hack 1997). Endotoxin, TNF-α and IL-1β are major activators of IL-8 production. In addition to the inflammatory mediators, also thrombin, ischemia and reperfusion can activate IL-8 release (Colotta 1994, Metinko 1992). IL-8 regulates leukocyte activation and migration during inflammation. Neutrophils are highly specific target cells for IL-8, but the role of IL-8 to the neutrophils is pivotal. High local concentrations of IL-8 induce neutrophil infiltration, endothelial damage, plasma leakage, and the development of local tissue injury (Colditz 1989). In contrast, high circulating intravascular IL-8 levels inhibit the migration of neutrophils in the tissues and IL-8 therefore has both anti- and proinflammatory properties, depending mainly on the site of its production (Hechtman 1991). Administration of IL-8 causes transient leukopenia, but it does not induce hemodynamic or metabolic alterations of sepsis, and it cannot induce septic shock state (Hack 1997).

Anti-inflammatory cytokines

In addition to proinflammatory cytokines, sepsis also activates the production and release of specific anti-inflammatory substances, including cytokine receptor antagonists, soluble cytokine receptors and anti-inflammatory cytokines (Granowitz 1991, Goldie 1995, Opal 2000). Interleukin-1 receptor antagonist (IL-1ra) is a naturally occurring inhibitor of IL-1, which competitively binds to the IL-1 receptor and inhibits the actions of IL-1 (Dinarello 1991). IL-1ra attenuates endotoxin effects in animal
models of sepsis, and it also reduces mortality (Fischer 1992). IL-1ra is produced mainly by macrophages. In experimental sepsis, the concentrations of circulating IL-1ra are 100-fold higher than those of IL-1β (Granowitz 1991). Although IL-1ra reduces mortality in experimental endotoxemia, its clinical relevance of IL-1ra production in sepsis is still unclear. Trials using exogenous IL-1ra in clinical sepsis have failed to demonstrate a definitive improvement in mortality (Fisher 1994, Opal 1997).

Interleukin-10 (IL-10) is considered to be a central anti-inflammatory cytokine. IL-10 was initially characterized as a cytokine that inhibited interferon (IFN)-γ synthesis, but IL-10 also has other important down-regulatory functions in relation to other proinflammatory cytokines (Fiorentino 1991). IL-10 inhibits the production of TNF-α, IL-1β, IL-6 and IL-8 (Moore 1993, Asadullah 2003). IL-10 suppresses free oxygen radical release and nitric oxide activity of macrophages and the production of prostaglandins (Goldman 1996). A major stimulus for the production of IL-10 is inflammation itself, and IL-1β and TNF-α can stimulate IL-10 production directly (Hack 1997). Several cell types can produce IL-10, including CD4+ and CD8+ T cells, macrophages, monocytes, B cells, dendritic cells and epithelial cells (Moore 1993). In septic shock, monocytes are a major source of IL-10 (Goldman 1996).

IL-10 not only limits the magnitude of the inflammatory response, but it also regulates the proliferation of T cells, B cells, natural killer cells, antigen-presenting cells, mast cells, and granulocytes (Asadullah 2003). IL-10 is a pluripotent cytokine that is considered to be an important molecule in immunoregulation and modulation of host defence reactions. IL-10 mainly mediates suppressive functions, but IL-10 also has stimulatory properties of innate immunity and of Th2-related immunity (Asadullah 2003). In animal models of septic shock, administration of IL-10 has prevented endotoxin-induced mortality (Howard 1993). Several studies have documented elevated plasma IL-10 concentrations in sepsis (Marchant 1994, Derx 1995, Gogos 2000). In septic shock IL-10 levels are higher than in sepsis (Derx 1995). Moreover, IL-10 levels have correlated positively with levels of proinflammatory cytokines and the severity of the septic shock (Friedman 1997).
2.3 Immunomodulatory trials

After the discovery of proinflammatory cytokines, sepsis was considered to be fundamentally a disease caused by uncontrolled inflammation. In several studies increased levels of proinflammatory cytokines were reported to correlate with the severity of sepsis (Hack 1989, Damas 1992, Martin 1994, Goldie 1995), and especially persistently high levels of IL-6 were associated with the development of MOF and poor prognosis (Pinsky 1993).

In 1985 Beutler, Milsark and Cerami demonstrated for the first time that neutralization of endogenous TNF by infusing antibodies against TNF was protective in experimental septic shock (Beutler 1985). This finding led to substantial boost in investigations that were able to demonstrate that the inhibition of various inflammatory mediators had beneficial effects in animal models of sepsis. After successful experimental studies, several randomized double-blind placebo-controlled trials were carried out to modify inflammatory response by specific anti-inflammatory agents in clinical sepsis (Marshall 2000). These included studies of administering monoclonal antibodies against endotoxin, interleukin-1 receptor antagonist (IL-1ra), monoclonal antibodies against TNF-α and soluble TNF-α receptors. Despite encouraging results in the experimental and preliminary clinical trials, the large phase III trials could not confirm beneficial effects on patient outcome (Table 4).
Table 4. Randomized, placebo controlled phase III immunomodulatory trials in severe sepsis and septic shock.

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Number of patients</th>
<th>28-day mortality placebo group</th>
<th>28-day mortality treatment group</th>
<th>Absolute risk reduction (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antiendotoxin therapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ziegler 1991</td>
<td>531</td>
<td>43%</td>
<td>39%</td>
<td>- 4% (-12% - +5%)</td>
</tr>
<tr>
<td>McCloskey 1994</td>
<td>2199</td>
<td>36%</td>
<td>38%</td>
<td>+ 3% (-1% - +7%)</td>
</tr>
<tr>
<td><strong>IL -1 receptor antagonist therapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fisher 1994</td>
<td>893</td>
<td>34%</td>
<td>30%</td>
<td>- 4% (-8% - +1%)</td>
</tr>
<tr>
<td>Opal 1997</td>
<td>696</td>
<td>41%</td>
<td>39%</td>
<td>- 2% (-9% - +5%)</td>
</tr>
<tr>
<td><strong>Soluble TNF-receptor fusion protein therapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abraham 1997</td>
<td>498</td>
<td>39%</td>
<td>35%</td>
<td>- 4% (-14% - +6%)</td>
</tr>
<tr>
<td>Abraham 2001</td>
<td>1342</td>
<td>28%</td>
<td>27%</td>
<td>- 1% (-6% - +4%)</td>
</tr>
<tr>
<td><strong>Monoclonal TNF-antibody treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abraham 1998</td>
<td>1878</td>
<td>43%</td>
<td>40%</td>
<td>- 3% (-7% - +2%)</td>
</tr>
<tr>
<td>Reinhart 2001</td>
<td>944</td>
<td>58%</td>
<td>54%</td>
<td>- 4% (-13% - +6%)</td>
</tr>
<tr>
<td>Panacek 2004</td>
<td>998</td>
<td>48%</td>
<td>44%</td>
<td>- 4% (-10% - +2%)</td>
</tr>
</tbody>
</table>

CI: confidence interval.
A common feature in these trials was that anti-inflammatory treatments usually demonstrated marginally beneficial effects on survival, but this difference did not reach the statistical significance (Marshall 2000). A retrospective subgroup analysis in the first phase III clinical trial of IL-1ra suggested that the treatment with IL-1ra caused a dose-related increase in survival among patients with highest risk of death (Fisher 1994). The second phase III trial, however, could not confirm these beneficial findings, and the study was terminated after an interim analysis found that it was unlikely that the primary efficacy endpoints would be met (Opal 1997). Studies which aimed to TNF neutralization generally showed small nonsignificant survival benefit in the treatment group and a pooled data revealed 3.5% reduction in mortality (Marshall 2000). A striking exception was obtained in a phase II clinical trial using a TNF inhibitor, in which a significant dose-related increase in mortality was observed in those patients who received TNFR:Fc therapy (Fisher 1996).

Proinflammatory cytokines are considered to have a major cytotoxic effect in sepsis, but they also have beneficial effects. An adequate inflammatory response is needed to cope with an infectious insult, and a complete blocking of the inflammatory cascade is detrimental (Opal 1996). Proinflammatory cytokines are involved in the immunological response devoted to the elimination of the invading organism. In experimental studies, there are several reports in which worsening of an infection has been demonstrated by the complete blocking the actions of TNF (Grau 1997). Blocking the actions of one key cytokine disturbs the balance between proinflammatory and anti-inflammatory response. An ideal immunomodulatory treatment should be able to block the toxic effects of cytokines while preserving the beneficial effects. Failure of the immunomodulatory trials may be due to fact that patients with sepsis represent a very heterogeneous group of patients (Marshall 2000). It has been suggested that the immunomodulatory therapies could be beneficial in a subgroup of patients who have an apparent hyperinflammatory response during sepsis (Reinhart 1996, Reinhart and Karzai 2001). For example, in the MONARCS trial monoclonal antibodies to TNF-α seemed to reduce mortality in the subgroup of patients with IL-6 levels above 1000 pg/ml (Panacek 2004).
2.4 Compensatory anti-inflammatory response

Compensatory anti-inflammatory response in clinical sepsis

After the negative results obtained from immunomodulatory trials, it has become evident that sepsis-triggered immunological cascades are bidirectional. In addition to the systemic inflammatory response, the compensatory anti-inflammatory response (CARS) is also activated in sepsis (Bone 1996). The early proinflammatory period is progressively suppressed by the development of the anti-inflammatory response, which probably has an important down-regulating role of various inflammatory reactions. To ensure that the effects of proinflammatory mediators do not become destructive, the body launches anti-inflammatory substances, including IL-4, IL-10, IL-11, IL-13, soluble tumor necrosis factor receptors, interleukin-1 receptor antagonists and transforming growth factors (Bone 1996, Opal 2000). Theoretically, these anti-inflammatory substances may have an important regulatory function in controlling and attenuating the systemic inflammatory response in sepsis, but the exact role of the compensatory anti-inflammatory response is not completely understood (Goldie 1995, Bone 1996).

Endogenous IL-10 production may represent an important regulatory mechanism in CARS, which controls the intensity of the inflammatory reactions. In experimental gram-negative sepsis, IL-10 production has shown to have an important protective function (Goldman 1996). Similar favourable effects of IL-10 production were observed in gram-positive sepsis (Floriquin 1994). In an animal model of septic peritonitis IL-10 has prevented lethal complications and several clinical reports suggest that endogenous IL-10 production may have important protective effects in ARDS, acute pancreatitis and in SIRS (van der Poll 1995, Donelly 1996, Armstrong 1997, Simovic 1999, Taniguchi 1999).

In certain circumstances, the anti-inflammatory response may also have detrimental immunosuppressive effects. First evidence of exaggerated immunosuppression in sepsis
was obtained as early as in 1977, when Meakins and coworkers demonstrated a loss of delayed hypersensitivity as a marker of anergy (Meakins 1977). Later Ertel et al. demonstrated that lipopolysaccharide-stimulated whole blood from patients with sepsis released markedly smaller quantities of the proinflammatory cytokines than blood from control patients (Ertel 1995). Other features which indicate excessive immunosuppression include defects in antigen presentation (Oberholzer 2001), decreased macrophage activation (Hotchkiss 2003), defective T-cell proliferation (Heidecke 1999), decreased monocyte HLA-DR expression (Kox 2000, Keh 2003, Hynninen 2003) and increased T-cell and B-cell apoptosis (Hotchkiss 2001). The disproportionate release of anti-inflammatory mediators may manifest clinically as an increased susceptibility to nosocomial infections (O'Sullivan 1995).

