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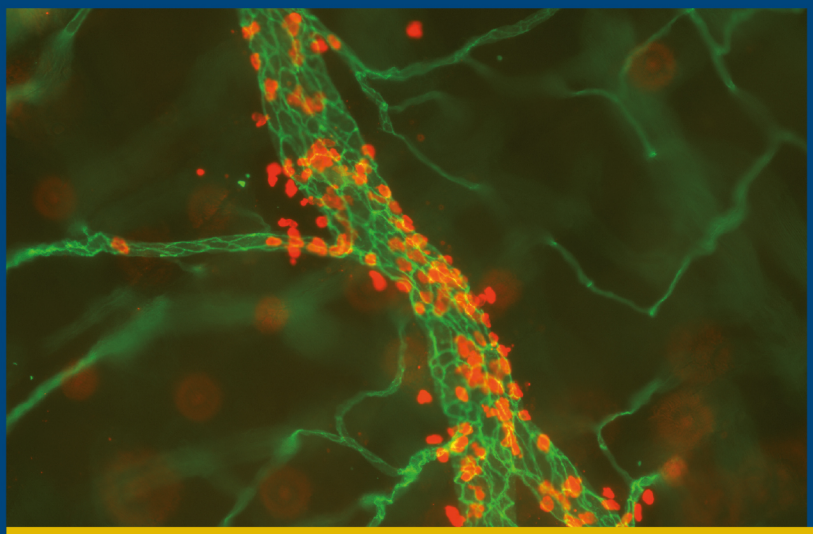
Sepsis – A Translational Approach

Hugo Van Aken and Dietmar Vestweber (Eds.)



Deutsche Akademie der Naturforscher Leopoldina –
Nationale Akademie der Wissenschaften, Halle (Saale) 2013

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Sepsis – A Translational Approach

Leopoldina Symposium

Münster
Friday, November 25, 2011

Editors:

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Member of the Academy

With 3 Figures and 1 Table

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Whole mount staining of a cremaster muscle of the mouse. Blood vessels are visualised in green by staining for the endothelial antigen PECAM-1 and neutrophil granulocytes are stained in red for MRP-14. The cremaster tissue was stimulated by TNF-α, which triggers inflammation and the extravasation of granulocytes. Reprinted from: Trends in Cell Biology *19*/1, VESTWEBER, D., WINDERLICH, M., CAGNA, G., and NOTTEBAUM, A. F.: Cell adhesion dynamics at endothelial junctions: VE-cadherin as a major player, pp. 8–15, 2009 (doi: 10.1016/j.tcb.2008.10.001), with permission from Elsevier.

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Inhalt

Foreword

Dear colleagues,
Ladies and gentlemen,

On November 25, 2011 the Leopoldina-Symposium on sepsis 2011 – a translational approach took place in Münster (Germany).

The topic is obviously timely. In the United States, it is estimated that > 751,000 cases of severe sepsis occur annually, resulting in an estimated 215,000 deaths. A rapid progression of illness severity from sepsis to severe sepsis to septic shock frequently occurs, driven by the body's inflammatory and anti-inflammatory responses to a pathogen, making sepsis a condition requiring timely intervention. The clinical management of severe sepsis and septic shock has evolved dramatically over the past decade and these new therapeutic approaches have been built on a deeper understanding of the natural evolution of sepsis. The aim of this symposium was to bring basic science and clinical research together to learn from each other and improve patient care.

Due to the incidence and the expensive effort of intensive care therapy of such patients, sepsis consumes considerable recourses and results in significant hospital costs. Adding indirect costs due to temporary or permanent morbidity severe sepsis imposes annual costs between 3.6 and 7.8 billion Euros in Germany in total. The treatment costs of sepsis in intensive care units amount to 1.7 billion which is about 1/3 of the total costs for intensive care in Germany. Thus, sepsis is a tremendous cost driver not only from the ICU but also from hospital or society's perspective. There is an urgent need for therapeutic standards and innovative therapies in the treatment of sepsis to improve outcome as well as for further cost studies to improve our knowledge.