Excessive production of IL-10 may mediate detrimental immunosuppressive actions in sepsis (Perl 2006). Although other studies have documented beneficial effects associated with adequate IL-10 production, there are studies where highest IL-10 levels have been observed among the nonsurvivors, and it has been proposed that the sustained overproduction of IL-10 is the major predictor of poor outcome in sepsis (van Dissel 1998, Gogos 2000). The relationship between the proinflammatory and anti-inflammatory cytokine responses in sepsis is inconsistent and varies between the studies. The conflicting results can be explained by the differences in the study populations and the short half-life of cytokines in the circulation. In clinical sepsis tissue concentrations of cytokines can be significant, but the plasma concentrations may be extremely low due to rapid elimination from circulation (Hack 1997).

Modulation of SIRS / CARS balance

Genetic factors modify both intensity and nature of the individual inflammatory response (Westendorp 1997). Genetic predisposition alters inflammatory response because genomic polymorphisms influence to the capacity of immune cells to produce cytokines. Multiple genomic polymorphisms within the genes encoding proinflammatory and anti-inflammatory cytokines, as well as cytokine receptor
antagonists have been identified (Holmes 2003). Genetic factors have been shown to have a significant impact on TNF-α production and monocytes of healthy individuals show large differences in TNF-α production after standardized stimulation (Jacob 1991). In clinical studies, TNF-α genomic polymorphism have been found to influence patient outcome in severe sepsis and certain alleles have been associated with significantly elevated TNF-α levels, development of multiple organ failure and increased mortality (Stuber 1996, Nadel 1996). Polymorphisms within anti-inflammatory cytokine genes have also been reported to have an impact on cytokine production (Schaaf 2003).

An elementary feature in the modulation of the individual immune response is the functional diversity of T helper lymphocytes (Abbas 1996). CD4+ T-helper (Th) lymphocytes can differentiate into functionally two different subsets of Th cells depending on the microenvironment of the cell. Precursor T helper (Th0) cells can develop either to Th1 or Th2 cells, which produce distinct patterns of cytokines (Mossman 1996). Th1 cells secrete interleukin-2 (IL-2) and interferon-γ (IFN-γ), thus creating a proinflammatory response, whereas Th2 cells produce anti-inflammatory response by secreting IL-4, IL-5, IL-6, IL-10, and IL-13 (Mosmann 1996).

In sepsis, site and type of infection modulate Th1/Th2 balance and local cytokine concentrations have substantial influence on T cell differentiation (van Deventer 2000). IL-4, IL-10 and IFN-γ are considered to be central cytokines that modulate this balance (Mosmann 1996, Abbas 1996). Both IL-10 and IFN-γ cross-regulate T cell differentiation. IFN-γ produced by Th1 cells amplifies the growth of Th1 cells and inhibits proliferation of Th2 cells, whereas IL-10 produced by Th2 cells blocks the activation of Th1 cells (Sher 1991, Asadullah 2003). IL-10 and IFN-γ induce self-amplification in Th cell maturation and once immune response begins to develop along one pathway, it becomes progressively polarized in that direction (Abbas 1996). The Th1/Th2 balance may be disturbed in severe sepsis, and in flow cytometry analysis alterations in T helper cell subset favouring Th2 response have been detected (Ferguson 1999). In addition to IL-10, IL-4 and IFN-γ, endogenous cortisol production modulates Th1/Th2 response in individual patients (Gonzalez 2006).
2.5. Hormonal regulation of the inflammatory process

Hypothalamic-pituitary adrenal activation in sepsis

Activation of the immune system in critical illness is accompanied by endocrinological alterations to provide optimal conditions to cope with acute stress. In the clinical setting neuroendocrine and immune system are linked together, and especially the activation of the hypothalamic-pituitary-adrenal (HPA) axis has significant effect on immune-mediated inflammatory reactions (Chrousos 1995). Although immunosuppressive effects of cortisol have been known for decades, it has only recently become apparent that immuno-neuroendocrine interactions are bidirectional (Beihuizen 2004).

In severe sepsis and septic shock serum cortisol levels are substantially elevated (Schein 1990). This activation of HPA axis and subsequent increase in cortisol production is considered to be essential for survival (Melby 1958, Finlay 1982, Rothwell 1991). Cortisol has a vital role in the maintenance of vascular tone, endothelial integrity, vascular permeability and the distribution of total body water within the vascular compartments (Lamberts 1997, Zaloga 2001). Adequate cortisol production has a crucial role in the maintenance of cardiovascular homeostasis during acute stress. In experimental sepsis adrenalectomy leads to fatal circulatory collapse, which can be prevented by replacement of corticosteroids (Hinshaw 1985).

Further evidence for the vital role of intact adrenal activity in critical illness was obtained by the etomidate-induced hypocortisolism. Etomidate blocks cortisol synthesis by inhibiting 11-β-hydroxylase activity. In 1979-1982 ICU mortality among trauma patients increased from 25% to 44% when etomidate was used as an anaesthetic agent in critical care units (Ledingham 1983). Later the inhibitory effects of etomidate were confirmed in a randomized prospective trial (Absalom 1999). In prolonged septic shock reduced adrenoreceptor sensitivity to vasopressors may be restored by corticosteroids, and cortisol can potentiate the vasoconstrictive action of catecholamines by increasing beta-adrenergic receptor synthesis and density (Saito 1995, Saito 1996, Annane 1998).
Glucocorticoids have potent anti-inflammatory and immunomodulatory effects. Cortisol inhibits transcription of the genes encoding pro-inflammatory cytokines by reducing nuclear factor kappa (NF-κB) activity (Auphan 1995). As a result, corticosteroids block the synthesis or the action of most pro-inflammatory cytokines (IL-1β, IL-2, IL-3, IL-6, IFN-γ and TNF-α) (Auphan 1995, Zuckerman 1989). Although the majority of the anti-inflammatory effects of corticosteroids are due to direct suppression of proinflammatory cytokine synthesis, part of the effects are due to the enhanced production of anti-inflammatory cytokines, like IL-10 (Tabardel 1996). Glucocorticoids induce a shift from a proinflammatory Th1 response to a Th2 response, which enhances the production of IL-4, IL-10 and IL-13 (Ramirez 1996). Glucocorticoids limit inflammatory reactions by decreasing expression of adhesion molecules, suppressing the release of proteolytic enzymes and inhibiting cyclo-oxygenase and inducible nitric oxide synthase activity (Di Rosa 1990, Cronstein 1992). The inhibition of nitric oxide synthase and cyclo-oxygenase-2 activity not only down-regulate inflammatory reactions, but also exert positive effects on hemodynamics by limiting the production of vasodilatory and procoagulant factors (Keh 2003). The main physiological actions of glucocorticoids in septic shock are presented in Table 5.
Table 5. Main effects of glucocorticoids in septic shock.

<table>
<thead>
<tr>
<th>Cardiovascular effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maintenance of vascular tone</td>
</tr>
<tr>
<td>Regulation of vascular permeability</td>
</tr>
<tr>
<td>Increase vascular sensitivity to catecholamines</td>
</tr>
<tr>
<td>Regulation of sodium and potassium excretion</td>
</tr>
<tr>
<td>Regulation of water excretion</td>
</tr>
<tr>
<td>Increase beta adrenergic receptor synthesis and affinity</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anti-inflammatory effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduction in the proinflammatory cytokine production (TNF, IL-1β, IL-6)</td>
</tr>
<tr>
<td>Increase in the anti-inflammatory cytokine synthesis (IL-10, IL-1ra)</td>
</tr>
<tr>
<td>Decrease in the adhesion molecule expression</td>
</tr>
<tr>
<td>Inhibition of chemokine (IL-8) synthesis</td>
</tr>
<tr>
<td>Inhibition of soluble phospholipase-A2 synthesis</td>
</tr>
<tr>
<td>Inhibition of inducible cyclooxygenase-2 synthesis</td>
</tr>
<tr>
<td>Inhibition of inducible nitric oxide synthase synthesis</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Metabolic effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulation of gluconeogenesis</td>
</tr>
<tr>
<td>Inhibition of peripheral tissue glucose uptake</td>
</tr>
<tr>
<td>Stimulation of hepatic glycogenolysis</td>
</tr>
<tr>
<td>Activation of lipolysis</td>
</tr>
<tr>
<td>Exacerbation of insulin resistance</td>
</tr>
</tbody>
</table>

The conventional activation of the cortisol production occurs via corticotropin releasing hormone (CRH) – adrenocorticotropic hormone (ACTH) – activation. Hypothalamic CRH activates the pituitary release of ACTH, which in turn stimulates cortisol and dehydroepiandrosterone secretion in adrenal cortex (Feek 1983). Secretion of CRH is pulsatile and is followed by the pulsatile release of ACTH (Voerman 1992). The central sympathetic nervous system stimulates hypothalamus to secrete CRH. The adrenal glands also receive a direct sympathetic nerve supply, and the activation of the sympathetic nervous system activates also directly cortisol production (Stewart 2003). In addition to CRH, hypothalamic vasopressin (AVP) stimulates ACTH secretion.
normal conditions AVP alone has a minor effect on ACTH secretion, but it acts synergistically with CRH (Chrousos 1995). In healthy subjects cortisol production is regulated by a negative feedback mechanism exerted by secreted cortisol on CRH and ACTH synthesis (Feek 1983). Figure 1 demonstrates the normal physiological activation of the HPA-axis.

**Figure 1.** Normal activation of the hypothalamic-pituitary-adrenal axis. Continuous arrows indicate activation, broken arrows indicate inhibitory effects. AVP: vasopressin; CRH: corticotropin-releasing hormone; ACTH: adrenocorticotropic hormone.
In sepsis and septic shock, significant functional alterations occur in the HPA axis. Typically, a biphasic pattern of HPA activation is observed (Beishuizen 2004). In the acute phase high cortisol concentrations are associated with elevated ACTH levels, which reflect normal physiological activation (Bornstein 1998). In the second phase, a discrepancy between high cortisol levels and low ACTH levels is observed (Vadas 1988, Vermes 1995). In this chronic or prolonged phase of critical illness non-ACTH mediated pathways become major regulators of cortisol production. The inflammatory cytokines, TNF-α, IL-1, IL-2 and IL-6 can activate the hypothalamic-pituitary-adrenal axis independently, and in combination they have a synergistic effect (Darling 1989, Imura 1991, Mastorakos 1993, Chrousos 1995). These cytokines exert their effects on cortisol production by increasing CRH and ACTH release but they also have direct effects on adrenal glands. Especially IL-6 is a powerful stimulator in the non-ACTH mediated activation of the adrenal function during critical illness (Mastorakos 1993, Soni 1995). In addition, IL-10 and its receptors are produced in pituitary and hypothalamic tissues, and IL-10 has been shown to enhance CRH and ACTH production in hypothalamus and pituitary gland (Rady 1995, Smith 1999). In normal subjects cortisol secretion follows a circadian pattern, but in critical illness these circadian changes are typically diminished or even lost (Voerman 1992, Schuetz 2006).

In addition to cytokines, vasoactive peptides activates HPA axis in sepsis. Vasopressin-mediated activation of V3 receptors in the hypophysis facilitates the release of ACTH (Feek 1983, Chrousos 1995). Other vasoactive peptides, such as endothelin, atrial natriuretic peptides and pro-adrenomedullin, are all capable of modulating adrenocortical function, but the exact role of these substances in the activation of the HPA axis is not fully established (Vermes 1995, Chirst-Crain 2005).

Another cause for the elevation of cortisol in critical illness is a shift from adrenal androgen and mineralocorticoid production towards glucocorticoid biosynthesis (Vermes 2001). In normal situations, dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) are most abundantly secreted steroids by the adrenal cortex, but in critically ill patients especially the serum levels of DHEAS levels are significantly decreased while cortisol levels are elevated (Beishuizen 2002).
DHEA/DHEAS are potent proinflammatory modulators of the immune response (Beishuizen 2004). DHEA stimulates the Th1-cell function, and the increase in cortisol production and concomitant decrease in DHEA/DHEAS synthesis may aggravate immunosuppression in sepsis (Schuetz 2006).

The metabolism of cortisol is changed in sepsis. The half-life of cortisol is increased during septic shock (Melby 1958). This increase of half-life is due to decreased rate of hepatic extraction and decreased renal enzymatic inactivation. This is explained by the changes in the 11β-hydroxysteroid dehydrogenase type I and type II activities, which modulate the cortisol/cortisone balance (Venkatesh 2007). In critical illness, cortisol binding globulin levels show remarkable changes and extremely low CBG levels have been observed in patients with septic shock (Beishuizen 2001). Since cortisol is bound to a large extent to CBG, and only the free hormone is considered to be biologically active, changes in CBG concentration affects the bioavailability of cortisol (Stewart 2003).

Relative adrenal insufficiency in sepsis

Although cortisol production is usually enhanced in sepsis, some patients may have relative or functional adrenocortical dysfunction, a concept introduced by Schein and Rothwell (Schein 1990, Rothwell 1991). Relative adrenal insufficiency is characterized by situations where measured cortisol levels are normal or even elevated, but they are still considered to be inadequate, and the patients may not be able to respond to any additional stress. In these situations cortisol demand is substantially increased, and therefore normal levels of cortisol may be inappropriate. Several approaches have been introduced to evaluate the adequacy of adrenal function in critically ill patients. Basal cortisol measurements, the low-dose (1 µg) ACTH test and the conventional (250 µg) ACTH test have been used in the assessment of cortisol production and adrenal reserve.

The standard ACTH stimulation test is most commonly used method for identifying adrenocortical hyporesponsiveness in critically ill patients (Lamberts 1997). In the
standard test, a cortisol response to exogenous 250 μg ACTH is measured 30 and 60 minutes intervals after corticotropin injection. Relative adrenal insufficiency is typically characterised by a supra-normal basal, but deficient post-stimulation increase in cortisol concentration.

Rothwell et al. demonstrated that basal cortisol levels were identical in survivors and nonsurvivors in septic shock, but all nonsurviving patients demonstrated a poor cortisol increment (< 250 nmol/l) in the standard ACTH stimulation test (Rothwell 1991). Later in a large prospective study Annane et al. confirmed that this cortisol increment of 250 nmol/l discriminated survivors and nonsurvivors well. Annane and coworkers developed a 3-level classification system of adrenal function based on the results of multivariate analysis (Annane 2000). The prognosis was good in those patients whose basal cortisol was below 937 nmol/l and the stimulation response was good (> 250nmol/l); mortality in this group was 26%. In contrast, the prognosis was poorest in those patients who had high basal cortisol levels and a blunted ACTH response (baseline > 937 nmol/l and increment < 250 nmol/l) with a mortality rate of 82%. Other investigators have also demonstrated that a blunted adrenocortical response in ACTH test is associated with a poor prognosis in septic shock (Sibbald 1977, Soni 1995). However, not all studies confirm these findings and in a study by Bouachour et al. there was no correlation between cortisol response and mortality (Bouachour 1995).

The standard ACTH stimulation test has been criticized to be insensitive in detecting clinically relevant changes in adrenal function. The standard ACTH stimulation test uses a corticotropin dose, that is 200-fold greater than ACTH levels produced during physiological stress (Marik 2000). It has been suggested that the low-dose (1μg) ACTH stimulation test would be more sensitive in detecting adrenal insufficiency (Dickstein 1991). In postoperative patients the low-dose test results were considered to be valid after uncomplicated surgery, but the test was more difficult to interpret in more severely ill postoperative patients (Richards 1999). In ICU patients the improved sensitivity of the low-dose ACTH test in detecting adrenal insufficiency has not been confirmed unambiguously (Soni 1995, Siraux 2005, Salgado 2006).
Random cortisol measurements have been suggested to replace the ACTH stimulation tests in the assessment of adrenal function. Marik performed both the high and low-dose test in 59 patients with septic shock to determine the sensitivity of each test in establishing a diagnosis of adrenal insufficiency (Marik 2003). In this study, a baseline cortisol concentration below 680 nmol/l predicted a beneficial clinical response to corticosteroids very accurately. In contrast, the sensitivity of the ACTH tests was poor. The conclusion in Marik’s study was that random cortisol measurements are more suitable than the ACTH stimulation tests in the assessment of adrenal function in septic shock patients. Table 6 summarizes the incidence of adrenal insufficiency in septic shock.
Table 6. Incidence of adrenal insufficiency (AI) in septic shock.

<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>ACTH test (μg)</th>
<th>Criteria for AI (nmol/l)</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rothwell 1991</td>
<td>32</td>
<td>250</td>
<td>increment &lt; 250</td>
<td>41</td>
</tr>
<tr>
<td>Moran 1994</td>
<td>68</td>
<td>250</td>
<td>increment &lt; 200 peak level &lt; 500</td>
<td>67 32</td>
</tr>
<tr>
<td>Bouachour 1995</td>
<td>40</td>
<td>250</td>
<td>increment &lt; 250 peak level &lt; 500</td>
<td>75 6</td>
</tr>
<tr>
<td>Soni 1995</td>
<td>21</td>
<td>1 250</td>
<td>peak level &lt; 500</td>
<td>29 24</td>
</tr>
<tr>
<td>Oppert 2000</td>
<td>20</td>
<td>250</td>
<td>increment &lt; 200</td>
<td>55</td>
</tr>
<tr>
<td>Anane 2000</td>
<td>189</td>
<td>250</td>
<td>increment &lt; 250</td>
<td>54</td>
</tr>
<tr>
<td>Bollaert 2003</td>
<td>82</td>
<td>250</td>
<td>increment &lt; 200 increment &lt; 250</td>
<td>34 38</td>
</tr>
<tr>
<td>Marik 2003</td>
<td>59</td>
<td>1 249 —</td>
<td>peak level &lt; 500 baseline &lt; 680</td>
<td>22 8 61</td>
</tr>
<tr>
<td>Manglik 2003</td>
<td>100</td>
<td>250</td>
<td>peak level &lt; 550</td>
<td>9</td>
</tr>
<tr>
<td>Siraux 2005</td>
<td>46</td>
<td>1 250</td>
<td>increment &lt; 250 increment &lt; 250</td>
<td>67 35</td>
</tr>
<tr>
<td>Salgado 2006</td>
<td>102</td>
<td>1 249</td>
<td>increment &lt; 250 increment &lt; 250</td>
<td>54 23</td>
</tr>
</tbody>
</table>
Etiology and risk factors of adrenal insufficiency

Several mechanisms are involved in the development of relative adrenal insufficiency in septic shock. Insufficient blood flow to the adrenal cortex and specific substances that either inhibit ACTH secretion or directly depress adrenal function may induce adrenal failure. Necrosis or haemorrhage of the pituitary gland or adrenal cortex has been reported in sepsis as a result of prolonged hypotension or severe coagulopathy, but the destruction of the adrenal glands must be very extensive to produce cortisol insufficiency (Zaloga 2001). In most cases, autopsy findings of patients with documented adrenal insufficiency have revealed intact adrenal glands (Soni 1995, Annane 1998).

Functional changes are probably more important determinants of adrenal insufficiency in sepsis. This concept is supported by findings in which impaired adrenal function during septic shock has normalized after recovery (Briegel 1996). In clinical sepsis cytokines stimulate HPA function, but also inhibitory effects are mediated by the cytokines. Especially local actions of TNF-α are widely different depending on the site of action. TNF-α can activate the HPA axis via hypothalamic CRH or pituitary ACTH release, but in the adrenal cells TNF-α reduces the ability of adrenocortical cells to respond to ACTH stimulation (Jäättelä 1991, Chrousos 1995). IL-6 is a very potent stimulus for both ACTH and cortisol secretion. Low IL-6 levels may contribute to adrenocortical insufficiency in sepsis because of understimulation of the pituitary-adrenal axis (Soni 1995). Corticostatin, a peptide produced by immune cells, may also impair adrenocortical function by competing with ACTH through binding to its receptor (Zhu 1992).

Together with TNF-α and IL-6, macrophage migration inhibitory factor (MIF) is a central cytokine that modulates adrenal function in sepsis (Baugh 2002). MIF is a potent proinflammatory cytokine that is released from macrophages and T lymphocytes that have been stimulated by glucocorticoids (Calandra 1995). MIF is able to antagonize the inhibitory effects of glucocorticoids on proinflammatory cytokine production, and it is
also able to overcome the glucocorticoid-induced inhibition of T-cell proliferation by restoring IL-2 and IFN-γ production (Calandra 1997). MIF is produced by pituitary cells (Bernhagen 1993), and elevated MIF levels have been observed in adrenal glands (Baugh 2002). Since the main action of MIF is to counteract the effects of glucocorticoids, it is possible that MIF is a central cytokine in mediating bidirectional communication between the immune and neuroendocrine systems in sepsis (Beishuizen 2001).

Certain subgroups of septic shock patients may be at an increased risk for developing relative adrenal insufficiency. Especially patients who have received etomidate are at greater risk for developing adrenal failure. A prospective observational study by Malerba and coworkers demonstrated that a single dose of etomidate increased by 12 times the risk of adrenal dysfunction (Malerba 2005). It has also been suggested that gender may have an influence on the development of adrenal insufficiency, but these results are conflicting. Malerba’s study proposed that relative adrenal insufficiency is more common in men, but a recent prospective study performed by Salgado et al. demonstrated that female sex was associated with a greater incidence of relative adrenal failure (Salgado 2008). Figure 2 presents the activation of the HPA axis during sepsis and septic shock.
Figure 2. HPA activation in sepsis. Continuous arrows indicate activation, broken arrows indicate inhibitory effects. AVP: vasopressin; ACTH: adrenocorticotropic hormone; CRH: corticotropin-releasing hormone; MIF: macrophage migration inhibitory factor.
2.6. Therapeutic aspects

High-dose corticosteroids in severe sepsis and septic shock

In theory, glucocorticoids have numerous beneficial effects in the treatment of severe sepsis. In a relatively small study performed by Schumer demonstrated that treatment with dexamethasone or methylprednisolone improved patients’ survival in severe sepsis (Schumer 1976). As a result, large well-designed randomised controlled studies were performed to find out whether the corticosteroid treatment has any benefit in the treatment of severe sepsis or septic shock (Sprung 1984, Bone 1987, VASSCS 1987, Luce 1988). In these studies patients received extremely high-dose corticosteroid treatment (up to 42 000 mg of hydrocortisone equivalent) in the early phase of sepsis (Lefering 1995). The treatment period was extremely short and typically patients received one, two or four dosages of corticosteroids within the first 24 hours. None of these studies could demonstrate any beneficial effects on shock reversal or mortality. In all studies corticosteroid treatment was either not useful (VASSCS 1987) or even harmful (Sprung 1984, Bone 1987, Luce 1988). These results were later confirmed in several meta-analyses, which concluded was that high-dose corticosteroids should not be used in the treatment of severe sepsis or septic shock (Lefering 1995, Cronin 1995, Annane 2004). In general, it was assumed that high doses of glucocorticoids failed to dampen the overactive inflammatory response, but instead induced immunosuppression (Keh 2006).