A lot of participants of this symposium were already members of the German National Academy of Sciences, which reflect and honor their scientific accomplishments. However, the admission into the academy is not only an honor, but is also associated with different commitments. For example:

(*1*.) The members of the German National Academy of Sciences are responsible for scientific advice to politicians. In case of sepsis, this is very important, because the incidence of sepsis increases and this disease consumes considerable recourses.
(*2*.) Representation of German scientists in international committees.
(*3*.) Support the education of young scientists through the Leopoldina-Support program and boost the scientific careers of young scientists through the "Jungen Akademie".

Hugo Van Aken ML Dietmar Vestweber ML

Nova Acta Leopoldina NF *117*, Nr. 395, 9–10 (2013)

Welcome by Leopoldina Executive Committee

Philipp U. Heitz ML (Au, Switzerland)

Member of the Executive Committee, Secretary of Class III

Dear organisers, dear colleagues,

On behalf of the German National Academy of Sciences Leopoldina I welcome you to this Leopoldina-Symposium "Sepsis 2011 – A Translational Approach". This welcome address is an opportunity to make you familiar with the Academy.

1. The History of the Academy is Long and Eventful: From the "Academia Naturae Curiosorum" to the German National Academy of Sciences Leopoldina

Shortly after the end of the devastating Thirty Year's War and of the signature of the Treaty of Westphalia in Münster and in Osnabrück in 1648, a series of academies have been founded in Europe.

Among them the Leopoldina is the oldest continuously existing Academy involved in natural sciences. It was founded on New Years Day 1652 in the Free Imperial City of Schweinfurt by four physicians, among them its first president Johann Laurentius Bausch, then the city physician. It was officially recognised in 1677 by the emperor Leopold I (*1640; 1658–1705). Ten years later it was elevated to the "Sacri Romani Imperii Academia Caesareo – Leopoldino Naturae Curiosorum", thereby vested with the privileges of an Imperial Academy (the freedom of censorship of all publications was the most important among them). For the first time "the Leopoldina" was the "Reichsakademie", which it remained until the dissolution of the "Holy Roman Empire" in 1806 by emperor Franz II after having been issued an ultimatum by Napoléon I. However, the Academy continued to enjoy its privileges and relinquished them only towards the end of the 19th century.

In 1991 the Leopoldina was given the legal status of a non-profit registered association, and, on July 14, 2008 it was nominated to the German National Academy of Sciences.

For more than two centuries the seat of the Academy was located in the city of residence of its presidents. Only in 1878, its permanent seat was established in Halle (Saale). By the end of this year the Academy will move to new headquarters, located in a completely restored old building on the Jägerberg in Halle.

The Academy is still based on the German speaking "home countries" Germany, Austria and Switzerland (approximately 75 % of members). However, it now includes members from

over 30 countries, thereby ensuring its supranational character. It is financed by the Federal Government (Federal Ministry of Education and Research, BMBF: 80 %) and the State Government of Saxony-Anhalt (20 %).

Although recognised as a nation separated from the "Holy Roman Empire" in 1648, Switzerland, and after 1806, also Austria were always considered "home countries" of the Academy. It is for this reason that a Swiss Member of the Executive Committee is here to welcome you.

2. The Academy Has Proven to be Resistant to Political Interventions

The Academy has successfully met the formidable challenges of survival and of maintaining its independence during the years 1933 to 1945 and from 1946 to 1989. Especially during the latter period it followed a strict policy of independence, successfully avoiding interventions by the government of the GDR. It thereby succeeded to become a most important link in the German and international exchange of scientific activities.

3. The Academy is Active

The Academy counts approximately 1350 researchers contributing to the progress of science by outstanding scientific achievements in natural, engineering, cultural, behavioral and social sciences, in humanities and medicine. It currently comprises 28 sections, which are grouped into four classes in order to promote interdisciplinary discussions. The Academy's most important missions are the following:

(*i*) Promoting science in national and international co-operation. For this purpose, it runs academic events, appoints commissions, and publishes the results obtained. It awards honors and prizes, and promotes junior scientists.
(*ii*) As the National Academy of Sciences, the Leopoldina officially assumes the representation of German scientists in international committees and it contributes to the science-based consulting of the public and politics.