Low-dose hydrocortisone therapy in septic shock

A renewed interest of corticosteroid therapy in septic shock occurred in 1990’s due to an increased understanding of functional adrenal insufficiency. The concept that relative adrenal dysfunction is associated with poor prognosis in sepsis led to the studies where the effects of low-dose hydrocortisone treatment were investigated in vasopressor-dependent septic shock. The hypothesis was that considerably lower replacement doses
of hydrocortisone in prolonged therapy could restore homeostasis, increase vasomotor tone, and have positive anti-inflammatory effects without causing exaggerated immunosuppression.

The first prospective double-blind study that evaluated low-dose glucocorticoids in the treatment of septic shock involved 41 patients from two intensive care units in France (Bollaert 1998). Patients received either hydrocortisone treatment (100 mg three times daily during 5-day period), or placebo, tapered over 6 days. In this study, it was calculated that a sample size of 80 patients would be needed to detect a 30% difference in the rate of shock reversal. However, in an interim analysis the results showed a striking difference in shock reversal between the study groups, and the study was discontinued after 41 patients. Shock reversal by day 7 was achieved in 68% of the treatment group versus 21% of the placebo group (p=0.007). There was also a trend towards lower 28-day mortality in the treatment group, but due to the small sample size this did not reach the statistical significance. In a second double-blind single-center study from Germany, 40 patients with septic shock were randomized to receive hydrocortisone by continuous infusion of 0.18 mg/kg/h or placebo and when shock was reversed, the hydrocortisone dosage was reduced to 0.08 mg/kg/h for 6 days (Briegel 1999). The primary end point was the time until shock reversal as defined by cessation of alpha-adrenergic support. In this study, it was calculated that a sample size of 40 patients was necessary to detect a 45% difference in shock reversal 48 hrs after starting treatment. This study showed a significant hemodynamic improvement in the treatment group as well. The time to reversal of shock was achieved at a median of 2 days in the hydrocortisone group, and in 7 days in the placebo group (p=0.005). The overall mortality was not affected by the treatment (Briegel 1999). In both studies hydrocortisone treatment was well tolerated, and no serious adverse events were observed. Later Keh and co-workers confirmed these findings in a crossover study where hydrocortisone therapy showed a clear effect on shock reversal (Keh 2003). Keh’s study demonstrated that hydrocortisone treatment increased blood pressure and systemic vascular resistance and decreased heart rate, cardiac index, and catecholamine requirement.
In addition to hemodynamic effects, Keh investigated immunological mechanisms underlying the beneficial effects of low-dose hydrocortisone therapy. Hydrocortisone treatment significantly decreased the levels of both proinflammatory (IL-6 and IL-8) and anti-inflammatory cytokines (IL-10 and soluble TNF-α receptors), whereas the monocyte-activating cytokine interleukin-12 levels were increased (Keh 2003). In vitro granulocyte function remained intact, indicating that low-dose hydrocortisone did not suppress innate defence mechanisms. The treatment thus modulated the immunologic response towards anti-inflammation rather than towards immunosuppression. Similar balancing effects on the immune response have also been reported by other investigators. Briegel and Oppert demonstrated decreased IL-6 and IL-8 levels, whereas IL-10 levels were unaltered (Briegel 2001, Oppert 2005). These findings suggest that hydrocortisone treatment may correct the imbalance between overactive proinflammatory response and inadequate anti-inflammatory response, and it may improve innate immunity in the early stage of septic shock. In contrast to high-dose methylprednisolone treatment which was believed to induce immunosuppression, the low-dose hydrocortisone therapy may actually have an immunobalancing and immunoenhancing role in sepsis (Briegel 2001, Bornstein 2003, Kaufmann 2007).

After Briegel’s and Bollaert’s trials, a relatively large multicenter study conducted in France was published in 2002 by Annane and co-workers (Annane 2002). This double-blind randomized study of 299 patients from 19 intensive care units investigated the effects of corticosteroid therapy in refractory septic shock. The treatment group received intravenous hydrocortisone 50 mg every 6 hours and fludrocortisone 50 μg every 24 hours for 7 days. The therapy was started within 8 hours of the onset of septic shock. Of the 299 patients, 229 were nonresponders in the ACTH test and only 70 were considered to have normal adrenal function. In this trial, the steroid replacement therapy showed a significant reduction in mortality in those patients who had adrenal insufficiency. In contrast, patients with normal adrenal function did not seem to benefit from the treatment. Also shock reversal was significantly enhanced only in those patients who had a poor response in the ACTH stimulation test, whereas in patients with normal ACTH response no beneficial effect was observed. The authors suggested that hydrocortisone-fludrocortisone combination treatment should be given to all patients.
with refractory septic shock after the ACTH stimulation test, and when the tests results are available the treatment should be discontinued to those patients whose adrenal function was normal.

Annané’s study faced considerable criticism in medical journals. Among the ACTH nonresponders, 28-day mortality was 63% in the placebo group and 53% in the treatment group. This reduction in mortality was statistically significant only after complex statistical maneuvers when the results were adjusted for baseline cortisol levels, cortisol response, McCabe classification, organ dysfunction score, arterial lactate levels and $\text{PaO}_2/\text{FiO}_2$ ratios using logistic models. In a conventional nonparametric test, however, a statistically significant reduction in mortality was not observed ($p=0.096$ Chi-square test between groups). In addition, 72 patients received etomidate within 24 hours after the randomization, and 68 of these patients (94%) developed adrenal insufficiency (Annane 2003). This drug-induced adrenal failure may have had a substantial influence on the results. The possible beneficial role of fludrocortisone in this therapy needs to be also further studied in detail.

**Coagulation inhibitors in sepsis**

In sepsis, inflammatory and procoagulant host responses to infection are closely related. Release of proinflammatory cytokines initiates not only the activation of the systemic inflammatory response, but it also activates coagulation pathways (Esmon 1999). Proinflammatory cytokines TNF-$\alpha$ and IL-6 activate coagulation by stimulating the release of tissue factor from monocytes and endothelium, which leads to the increased conversion of prothrombin to thrombin (Levi 2002). Concomitantly, natural anticoagulant and fibrinolytic activity are impaired (Levi 1999). Intravascular thrombin generation is considered to be a highly inflammatory stimulus and enhanced coagulation and inflammation are thought to from a vicious cycle in the pathophysiology of sepsis (Esmon 2001).
The pharmacological modification of the coagulation system has been an important target for treatment of sepsis because natural anticoagulant systems also have anti-inflammatory effects. In normal conditions, thrombin generation is limited by the tissue pathway inhibitor, antithrombin and the protein C system (Levi 1999). During severe sepsis, all three regulatory systems are defective as a result of endothelial dysfunction (Levi 2002). All of these systems have anti-inflammatory effects and also modulate intracellular signaling, cytokine secretion, cellular apoptosis and leukocyte-endothelial interactions. Numerous experimental and clinical studies have investigated whether these natural coagulation inhibitors could improve patient outcome in severe sepsis due to their anticoagulant and anti-inflammatory effects. Recombinant tissue factor pathway inhibitor (TFPI) and antithrombin seemed to improve patient prognosis in sepsis in experimental and phase 2 clinical studies (Eisele 1998, Abraham 2001), but in phase 3 studies these positive results could not be confirmed. TFPI has been evaluated in a large phase 3 clinical trial (OPTIMIST), which involved 1,987 patients. This trial found no substantial improvement in 28-day survival even a subgroup of patients not receiving concomitant heparin seemed to benefit from the treatment (Abraham 2003). Similar findings were observed in large antithrombin trial, which involved 2,314 septic patients (Warren 2001).

In 2001, recombinant human activated protein C (rhAPC) was approved for the treatment of severe sepsis after the results obtained in a prospective PROWESS study. In this randomized, controlled phase III clinical trial, 1,690 patients with severe sepsis were randomized to receive either a continuous infusion of APC (24 μg/kg/h) for 4 days, or placebo (Bernard 2001). Administration of rhAPC caused a significant reduction in 28-day mortality. The mortality rates of those patients randomized to APC was 24.7%, while patients receiving placebo had mortality rate of 30.8%. Treatment with rhAPC was associated with a 19.4% relative reduction in mortality (p = 0.0049). APC also reduced D-dimer and IL-6 plasma levels, suggesting diminished activation of the coagulation pathway and reduced cytokine production (Bernard 2001). A post hoc subgroup analysis revealed that the treatment effect was only evident in the most severely ill patients with a higher risk of death, defined by an APACHE II score > 25 (Angus 2004).
After positive results obtained from the PROWESS trial, subsequent studies have not confirmed similar beneficial effects in other study populations, such as children or adults with lower severity of illness (Abraham 2005, Eisenberg 2005). A prospective ADDRESS trial was carried out in septic patients with lower risk of death in order to clarify the efficacy and safety of rhAPC in this specific population (Abraham 2005). For the primary end-point in the ADDRESS study, 28-day mortality, no difference between treatment and placebo group was observed. The mortality rate in the placebo group was 17% and in the treatment group 18.5%. Furthermore, 321 patients in the ADDRESS study had APACHE II scores > 25 at the enrolment, and in this patient population the mortality rates in the rhAPC group was 29.5% and 24.7% in the control group. The pediatric sepsis rhAPC study was discontinued prematurely because lack of efficacy (Eisenberg 2005). These conflicting results concerning the use of rhAPC have created substantial controversy regarding efficacy, safety and cost-benefit profile of APC in the treatment of sepsis (Costa 2007). It has been suggested that the risks of rhAPC therapy may outweigh its benefits (Dellinger 2006, Mackenzie 2006, Eichacker 2007).

**Intensive insulin therapy**

Intensive insulin therapy and strict normoglycemia has been shown to reduce morbidity and mortality among critically ill surgical patients (Van den Berghe 2001). Intensive insulin therapy exerts anti-inflammatory effects and tight glycemic control has been reported to prevent excessive inflammation as illustrated by lower C-reactive protein and mannose-binding lectin levels (Hansen 2003). Persistently elevated blood glucose levels may be harmful due to intrinsic proinflammatory effects of hyperglycemia, which may aggravate systemic inflammatory response (Yu 2003). In addition, insulin itself has potent anti-inflammatory properties (Marik 2004). Although intensive insulin therapy has shown to reduce mortality in critically ill surgical patients, there in no clinical study that has confirmed these beneficial effects in sepsis. A recent prospective randomized study performed in Germany in patients with severe sepsis was discontinued prematurely because of identical mortality rate in the intensive insulin and control
group, and a greater incidence of hypoglycemia in the tight blood sugar group (Brunkhorst 2008).
3. AIMS OF THE STUDY

The objective of the present study was to evaluate the clinical significance of the compensatory anti-inflammatory response in severe sepsis and septic shock. The specific objectives were:

1. To investigate the role of adrenal insufficiency in the development and resolution of multiple organ failure (Study I).

2. To study the role of the anti-inflammatory cytokine response in the pathogenesis of multiple organ failure (Study II).

3. To investigate the dynamic changes in adrenocortical function in critically ill patients and to assess the reproducibility of the ACTH test in patients with or without sepsis (Study III).

4. To study the effects of hydrocortisone therapy on glycemic control and hemodynamic changes in septic shock (Study IV).
4. PATIENTS AND METHODS

4.1. Patients

Patient characteristics

One-hundred-seventy-three critically ill patients were enrolled in the study. Of these, 120 had septic shock and 33 severe sepsis. In addition, 20 nonseptic critically ill patients in study III served as a control group. Studies I and II were performed in the Surgical Intensive Care Unit of Tampere University Hospital and studies III and IV were multicenter studies conducted in the Intensive Care Units of Tampere University Hospital, Kuopio University Hospital, Päijät-Häme Central Hospital and South-Carelian Central Hospital. Fourteen patients from the study I also participated in study II.

Sepsis was defined according to the American College of Chest Physicians / Society of Critical Care Medicine Consensus Conference as the presence of systemic inflammatory response syndrome and a documented source of infection (Bone 1992). In studies I and II severe sepsis was defined as a sepsis associated with organ dysfunction, hypoperfusion abnormality or sepsis-induced hypotension (Bone 1992). The criteria for organ dysfunction and hypoperfusion abnormality were defined as the presence of one or more of the following manifestations: hypoxemia (PaO₂ < 10 kPa), elevated plasma lactate level (> 2 mmol/l), or oliguria (urine output < 30 ml/h) (Bone 1987). Septic shock was defined as a sepsis associated with hypotension despite adequate fluid resuscitation (systolic blood pressure < 90 mmHg or a decrease of systolic blood pressure by 40 mmHg or more from the baseline) (Bone 1992). The patient data are summarized in the Table 7.
Table 7. Demographics of the patients at ICU admission.

<table>
<thead>
<tr>
<th></th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inclusion criteria</td>
<td>severe sepsis</td>
<td>severe sepsis</td>
<td>ICU patients</td>
<td>septic shock</td>
</tr>
<tr>
<td>No of patients</td>
<td>41</td>
<td>38</td>
<td>60</td>
<td>48</td>
</tr>
<tr>
<td>Age</td>
<td>53.0 ± 15.8</td>
<td>52.8 ± 14.4</td>
<td>56.9 ± 13.3</td>
<td>60.6 ± 16.6</td>
</tr>
<tr>
<td>APACHE II</td>
<td>13.5 ± 5.7</td>
<td>13.3 ± 5.8</td>
<td>20.4 ± 6.3</td>
<td>22.6 ± 6.8</td>
</tr>
<tr>
<td>SAPS II</td>
<td>34.5 ± 12.2</td>
<td>32.9 ± 10.4</td>
<td>44.1 ± 12.2</td>
<td>51.9 ± 12.9</td>
</tr>
<tr>
<td>SOFA score</td>
<td>8.5 ± 2.8</td>
<td>8.4 ± 2.9</td>
<td>8.5 ± 2.9</td>
<td>10.2 ± 2.3</td>
</tr>
<tr>
<td>ICU mortality</td>
<td>31.7%</td>
<td>31.6%</td>
<td>20%</td>
<td>22.9%</td>
</tr>
</tbody>
</table>

Exclusion criteria

Patients under 18 years of age and patients receiving glucocorticoids were excluded from the study. Patients who died within 24 hours of the onset of sepsis were also excluded (two in the study I, one in the study II and three in the study IV). In study I, one patient was excluded because of absolute adrenocortical insufficiency (stimulated cortisol level < 500 nmol/l). In study IV, pre-existing diabetes was an additional exclusion criterion.
4.2. Methods

Study designs

**Study I.** Study I was a prospective observational study. The purpose of the study was to investigate the incidence of adrenal insufficiency in severe sepsis and its relation to the development and resolution of multiple organ failure. In all, 41 patients who fulfilled the criteria of severe sepsis or septic shock were enrolled in the study. A short ACTH stimulation test was performed within 24 hours of the diagnosis of severe sepsis. A peak cortisol level < 680 nmol/l and a rise in serum cortisol level less than 260 nmol/l were used as the criteria for relative adrenocortical insufficiency (Seppälä 1996). After the ACTH test the serum cortisol values were followed on the third and fifth day of the intensive care (ICU) period. The severity of organ dysfunction was assessed daily using the Sequential Organ Failure Assessment (SOFA) score (Vincent 1996). At the time of ACTH test, plasma ACTH measurements were also performed. Length of ICU stay, duration of mechanical ventilation, ICU mortality and hospital mortality rates were assessed in study I.

**Study II.** Study II was a prospective observational study that investigated the relationship between the pro- and anti-inflammatory cytokine response in severe sepsis and their role in the development of multiple organ failure (MOF). Thirty-eight patients who fulfilled the criteria of severe sepsis were prospectively enrolled in the study. Serial cytokine analyses were performed in two separate study groups: one in patients who developed severe multiple organ dysfunction and the other with no evidence of severe organ dysfunction. The severity of organ dysfunction was assessed daily by the SOFA-scoring system. Severe multiple organ failure was defined prospectively as a maximum SOFA score of 10 or higher, which indicates severe dysfunction (grade 3 or 4) at least in three organ systems (Vincent 1998). Serial measurements of the plasma IL-6, IL-1β, IL-10 and IL-1ra concentrations were performed. Cytokine samples were taken at the onset of severe sepsis followed by subsequent samples on the third and fifth day of the intensive care period.
Study III. Study III was a multicenter prospective cohort study that included 60 critically ill patients. In this study, the reproducibility of the ACTH stimulation test was investigated. Two consecutive short ACTH stimulation tests were performed in three separate study groups. The first group consisted of patients with sepsis (n=20), a second group of patients with septic shock (n=20) and a third group were critically ill patients without sepsis (n=20). The first ACTH stimulation test was performed within 24 hours of the diagnosis of sepsis or septic shock in groups 1 and 2 or within 24 hours of ICU admission in group 3 (day 1). A short corticotropin stimulation test was repeated 24 hours after the first ACTH stimulation test (day 2) and individual cortisol responses in two ACTH tests were compared. Absolute adrenal insufficiency was defined as a maximum cortisol concentration of less than 500 nmol/l (18 μg/dl) after the ACTH stimulation test (Bouachour 1995, Lamberts 1997) and an increase in the serum cortisol concentration of less than 248 nmol/l (9 μg/dl) irrespective of basal cortisol level was used as the criterion for relative adrenal insufficiency (Annane 2000).

Study IV. Study IV was a multicenter prospective randomized study. In this study, metabolic and hemodynamic effects of two corticosteroid treatment modalities (bolus versus continuous hydrocortisone infusion) were compared. Special attention was focused on the blood glucose profiles, and the amount of nursing workload needed to maintain tight glycemic control during intensive insulin therapy. Serial hemodynamic measurements and the reversal of shock were also compared between the study groups. When patients were considered to benefit from the corticosteroid treatment, they were randomly assigned to receive hydrocortisone either by a conventional bolus therapy (50 mg bolus of hydrocortisone every six hours intravenously) or by continuous infusion of the equivalent dose (200 mg/day). Hydrocortisone treatment was started according clinical judgement when patients required high-dose or increasing norepinephrine support (Keh 2004). Hydrocortisone was given as hydrocortisone sodium succinate, (Solu-Cortef®, Pharmacia, Puurs, Belgium). When continuous infusion was used hydrocortisone was diluted with physiologic saline. Randomization was performed in blocks of four patients by means of sequentially numbered opaque envelopes. Duration of hydrocortisone treatment was 5 days. After the randomization a maintenance infusion
of 5% glucose was started at the rate of 30 ml/kg/day. At the same time a protocol-based enteral nutrition with standard formulas (1 kcal/ml) was initiated. Enteral feeding was started at 500 ml/day with daily increments of 500 ml if possible. The maximum amount of enteral nutrition was set at 1500 ml/day. Blood glucose levels were monitored every two hours. The goal was to maintain plasma glucose levels between 4 and 7 mmol/l. When the plasma glucose level exceeded 7 mmol/l, an insulin infusion of 1 IU/ml (Actrapid®, Novo Nordisk A/S, Baksvaerd, Denmark) was started, and the dose was adjusted according to a strict algorithm (Table 8).

Table 8. Algorithm for glucose control in study IV.

1. Initial infusion

<table>
<thead>
<tr>
<th>Blood glucose (mmol/l)</th>
<th>Insulin infusion rate (IU/hour)</th>
<th>Control interval (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.0-9.9</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>10-11.9</td>
<td>2</td>
<td>1-2</td>
</tr>
<tr>
<td>&gt;12</td>
<td>4</td>
<td>1-2</td>
</tr>
</tbody>
</table>

2. Maintenance infusion

<table>
<thead>
<tr>
<th>Blood glucose (mmol/l)</th>
<th>Insulin infusion rate</th>
<th>Control interval (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2.5</td>
<td>10% glucose 150 ml iv.</td>
<td>0.5</td>
</tr>
<tr>
<td>&lt;3.0</td>
<td>Stop insulin</td>
<td>1</td>
</tr>
<tr>
<td>3.1-3.9</td>
<td>Reduce insulin dose by half</td>
<td>1</td>
</tr>
<tr>
<td>4.0-4.9</td>
<td>Reduce by 0.5 IU/hour</td>
<td>2</td>
</tr>
<tr>
<td>5.0-6.9</td>
<td>Insulin dose unchanged</td>
<td>2</td>
</tr>
<tr>
<td>7.0-9.9</td>
<td>Increase by 0.5-1 IU/hour</td>
<td>2</td>
</tr>
<tr>
<td>10-11.9</td>
<td>Increase by 1 IU/hour</td>
<td>2</td>
</tr>
<tr>
<td>&gt;12.0</td>
<td>Increase by 2 IU/hour</td>
<td>2</td>
</tr>
</tbody>
</table>
**Laboratory assays**

**Short ACTH stimulation tests (I, III).** In the studies I and III, a short corticotropin stimulation test was performed within 24 hours of the diagnosis of sepsis or septic shock or within 24 hours of ICU admission in nonseptic patients. Tetracosactin 0.25 mg (Synacthen®, Ciba-Geigy, France) was infused, and blood samples were taken immediately before the test for the determination of basal serum cortisol concentration and 30 and 60 minutes thereafter. After centrifugation serum samples were stored at 4 °C and analysed within 24 hrs. If the assay was delayed, samples were stored frozen at –20 °C. Cortisol was measured by fluoroimmunoassay in Tampere University Hospital and in Päijät-Häme Central Hospital; an enzyme immunoassay was used in Kuopio University Hospital. ACTH stimulation tests were obtained in the morning. Random cortisol measurements were also obtained 9.00 am.

**Plasma ACTH measurements (I).** Plasma ACTH levels were measured in patients with severe sepsis. Blood samples for the ACTH measurements were taken immediately before the ACTH stimulation test. Blood samples were drawn into EDTA tubes, centrifuged and stored frozen at –20 °C. Adrenocorticotropic hormone analyses were performed using an immunoluminometric assay.

**Plasma concentrations of IL-1, IL-6, IL-10 and IL-1 receptor antagonist (II).** In study II blood samples for cytokine analyses were drawn into EDTA tubes, centrifuged and stored at –70°C. The cytokine analyses (IL-1, IL-10, IL-6) were performed using an ELISA immunoassay technique (Pelikine Compact™, Central Laboratory of the Netherlands Red Cross Transfusion Service, Amsterdam, Netherlands). The IL-1ra assays were also performed with an enzyme immunoassay method (Quantikine®, R&D Systems Inc, Minneapolis, USA). The detection limits for the cytokine assays were 0.4 pg/ml for IL-1β, 0.6 pg/ml for IL-6, 1.2 pg/ml for IL-10, and 14 pg/ml for IL-1ra.

**Plasma glucose measurements (IV).** In study IV, blood glucose levels were monitored every two hours from the arterial line during the study period. Blood glucose measurements were performed with blood gas analyzers, and the results were expressed as plasma glucose levels.
Scoring methods for the severity of illness

Acute Physiology and Chronic Health Evaluation (APACHE) II scores (Knaus 1985) and Simplified Acute Physiology Scores (SAPS) II (Le Gall 1993) were calculated and the severity of organ dysfunction was assessed using Sequential Organ Failure Assessment (SOFA) scores (Vincent 1996) at the time of ICU admission. In studies I and II SOFA score were calculated daily during the 10-day study period to assess the development and the resolution of organ failure. In sedated patients, the SOFA scores for neurological system were not graded.