Compared to the age of the National Academy of Sciences anesthesia with its 170 years or so is still relatively young. However, the progress achieved in this discipline is striking – without this enormous progress major surgery carried out today would not be feasible.

On behalf of the Leopoldina I wish you a successful conference with relevant new information, fascinating talks, lively and stimulating discussions, and successful networking.

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Sepsis: A Challenge for Politicians, Researchers, and Clinicians

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Abstract

The first decade of the new millennium started with a series of high impact publications demonstrating new medical strategies which could improve survival in patients with sepsis. However, most of the confirming studies for these new strategies failed to reproduce the results of the initially positive clinical trials.

Due to the high incidence of sepsis (at present 76–110 per 100,000 adult inhabitants per year in Germany), which will further increase in the next decades as a consequence of the demographic change with more elderlies, and due to the mortality of > 45 % for severe sepsis and septic shock, sepsis constitutes a challenge for politicians, researchers, and clinicians.

The high direct and indirect costs of sepsis cause a high economic burden for the health care system challenging the politicians to set up the resources to efficiently fight against this disease. At the same time, researchers should aim at a better understanding of the pathophysiology of sepsis allowing for new drug development and a better translation from basic research results into the clinical practice. In combination with more preclinical and pre-marketing studies this could identify more precise indications and contraindications for new drugs and even avoid or at least limit the failure of expensive clinical trials. Besides the need for an improved understanding of the pathophysiology of sepsis the clinicians have to improve their implementation of the guidelines for prevention of nosocomial infections. Moreover, it is essential that all involved physicians are diagnosing sepsis as soon as it occurs in order to treat sepsis according the current guidelines. In addition, beside the needs which results from acute medical treatment, the clinicians have to learn to deal with the long-term consequences for the patients and their relatives.

Zusammenfassung

Die erste Dekade des neuen Jahrtausends begann mit einer Reihe von aufsehenerregenden Publikationen von neuen Behandlungsstrategien, die das Überleben von Patienten mit Sepsis verbessern können. Viele der Kontrollstudien für diese neuen Strategien konnten jedoch die Resultate der ersten positiven klinischen Versuche nicht bestätigen.

Aufgrund der hohen Inzidenz der Sepsis (zurzeit 76–110 auf 100 000 erwachsene Einwohner pro Jahr in Deutschland), welche in den nächsten Jahrzehnten als Konsequenz des demographischen Wandels mit mehr älteren Personen noch ansteigen wird, und der großen Mortalität von mehr als 45 % bei schwerer Sepsis und septischem Schock sind septische Erkrankungen eine Herausforderung für Politiker, Forscher und Kliniker.

Die hohen direkten und indirekten Kosten der Sepsiserkrankungen verursachen eine bedeutende Belastung für die Gesundheitssysteme, und die Politik ist gefordert, ausreichende Ressourcen für eine effiziente Bekämpfung dieser

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Krankheit bereitzustellen. Gleichzeitig sollten die Forscher auf ein besseres Verständnis der Pathophysiologie der Sepsis hinarbeiten, das die Entwicklung neuer Medikamente und eine bessere Umsetzung von Erkenntnissen der Grundlagenforschung in die klinische Praxis erlaubt. In Verbindung mit mehr vorklinischen Studien und Analysen vor der Markteinführung könnten auf diese Weise die präzisen Indikationen und Kontraindikationen für neue Medikamente ermittelt und das Risiko des Scheiterns großangelegter und teurer klinischer Versuche eingeschränkt werden. Die Kliniker sollten – neben der Notwendigkeit, die Pathophysiologie der Sepsis besser zu verstehen – vor allem die Richtlinien zur Vermeidung von Hospitalinfektionen weiterentwickeln. Es ist besonders wichtig, dass alle beteiligten Ärzte eine Sepsis so früh wie möglich diagnostizieren, um eine solche Erkrankung gemäß den aktuellsten Richtlinien zu behandeln. Neben den Erfordernissen der akuten Behandlung müssen die Ärzte es auch lernen, mit den langzeitigen Konsequenzen für die Patienten und ihre Verwandten umzugehen.

The first decade of the new millennium started with a series of high impact publications demonstrating medical strategies which could improve survival in patients with sepsis. At the beginning of the decade the ARDS-Network published results showing that a protective ventilation strategy using a tidal volume of 6 ml/kg ideal bodyweight resulted in a 9 % survival benefit (*The ARDS Network* 2000). One year later VAN DE BERGHE et al. (2001) published a monocentric study revealing that an intensive insulin therapy with blood glucose levels between 80–110 mg/dl was associated with an 4 % increase in over-all survival rate in general surgical intensive care patients in comparison to conventional insulin therapy which was only initiated if blood glucose levels were higher than 215 mg/dl. In the same year, BERNARD et al. (2001) published a multicenter study on the use of activated protein C in patients with severe sepsis (PROWESS-Study) which resulted in an increase in survival of 6 % in the intervention group. In 2002, two further interesting studies were published: RIVERS et al. (2002) showed that early and aggressive resuscitation in sepsis aiming at a mean arterial blood pressure of > 65 mmHg, a central venous oxygen saturation of > 70 % and a central venous pressure of > 8–12 mmHg resulted in a 16 % survival benefit. ANNANE and coworkers (2002) found a 6 % increase in survival using low hydrocortisone therapy in septic shock.

Theoretically, combining all these new strategies should have resulted in a cumulative 41 % increase in survival rate. However, no one expected such an increase in sepsis survival. Nevertheless, inspired from the positive findings of the above studies, the International Sepsis Campaign published the so-called Barcelona Declaration: "In view of the urgent need for action to deal with the growing burden of sepsis we […] call on healthcare professionals, health agencies and the public to support our initiative to reduce the incidence of sepsis mortality by 25 % within 5 years."

Unfortunately, we had to realise that our hopes were not fulfilled. Very recently, VILLAR et al. (2011) published the ALIEN-study demonstrating that despite the use of lung protective ventilation, overall ARDS intensive care unit (*ICU*) and hospital mortality was still as high as 42.7 % and 47.8 %, respectively. A second study on intensive insulin therapy in general medical ICU patients could not demonstrate the previously shown survival benefit. The first study analyzing this approach in a population of septic patients, the so-called VISEP trial by BRUNKHORST et al. (2008) even revealed a trend towards a lower survival rate in the intensive insulin group. In addition, the incidence rate of severe hypoglycemia episodes (≤ 40 mg/dl) was higher in the intensive-therapy group than in the conventional-therapy group (17.0 % vs. 4.1 %), as was the rate of serious adverse events (10.9 % vs. 5.2 %). Moreover, further studies on the use of activated protein C in severe sepsis in adults (ABRAHAM et al. 2005) and children (NADEL et al. 2007) could not confirm the positive results of the PROWESS-Study. Since also the PROWESS-Shock study, which assessed the efficacy of activated protein C in septic shock,

demonstrated negative results, the company decided in October 2011 to withdraw activated protein C from the market worldwide. Finally, a second multicenter study on the use of low dose hydrocortisone also failed to confirm a survival benefit in patients with septic shock (SPRUNG et al. 2008).

All these studies failing to reproduce the results of initially positive clinical trials lead to the prognosis that sepsis will constitute a challenge also for the next decades for politicians, researchers and clinicians.