Statistical analysis

The descriptive statistics are reported as mean values ± SD. The patient groups were compared using the unpaired Student’s t-test for the continuous variables, and the Chi-square test or the Fisher’s exact test for categorical data as appropriate. In study III, a one-way analysis of variance with Bonferroni’s test was used to compare the continuous variables between the groups. Analysis of variance (ANOVA) for repeated measurements was used in study I to compare serial SOFA scores between the groups and in study IV when blood glucose profiles, insulin requirements and serial hemodynamic data were compared. In study III, Pearson’s correlation coefficient was used to assess the correlation between the results of the ACTH stimulation tests on day 1 and day 2. In study IV, Kaplan-Meier curves were calculated for shock reversal, and the comparison between the groups was performed with the log rank test.

In study II, the cytokine measurements were not normally distributed, and therefore nonparametric tests were used. The within-group variations were analysed using Friedman’s test, and the differences between the groups were compared using the Mann-Whitney U-test. The cytokine levels are expressed as median values (range). In study IV, a sample size was calculated on the basis of detecting a difference of 1 mmol/l in mean blood glucose levels between the study groups. A standard deviation of 1 mmol/l in blood glucose level was assumed when calculating a sample size based on previous studies (Van den Berghe 2001). A minimum of 17 patients was required in
each group ($\alpha = 0.05$, power = 80%). ICU mortality was expected to be 30%, and therefore 24 patients were randomized in both groups. P-values below 0.05 were considered significant. All Statistical analyses were performed using the SPSS Software (SPSS Inc. Chicago, USA).

**Ethical considerations**

All study protocols were approved by the local Ethics Committees. Informed consent was obtained from the patients or their first-degree relatives. Studies I, II, III were prospective observational studies and no specific interventions were performed. Study IV was randomized, but not placebo-controlled study, and all patients who were considered to benefit from the hydrocortisone received the actual treatment.
5. RESULTS

5.1. Incidence of adrenal insufficiency

The incidence of adrenal dysfunction was investigated in patients with severe sepsis (I) and in patients with septic shock (III). In severe sepsis, six patients (15%) had relative adrenal insufficiency. One patient demonstrated absolute adrenal insufficiency (stimulated cortisol level < 500 nmol/l), and a total incidence of adrenal dysfunction was 17% (I). In septic shock, the incidence of relative adrenal dysfunction was considerably higher: 16 patients (40%) with septic shock had impaired adrenal function on the first ACTH stimulation test, two of these patients demonstrated absolute adrenal insufficiency. The diagnostic criteria for relative adrenal insufficiency were slightly different in study I and III. If the same specific diagnostic criteria for adrenal insufficiency for septic shock patients used in the study III were applied to the study I population, two additional patients with relative adrenal insufficiency would be identified. Therefore, the total incidence of adrenal insufficiency would be 22% in severe sepsis.

5.2. Impact of adrenal function on the development and resolution of MOF

The impact of adrenal function on the development and resolution of multiple organ failure was assessed in severe sepsis (I). In nonsurvivors, the SOFA scores remained elevated in both study groups, and no differences were detected during the ICU stay. In survivors, the SOFA scores declined in both study groups, but resolution of organ failure was more rapid in those patients who demonstrated an adequate adrenal response compared with patients with an inadequate response. The difference in the SOFA scores between study groups was statistically significant (p=0.029, ANOVA). The daily SOFA scores of the non-survivors are presented in Figure 3 and the corresponding scores for survivors in Figure 4.
Figure 3. Daily SOFA scores (mean ± SD) of nonsurvivors in the inadequate adrenal response (IAR) and in the adequate adrenal response (AAR) groups (Figure 1 in the original article [study I]). There were no statistical differences between groups (p=0.902, ANOVA).

Figure 4. Daily SOFA scores (mean ± SD) of survivors in the inadequate adrenal response (IAR) and adequate adrenal response (AAR) groups (Figure 2 in the original article [study I]). The difference between the study groups during the study period was significant (p=0.029, ANOVA).
5.3. Impact of anti-inflammatory cytokines on the development of MOF

The impact of the anti-inflammatory cytokine profile on the development and resolution of multiple organ failure was evaluated in severe sepsis (II). Elevated concentrations of IL-6 and IL-1ra were detected in all patients, whereas IL-10 levels were elevated in 68% of the patients, and elevated IL-1β concentrations were found only in 38% of the patients. Fifteen (40%) of these patients developed severe MOF. The IL-6 levels were significantly higher in those patients who developed severe multiple organ failure and in the IL-6 levels remained elevated in these patients. In contrast, in those patients who did not develop MOF IL-6 concentrations decreased during the study period (Figure 5). The initial IL-10 levels were similar in both groups. In patients without severe MOF the IL-10 concentrations decreased over the time, but in MOF patients IL-10 levels were elevated on day 5 (Figure 6). To compare the relations between the pro- and anti-inflammatory responses, the IL-6/IL-10 and the IL-1β/IL-1ra ratios were studied. The median concentrations of IL-1ra were 2,200 times higher than those of IL-1β and no significant changes were seen in the IL-1β/IL-1ra ratios between the study groups. In contrast, the IL-6/IL-10 ratio remained low in patients who did not develop MOF, whereas in the MOF patients the IL-6/IL-10 ratio was significantly higher in the early phase of sepsis on days 1 and 3 (Figure 7).
Figure 5. IL-6 levels in patients with severe sepsis. No MOF represents the patient group without multiple organ failure (n=22) and MOF indicates patients who developed severe multiple organ failure (n=15). All patients were alive at day 5 and there are no missing cytokine data in the figure. IL-6 levels remained elevated in the MOF patients, whereas in the no MOF group a significant decrease was observed (p<0.001). The p-values in the figure represent the differences in cytokine levels between the study groups at each time point. The horizontal bars represent the median values (Figure 1 in the original article [study II]).
Figure 6. IL-10 levels in patients with severe sepsis. No MOF represents the patient group without multiple organ failure (n=22). MOF indicates patients who developed severe multiple organ failure (n=15). All patients were alive at day 5 and there are no missing cytokine data in the figure. In the MOF group, no significant changes were observed in IL-10 levels, whereas in the no MOF patients the levels declined (P=0.02 within the group). The P-value in the figure represents the difference in cytokine levels between the groups at the day 5. The horizontal bars represent the median values (Figure 2 in the original article [study II]).
Figure 7. IL-6/IL-10 ratios in patients with severe sepsis. No MOF represents the patient group without multiple organ failure (n=22). MOF indicates patients who developed severe multiple organ failure (n=15). IL-6/IL-10 levels were elevated in the MOF patients at day 1 and 3, whereas in the no MOF group the IL-6/IL-10 ratios remained low. The P-values in the figure represents the difference in cytokine levels between the groups at day 1 and 3. The horizontal bars represent the median values (Figure 5 in the original article [study II]).
5.4. Reproducibility of the ACTH test

The reproducibility of the ACTH stimulation test was investigated in 60 critically ill patients (III). The reproducibility of the test was good in patient with sepsis and in nonseptic patients. There was a good correlation in cortisol responses on day 1 and day 2 among the nonseptic critically ill patients (Pearson’s correlation coefficient 0.69, p=0.001) (Figure 8). In patients with sepsis the correlation was rather good (Pearson’s correlation coefficient 0.54, p=0.014) (Figure 9). In septic shock patients no correlation between the two cortisol responses was observed (Pearson correlation coefficient 0.40, p=0.080) (Figure 10).

Figure 8. Cortisol responses in the ACTH stimulation tests in nonseptic critically ill patients on days 1 and 2 (Figure 2 from Study III). Correlation between cortisol responses was good (p=0.001). Dotted lines indicate the threshold of 248 nmol/l.
Figure 9. Cortisol responses in the ACTH stimulation tests in patients with sepsis on days 1 and 2 (Figure 2 from Study III). Correlation was good in patients with sepsis (p=0.014). Dotted lines indicate the threshold of 248 nmol/l.

Figure 10. Cortisol responses in the ACTH stimulation tests in patients with septic shock on days 1 and 2 (Figure 2 from Study III). No correlation was observed (p=0.080). Dotted lines indicate the threshold of 248 nmol/l.
In patients with septic shock 5 out of 8 patients who had impaired adrenal response on the first day demonstrated a normal adrenal response on the second day, and six patients with septic shock (30%) who had a normal adrenal function on the first day demonstrated impaired adrenal function on the second day. An impaired adrenal response was seen in both stimulation tests only in three patients with septic shock; two of these patients had absolute adrenal insufficiency.
5.5. Comparison between continuous vs. bolus hydrocortisone infusion in septic shock

Blood glucose profiles, insulin requirements, amount of nursing workload needed and shock reversal was compared in septic shock patients who were randomized to receive hydrocortisone treatment either by bolus or by continuous infusion. Mean daily blood glucose levels, insulin requirements and intake of calories were similar in both groups (Figures 11, 12 and 13). When insulin requirements were adjusted to administered calories, there was a trend for lower insulin requirements in the infusion group throughout the study period, but this difference was not statistically significant.

Figure 11. Blood glucose levels (mean ± SD) in the study groups (Figure 2 in the original article [study IV]). No statistical difference between the study groups (p= 0.339, ANOVA).
Figure 12. Insulin requirements (mean ± SD) in the study groups (Figure 3 in the original article [study IV]). No statistical difference between the study groups (p=0.908, ANOVA).

Figure 13. Intake of calories (mean ± SD) in the study groups (Figure 4 in the original article [study IV]). No statistical difference between the study groups (p=0.169, ANOVA).
The data concerning glycemic control is presented in Table 9. Although the mean blood glucose levels were quite identical, the hyperglycemic (> 7 mmol/l) episodes were more common in bolus group than in infusion group (p=0.039). Severe hyperglycemia (blood glucose >8.3 mmol/l) was rare in both study groups, and also hypoglycemic episodes were uncommon. Three hypoglycemic (blood glucose < 3 mmol/l) episodes were observed in bolus group and only one in infusion group. Severe hypoglycemia (blood glucose < 2.2 mmol/l) was not observed in either study groups. The amount of nursing workload needed to maintain normoglycemia was higher in the bolus group: more insulin infusion rate adjustments were needed in the bolus group than in infusion treated patients (p = 0.038).

Table 9. Glycemic control in the study groups (Table 3 in the original article [study IV]).

<table>
<thead>
<tr>
<th></th>
<th>Bolus group N = 23</th>
<th>Infusion group N = 22</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean blood glucose (mmol/l)</td>
<td>6.4 ± 0.7</td>
<td>6.2 ± 0.7</td>
<td>0.040</td>
</tr>
<tr>
<td>Blood glucose variation coefficient (%)</td>
<td>20.2 ± 6.9</td>
<td>16.5 ± 4.8</td>
<td>0.063</td>
</tr>
<tr>
<td>Blood glucose &gt; 7 mmol/l (episodes / patient)</td>
<td>15.7 ± 8.5</td>
<td>10.5 ± 8.6</td>
<td>0.039</td>
</tr>
<tr>
<td>Blood glucose &gt; 8.3 mmol/l (episodes / patient)</td>
<td>3.6 ± 3.4</td>
<td>2.6 ± 3.2</td>
<td>0.383</td>
</tr>
<tr>
<td>Mean insulin dose (IU/day)</td>
<td>66 ± 43</td>
<td>61 ± 40</td>
<td>0.381</td>
</tr>
<tr>
<td>Insulin infusion adjustments (no / patient / day)</td>
<td>4.7 ± 2.2</td>
<td>3.4 ± 1.9</td>
<td>0.038</td>
</tr>
<tr>
<td>Blood glucose &lt; 3 mmol/l (episodes / group)</td>
<td>3 (13%)</td>
<td>1 (4.5%)</td>
<td>0.609</td>
</tr>
<tr>
<td>Blood glucose &lt; 2.2 mmol/l (episodes / group)</td>
<td>0</td>
<td>0</td>
<td>n.s.</td>
</tr>
</tbody>
</table>
The reversal of shock was similar in both study groups. The vasopressor support could be withdrawn within 48 hrs in 14 (58%) of the patients in bolus group and the corresponding figure in the infusion group was 12 (50%). After 5 days vasopressors were withdrawn in 20 patients (83%) in bolus group and in 15 patients (63%) in the infusion group. Serial hemodynamic data are presented in Table 10.