1. Challenges for the Politicians

The incidence and relevance of sepsis is often underestimated. In 2001, ANGUS and coworkers (ANGUS et al. 2001) estimated that in the US alone at least 750,000 cases of severe sepsis occur every year. Because this figure was based on data from the year 1995, the US will most probably have an even higher number of cases today. The above authors believe that the number of cases of severe sepsis will increase steadily with an annual rate of 1.5 %. Since this increase in the incidence of severe sepsis is greater than the anticipated population growth, it is believed that this reflects, to some extent, the demographic change and the increase in the number of elderly Americans, who are more often suffering from sepsis than younger people. It has been shown that the incidence of sepsis is age-dependent, and starts in the US with 5.3/1000 at the age < 1 year, decreases to 0.2/1000 at the age of 5–14 years and finally increases to 26.2/1000 at the age > 85 years. There are predictions that in 2050 the US will have a population of 550 million inhabitants and at the same time an incidence of sepsis of 1.6 million cases per year, which is double as high as today.

In 2003, a prospective observational, cross-sectional 1-day point-prevalence study was performed in Germany to determine the national incidence, mortality and treatment of sepsis, severe sepsis and septic shock (ENGEL et al. 2007). 454 ICUs from a representative nationwide sample of 310 hospitals stratified by size were visited. From the received data the expected number of newly diagnosed cases with severe sepsis in Germany was estimated to be between 76–110 per 100,000 adult inhabitants per year. Therefore, the German politicians as well as the national health insurances have to realise that the given numbers of the German ministry of health dating from the year 2002 with an anticipated number of 5700 sepsis cases annually is too low, and might be as much as 10-times higher and still increases due to the demographic change. With such a high incidence the significance/meaning for the population is the same as e. g. breast carcinoma or colon carcinoma, which are newly diagnosed every year in 110/100,000 inhabitants and 50/100,000 inhabitants, respectively. Sepsis might even be more dangerous since the ICU and hospital mortality of patients with severe sepsis was 48.4 % and 55.2 %, respectively, which is higher than the mortality in breast or colon carcinoma.

The high incidence in combination with the high mortality rate causes a high economic burden for the health care system as well as the society. This economic burden is not only due to direct costs, which are estimated to be about 5 billion € per year in Germany, but also due to 10.2 billion € annual indirect costs (BRUNKHORST et al. 2011). These costs may result from the expensive acute medical treatment, but also from the post-discharge phase. Most patients have persistent cognitive and functional limitations in different areas (IWASHYNA et al. 2010), which slow down or even forbid the return to their normal working life. Moreover, it has been shown that not only patients after having survived severe sepsis but also close relatives are

developing anxiety and a posttraumatic stress disorder syndrome, resulting in a need for treatment. Therefore, beside the urgent need to create more awareness for the disease "sepsis", the politicians are asked to generate funds for treatment and research programs.

2. Challenges for the Researchers

Reasons for the failure of the initially mentioned confirmatory clinical studies might be the incomplete understanding of the pathophysiology of sepsis, the insufficient translation from basic research into the clinical practice and the time pressure of companies to get drug approval in order to allow a reimbursement for their research investment.

Clinicians still treat sepsis, severe sepsis and septic shock more or less independent from the etiology, since the final pathway of this disease leading to cardio-pulmonary failure and multi-organ failure is believed to be the same. But this idea may be misleading.

On the one hand some major cornerstones of the inflammatory response are known and seem to represent the common pathway in most etiologies of sepsis. The activation of the complement system and the C5-convertase activity of thrombin in the coagulation cascade lead to the generation of the anaphylatoxin C5a. This anaphylatoxin causes the release of pro-inflammatory mediators, such as the macrophage migration inhibitory factor (*MIF*) and the high-mobility group box 1 protein (*HMGB1*), and induces the tissue-factor expression, which is part of the coagulation cascade. Today, we know that the complement system, the coagulation cascade and the fibrinolysis cascade are interacting. Each of these systems has its own way to influence the course of the sepsis, e. g. HMGB1 binds to Toll-like receptor 4 (*TLR4*) and increases the release of further pro-inflammatory mediators. The complex Toll-like receptor signaling with the associated downstream effectors of immune cell function plays a crucial role in the innate system as a first line of defense. However, the signaling of the different mediators may also cause a sustained inflammatory response which can result in severe tissue damage. The Toll-like receptors are one of three families of so-called pattern recognition receptors, which initiate the host response during sepsis or tissue injury responsible for the innate immune response and regulation of the adaptive immune response to infection. The other two families are the nucleotide-oligomerisation domain leucine-rich repeat proteins and the cytoplasmic caspase activation and recruiting domain helicases such as retinoic-acid-inducible gene I-like helicases. Without doubt, there is a lot more of knowledge on the common pathway of sepsis, which is, however, not the scope of this article.