### Table 10. Hemodynamic parameters in Study IV (Table 4 in the original article).

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heart rate (1/min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bolus group</td>
<td>107 ± 20</td>
<td>93 ± 20</td>
<td>85 ± 20</td>
<td>78 ± 22</td>
<td>86 ± 21</td>
<td>0.93</td>
</tr>
<tr>
<td>Infusion group</td>
<td>100 ± 21</td>
<td>95 ± 19</td>
<td>87 ± 23</td>
<td>86 ± 22</td>
<td>83 ± 15</td>
<td></td>
</tr>
<tr>
<td><strong>MAP (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bolus group</td>
<td>62 ± 7.5</td>
<td>75 ± 12</td>
<td>79 ± 13</td>
<td>85 ± 15</td>
<td>87 ± 15</td>
<td>0.06</td>
</tr>
<tr>
<td>Infusion group</td>
<td>65 ± 7.9</td>
<td>72 ± 9.4</td>
<td>72 ± 12</td>
<td>75 ± 13</td>
<td>79 ± 19</td>
<td></td>
</tr>
<tr>
<td><strong>Cardiac index (l/min/m²)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bolus group</td>
<td>3.8 ± 1.7</td>
<td>3.5 ± 1.1</td>
<td>3.5 ± 0.9</td>
<td>3.4 ± 1.0</td>
<td>3.4 ± 0.7</td>
<td>0.52</td>
</tr>
<tr>
<td>Infusion group</td>
<td>3.6 ± 1.5</td>
<td>3.9 ± 1.3</td>
<td>3.5 ± 1.1</td>
<td>3.6 ± 1.3</td>
<td>3.8 ± 1.5</td>
<td></td>
</tr>
<tr>
<td><strong>SvO₂ (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bolus group</td>
<td>61 ± 7.6</td>
<td>67 ± 7.2</td>
<td>69 ± 7.3</td>
<td>70 ± 7.6</td>
<td>69 ± 7.0</td>
<td>0.50</td>
</tr>
<tr>
<td>Infusion group</td>
<td>64 ± 12</td>
<td>65 ± 10</td>
<td>63 ± 11</td>
<td>66 ± 13</td>
<td>70 ± 13</td>
<td></td>
</tr>
<tr>
<td><strong>SVR (dyn.s/cm²)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bolus group</td>
<td>623 ± 221</td>
<td>770 ± 323</td>
<td>861 ± 249</td>
<td>999 ± 334</td>
<td>1061 ± 200</td>
<td>0.55</td>
</tr>
<tr>
<td>Infusion group</td>
<td>731 ± 254</td>
<td>705 ± 248</td>
<td>818 ± 274</td>
<td>836 ± 332</td>
<td>832 ± 214</td>
<td></td>
</tr>
<tr>
<td><strong>Shock reversal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bolus group</td>
<td>0 / 24 (0%)</td>
<td>3 / 24 (13%)</td>
<td>14 / 24 (58%)</td>
<td>18 / 24 (75%)</td>
<td>20 / 24 (83%)</td>
<td>0.48</td>
</tr>
<tr>
<td>Infusion group</td>
<td>0 / 24 (0%)</td>
<td>5 / 24 (21%)</td>
<td>12 / 24 (50%)</td>
<td>14 / 24 (58%)</td>
<td>15 / 24 (63%)</td>
<td></td>
</tr>
</tbody>
</table>

MAP: mean arterial pressure, SVR: systemic vascular resistance; SvO₂ mixed venous oxygen saturation. P-values represent difference between the study groups (ANOVA and log rank test).
6. DISCUSSION

6.1. Clinical significance of anti-inflammatory response

The main purpose of this study was to investigate anti-inflammatory mechanisms in severe sepsis, to evaluate the clinical significance of the anti-inflammatory response and relative adrenal insufficiency and the limitations of the current diagnostic methods, and to compare different hydrocortisone treatment modalities in vasopressor-dependent septic shock. The results of this study demonstrated that both adequate adrenal function and IL-10 response seemed to have an important protective function in the pathophysiology of sepsis. Inadequate cortisol production and impaired IL-10 production was associated with the development of severe MOF, poor resolution of organ dysfunction, longer ICU stay and increased mortality. This study also demonstrated that the current diagnostic methods are not optimal in the identification of patients who might benefit from hydrocortisone therapy. Especially in septic shock a single ACTH stimulation test could not accurately reveal patients who had impaired adrenal function, and the results of the two consecutive ACTH tests were poorly reproducible in patients with septic shock.

There is a general agreement that the activation of the HPA axis functions as an important mechanism sustaining homeostasis in sepsis, but there is much controversy regarding the absolute levels of cortisol that are considered to be adequate in septic shock. Conflicting results have been published on the correlation between isolated plasma cortisol levels and patient prognosis. Both very high and low cortisol levels have been associated with increased mortality in sepsis, but in other studies no correlation has been observed (Sibbald 1977, Finlay 1982, Jurney 1987, Span 1992, Annane 2000). In general, it has been suggested that single plasma cortisol measurements have no predictive value on patient outcome (Schein 1990, Lippiner-Friedman 2007). The results in this study are accordance with these findings. Neither single nor serial cortisol measurements were useful in the assessment of adrenal reserve in severe sepsis. Even in situations where plasma cortisol levels are high, the decreased number or affinity of glucocorticoid receptors may contribute to glucocorticoid resistance in septic shock, and
patients may have functional cortisol deficiency (Huang 1987, Molijn 1995). The intracellular activity of cortisol may also be impaired due to changes in the activity of the type I and II 11\(\beta\)-hydroxysteroid dehydrogenase enzymes, which catalyze the interconversion of active cortisol and inactive cortisone (Draper 2005).

In addition to adequate cortisol production, sufficient IL-10 production also seemed to have protective function in the pathophysiology of multiple organ failure. Study II could not confirm the previously presented hypothesis of the role of overproduction of IL-10 in the pathogenesis of MOF (Friedman 1997). In contrast, the findings in this study support the opposite concept that there is a relative IL-10 deficiency in MOF patients. Relatively low IL-10 levels may exacerbate the systemic inflammatory response. In zymosan-induced experimental MOF, the absence of endogenous IL-10 has enhanced the development of organ dysfunction (Malleo 2007). Several clinical reports also suggest that endogenous IL-10 production have protective effects during critical illness. High levels of IL-10 have been reported to be beneficial in critically ill patients with SIRS, ARDS and severe pancreatitis (Donelly 1996, Armstrong 1997, Simovic 1999, Taniguchi 1999). Taniguchi et al. have demonstrated that in patients with SIRS elevated IL-10 levels were observed both in survivors and nonsurvivors, but the IL-6/IL-10 ratio was significantly higher among the nonsurvivors (Taniguchi 1999), a similar finding which was observed in the present study. In addition to anti-inflammatory effects, IL-10 also may be an important endogenous regulator of the activity of the HPA axis (Smith 1999). An inadequate IL-10 response and relative adrenal insufficiency may therefore be linked together in sepsis.

The incidence of relative adrenal insufficiency in severe sepsis was 22% (Study I) and in septic shock 40% (Study III). At present, data concerning the incidence of adrenocortical dysfunction in sepsis have substantial variation, because the exact criteria for relative adrenal insufficiency in critical illness are lacking. Due to the heterogeneity of the diagnostic methods in different studies, the incidences of adrenocortical insufficiency have varied between 0% and 55% in sepsis (Schein 1990, Soni 1995, Annane 2000, Oppert 2000). The most widely used criteria for relative adrenal insufficiency is a cortisol increment of < 250 nmol/l in the conventional ACTH test.
irrespective of the basal cortisol concentration (Keh 2004). With this criterion, up to 77% of the patients with refractory septic shock were considered to have relative adrenal failure (Annane 2002). However, if the basal cortisol levels are extremely high, it is probable that the cortisol response is blunted. Moran et al. demonstrated that cortisol response in ACTH simulation test was negatively correlated with the basal cortisol levels and in Oppert’s study majority of the patients who had basal cortisol levels above 1000 nmol/l the increment in cortisol was <250 nmol/l (Moran 1994, Oppert 2000). Whether this represents a maximally stimulated and appropriately functioning adrenal cortex or true relative adrenocortical insufficiency is still a matter for debate (Lamberts 1997, Marik 2000).

Interpretation of the low-dose (1 μg) ACTH test has similar problems. Siraux and co-workers performed both the low-dose and conventional ACTH test in 46 consecutive patients with sepsis shock. In their study the low-dose test identified a subgroup of patients with inadequate adrenal reserve who had a worse outcome and who would have missed by the high-dose test (Siraux 2005). The incidence of relative adrenal insufficiency was 68% in the low-dose test and 35% in the conventional test (Siraux 2005). In a study by Salgado et al. the cortisol response was twice as high in the conventional test as in the low-dose test. Correspondingly, the incidence of relative adrenal insufficiency was significantly higher in the low-dose test (Salgado 2006). If the same diagnostic threshold is used for the diagnosis of adrenal dysfunction, it is evident that the incidence of adrenal dysfunction in the low-dose test is substantially higher. Similarly, there is no consensus which threshold for cortisol increment should be used in the low-dose ACTH test in critically ill patients.

The most relevant finding in the present study concerning clinical practise was the finding that the results of ACTH stimulation tests were poorly reproducible in septic shock. This finding further questions the whole concept of relative adrenal insufficiency. No correlation was seen between the cortisol responses in two consecutive ACTH stimulation tests. Moreover, the majority of the septic shock patients who had a poor cortisol response on the first day demonstrated preserved adrenal function on the second day. Conversely, in six patients an adequate adrenal response on
the first day turned to be inadequate on the second day. Only in three patients impaired adrenal function was seen in both tests. These findings imply that in septic shock cortisol production may change rapidly over a short period of time. Analogous findings have been reported previously (Voerman 1992, Rydvlall 2000). According to the present study, it is probable that the results of the ACTH stimulation test may also change very rapidly. The poor reproducibility of the ACTH test in septic shock has been previously observed by Bouachour, and the results in study III confirm this finding (Bouachour 1995). In contrast, the reproducibility of the ACTH stimulation test was good in nonseptic critically ill patients. It is therefore obvious that the rapid changes in adrenocortical function are related specifically to septic shock.

The results obtained in study III shows that a single ACTH stimulation test can not identify all those patients who might benefit from hydrocortisone therapy, and there may be additional patients who will respond to corticosteroid therapy even if the results of the ACTH stimulation test are normal at the onset of septic shock. The value of the ACTH test in the assessment of adequacy of cortisol production has been recently questioned by several other authors. It has been suggested that its use could be limited for cases where the diagnosis of adrenal insufficiency is of special importance (Marik 2003, Ligtenberg 2004, Morel 2006).

Another confounding factor in the diagnosis of adrenal insufficiency is a variation in laboratory analysis. Cortisol immunoassays vary substantially. In general, there is a trend to obtain higher cortisol values than measured using the reference gas chromatography method (Vogeser 2005). In the CORTICUS trial, there was a considerable difference in the diagnosis of patients as responders, depending on the methodology. As a result, in approximately 20% of the patients the diagnosis of relative adrenal insufficiency could be missed due to laboratory inaccuracies (Briegel 2005).

The concept of serum free cortisol has added further complexity to the diagnosis of adrenal dysfunction in septic shock. In healthy subjects more than 90% of circulating total cortisol is protein-bound to albumin and cortisol binding globulin (CBG), and only 10% of the cortisol is present in the free form (Stewart 2003). This free cortisol is
considered to be the active form of the hormone and responsible for the physiological actions. Changes in the concentration of its carrier proteins can affect the concentration of the bound and unbound fraction of cortisol. In sepsis, decreased CBG and albumin levels are frequently observed. In these situations serum free cortisol levels are increased, even though at the same time the total serum cortisol levels seem to be inappropriately low (Arafah 2006). In the presence of hypoproteinemia, the majority of the patients may be incorrectly considered to have adrenal insufficiency, even though free cortisol levels are actually adequate (Hamrahian 2004). The data concerning free cortisol measurements in sepsis is currently very limited, and the technique for direct measurements for free cortisol is not available for routine clinical practise (Hamrahian 2004, Ho 2006).
6.2. Therapeutic implications

Currently, there is no consensus about whether the hydrocortisone therapy can be detrimental for some patients with sepsis shock, and whether patients whose adrenal function is normal should be excluded from corticosteroid therapy. In Annane’s study, there was tendency towards increased mortality in the treatment group among those patients who were responders in the ACTH test. In critical illness, prolonged use of corticosteroids may increase the risk of metabolic and neuromuscular complications and hospital-acquired infections (Rady 2006). Especially the risk of critical illness polyneuropathy and myopathy is increased due to corticosteroid therapy, and the use of corticosteroids has been associated with protracted weaning from mechanical ventilation and increased need for tracheostomy (De Jonghe 2002, Rady 2006). Plasma concentrations of cortisol obtained during so-called low-dose hydrocortisone therapy are clearly higher than physiological plasma levels. The plasma cortisol concentrations by using constant hydrocortisone infusion are 2000–3000 nmol/l, and the peak levels achieved after intravenous boluses are even higher, reaching values of 3000-4000 nmol/l (Oppert 2000, Arafah 2006).

The major adverse event associated with corticosteroid therapy is uncontrolled hyperglycemia. Hydrocortisone is a potent glucocorticoid. Hydrocortisone stimulates gluconeogenesis both in liver and in peripheral tissues, and it also exacerbates insulin resistance. In critical illness corticosteroid treatment induces hyperglycemia, and the frequency of insulin use increases with corticosteroid exposure (Rady 2006). Impaired glycemic control has been associated with increased mortality in heterogeneous population of critically ill patients (Krinsley 2003). Van den Berghe at al. showed that preventing hyperglycemia with insulin substantially improved outcome in critically ill surgical patients (Van den Berghe 2001). This survival benefit was also observed in a recent prospective study in medical ICU population who required ICU treatment for more than three days (Van den Berghe 2006). Prolonged hyperglycemia is also one possible pathophysiologic mechanism behind the development of critical illness polyneuropathy and myopathy (Bercker 2005). So far however, there is no evidence for what glucose level would be optimal for septic shock patients, and there is no clinical
study that has confirmed the beneficial effects of strict normoglycemia in sepsis (Cariou 2004). A multicenter study in Germany was discontinued prematurely because of identical mortality rate in the intensive insulin and control group, and a greater incidence of hypoglycemia in the tight blood glucose group (Brunkhorst 2008). In severely ill ICU patients the risk of hypoglycemia seems to be higher than in postoperative patients, and patients with sepsis are especially vulnerable to hypoglycemia (Viresendorp 2006, Brunkhorst 2008).

The result obtained in study IV give novel information on combining intensive insulin and hydrocortisone therapy in sepsis management. The goal of tight glycemic control is made more complicated by steroid-induced hyperglycemia. Study IV is the first prospective randomized trial that has investigated the metabolic effects of hydrocortisone treatment in septic shock patients. The results in this study demonstrate that continuous hydrocortisone infusion decreases the number of hyperglycemic episodes during intensive insulin therapy. This approach also reduces the amount of nursing workload required to maintain tight blood glucose control compared with conventional bolus application.
6.3. Limitations of the study

These studies were performed over a relatively long period of time. The first two studies were performed in a surgical intensive unit between 1997 and 1999, whereas studies III and IV were carried out 2003-2006 in multidisciplinary intensive care units. A striking difference between the severity of illness and ICU mortality was observed in these studies. In the first two studies the severity of illness was substantially lower, but at the same time the ICU mortality was high. During these years various randomized controlled trials have shown that specific therapeutic measures can reduce morbidity and mortality in selected groups of critically ill patients. These therapeutic measures include early goal-directed therapy, low tidal volume ventilatory treatment, strict glycemic control, daily interruption of sedative agents and steroid replacement therapy in vasopressor-dependent septic shock (ARDS Network 2000, Kress 2000, Rivers 2001, Van den Berghe 2001, Annane 2002). In critically ill surgical patients, Hartl et al. showed that implementation of these therapeutic measures decreased ICU mortality. In this observational study, ICU mortality was unchanged between 1993 and 2001, but after 2001 mortality decreased markedly from 32% to 19% after the implementation of these evidence-based strategies (Hartl 2006). The mortality figures in Hartl’s study are very similar to the mortality figures obtained in the present study. Other studies have also reported that the survival of septic shock patients has substantially improved during these years (Dombrovskiy 2005).

In addition to the different mortality rates the severity of illness was also different in these studies. Both the APACHE II and SAPS II scores were substantially higher in studies III and IV. This difference is partly explained by the differences in the data collection methods between the studies. In the first two studies physiologic variables were recorded from manually kept ICU worksheets, whereas in studies III and IV clinical information management systems were in routine use. The increased sampling rate of hemodynamic values in clinical information systems results in higher severity scores, a higher risk of hospital death and lower standardized mortality ratio (Suistomaa 2000). The differences in ICU admission and discharge policies and variations in the quality of ICU care may explain the differences in ICU performances over time.
(Metnitz 2000). The relatively small sample size, especially in studies I and II, may have had a substantial influence on the results. The interpretation of the results in these studies should therefore be done with caution.
6.4. Future perspectives

In 2002 the Society of Critical Care Medicine, the European Society of Intensive Care Medicine, and the International Sepsis Forum developed in collaboration a three-phase Surviving Sepsis Campaign. The purpose of this campaign was to increase clinician and public awareness of the incidence of sepsis, to develop guidelines for the management of severe sepsis and to improve the standard of care and to decrease the mortality in severe sepsis by 25% in 5 years (Dellinger 2004). The Surviving Sepsis guidelines recommend low-dose corticosteroids in the treatment of vasopressor-dependent septic shock, and over the last few years the use of steroids for patients in septic shock has increased (Keh 2004). Current evidence shows that hydrocortisone therapy has a significant effect on shock reversal, but the question whether this treatment has a true effect on patients prognosis is not fully established. Although there might be short-term benefits in decreasing mortality in certain subgroups of patients, there is a risk that use of steroids is associated with greater functional disabilities in survivors of ICU. Moreover, there is a need for the standardization of diagnostic testing and indications for hydrocortisone therapy in patients with septic shock. The purpose of the recent randomized controlled CORTICUS study comparing hydrocortisone to placebo in septic shock was to develop a uniformly accepted diagnostic testing system for adrenal insufficiency, to create diagnostic criteria for adrenal insufficiency and to establish indications for corticosteroid therapy in septic shock. This study enrolled 500 patients over 3 years from 52 European centers before the study was prematurely suspended. There was no difference in the overall 28-day mortality rate, although the duration of shock was shorter in patients who received corticosteroids (Sprung 2008). There was no apparent benefit for hydrocortisone replacement therapy in the subgroup of patients with relative adrenal insufficiency, as defined by the criteria of Annane.

Evidence-based medicine is difficult to apply in critical care medicine and in the treatment of sepsis particular. Clear-cut positive results from large randomized controlled trials in severe sepsis are not abundant. Many of the interventions applied to the treatment of sepsis that have undergone testing in general ICU population but not specifically in population with sepsis or septic shock. The mortality rate in vasopressor-
dependent septic shock is still high. In this setting, many treatment strategies can not ethically be studied in a placebo-controlled trial. The current situation is that the most of the therapies applies to critically ill patients lack even grade B level of evidence, and in Surviving Sepsis Campaign guidelines, the evidence for most interventions the is only level E (Vincent 2004). Although the effect of a single therapeutic intervention on patient mortality is difficult to show in randomized controlled trials, several individual hospitals have successfully implemented Surviving Sepsis Campaign guidelines, and an improved outcome in septic shock has been reported in individual ICUs (Kortgen 2006, Micek 2006, Shapiro 2006). The findings of the present study also support the concept that the mortality from sepsis has been reduced in the 21st century.

The concept that death from sepsis is attributable to an overstimulated immune system in the early phase of disease based on animal studies does not reflect the real clinical picture in humans (Fink 1990). The experimental studies used large doses of endotoxin to trigger systemic inflammatory response (Deitch 1998). Consequently, levels of circulating cytokines were exponentially higher in animals than they are in critically ill patients (Deitch 1998). Studies have shown that the frequency of an exaggerated inflammatory response is considerably lower than what it was originally thought to be. In clinical studies, many patients with severe sepsis frequently demonstrate undetectable levels for some cytokines (Damas 1991, Goldie 1995). This phenomenon was observed also in this study. Only one third of the patients had elevated IL-1β levels during the whole study period, and in general, the cytokine concentrations were markedly lower than in experimental studies.

Although anti-inflammatory reactions seem to have an important protective function in sepsis, individual patients may undergo many stages during which either pro- or anti-inflammatory reactions may be dominant. Therefore therapies directed to attenuate inflammatory response may therefore be beneficial in some septic patients and deleterious in others (Marshall 2000). Age, immune status, type of infection and genetic predisposition modifies the inflammatory response during the intensive care period. One step forward to a better targeted therapy in the future would be a better characterization of both systemic inflammatory and compensatory anti-inflammatory reactions. So far,
however, the heterogeneous nature of sepsis and the complexity of the disease process make it difficult to characterize individual inflammatory response in everyday clinical practice.
7. CONCLUSIONS

Based on these studies the following conclusions can be drawn.

1. With the current diagnostic criteria, functional adrenal insufficiency is relatively common in sepsis. An impaired adrenal response at the onset of sepsis was observed in 22% of the patients with severe sepsis and 40% of the patients with septic shock. In contrast, absolute adrenal insufficiency was uncommon.

2. Endogenous anti-inflammatory reactions have an important role in the development and resolution of multiple organ failure. Both inadequate cortisol and IL-10 production were associated with the development of severe multiple organ failure, poor resolution of organ dysfunction, longer ICU stay and increased mortality.

3. In septic shock changes in adrenocortical function are very rapid. A single ACTH test is not a reliable method in detecting inadequate cortisol production. Dichotomous categorization of adrenal function seems to be artificial in septic shock, and a single ACTH stimulation test can not identify accurately patients who might benefit from hydrocortisone therapy. If the results of the ACTH stimulation test are normal at the onset of septic shock, it is still possible that patients may develop adrenocortical dysfunction later during the ICU period.

4. Strict normoglycemia is more easily achieved if hydrocortisone therapy is given by continuous infusion to septic shock patients. Continuous hydrocortisone infusion will reduce the number of hyperglycemic episodes, and reduce the nursing workload during intensive insulin therapy. The differences between the study groups were rather marginal, and in both groups the normoglycemic goal could be achieved quite successfully. Slightly more liberal glucose control than in the original intensive insulin therapy trials very effectively prevented severe hypoglycemic episodes.
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