On the other hand, there are data showing that, depending on the etiology, the pathophysiology of sepsis is very specific, e. g. in case of *Streptococcus A*. The leading invasive strain of group A *Streptococcus* produces the M1 protein, which is a major virulence factor inducing toxic-shock-like vascular leakage and tissue injury. Neither the M1 protein nor fibrinogen alone are able to cause neutrophil activation. However, the structural complex formed between streptococcal M1 and human fibrinogen is responsible for this part of the pathophysiology in *Streptococcus A* sepsis (Macheboeuf et al. 2011). This example shows that our current knowledge, especially with respect to structural complex buildings between different mediators and receptors, is still incomplete and may be responsible for etiology specific pathways of sepsis.

Moreover, we only begin to understand how the results of basic research can be translated into clinically effective therapeutic strategies. Sometimes the basic molecular findings justify

the hope for an effective new therapy of sepsis. Companies take up the new idea and try to get the necessary data to get market approval as fast as possible. However, due to this economic – and sometimes also clinical – pressure to get a drug approval as fast as possible, essential preclinical and premarketing research might be shortened or even missing. This could result in clinical studies in which basically effective drug therapies fail to show a benefit because the indication is not specifically enough defined. An example for such an insufficient premarketing research might be activated protein C. Although in the late 1980s and early 1990s several laboratory and animal studies revealed evidence for the involvement of activated protein C in the sepsis pathway, BERNARD and coworkers who had published in 2001 the first multicenter trial on activated protein C still had to entitle the figure explaining the mechanisms of action with "Proposed actions of activated protein C in modulating the systemic inflammatory, procoagulant, and fibrinolytic host responses to infection". Whereas the so far known mode of action gave reason to believe that children and adults with different degree of sepsis should benefit from activated protein C, the performed specific trials could not demonstrate a survival benefit in children (NADEL et al. 2007), in patients with sepsis and only one organ failure (ABRAHAM et al. 2005) or in patients with septic shock, which finally led to a worldwide withdrawal of this drug from the market. This clearly demonstrates our insufficient understanding of the mode of action of this drug and the insufficient preclinical studies to identify the correct indications and contraindications. On the long run, in order to avoid such disappointing clinical results it might be less expensive to invest more in basic research and preclinical studies.

3. Challenges for the Clinicians

The challenges for clinicians are manifold. Besides the need for a better understanding of the pathophysiology of sepsis the physicians have to prevent nosocomial infections, diagnose septic patients as early as possible, treat sepsis according the current guidelines and deal with the long-term consequences for the patients and their relatives.

Although sepsis mostly results from etiologies which cannot be influenced by the clinicians, we have to accept that in some cases hospital acquired infections are the cause of sepsis. Whereas endogenous caused hospital acquired infections are only partly avoidable, the exogenous caused hospital acquired infections are mostly preventable. It is estimated that out of the 400,000 to 600,000 nosocomial infections occurring in Germany every year about 80,000–180,000 are avoidable (GASTMEIER et al. 2010). The most important reason for the exogenous caused hospital acquired infection is the insufficient compliance with evidence-based infection control guidelines. Especially the hand hygiene control measures are insufficiently followed (BERENHOLTZ et al. 2004). It is estimated that the insufficient adoption of the infection control measures causes the death of 1500 to 4500 patients every year in Germany (GASTMEIER et al. 2010).

Since the early treatment of sepsis with antibiotics and volume resuscitation (RIVERS et al. 2001) seem to be key elements of the therapy, it is essential to diagnose sepsis as soon as sepsis occurs. Even if the used definition for sepsis – two or more SIRS-criteria in combination with proven or suspected infection – is not perfect, it is internationally accepted. Using an intensive training enabling the physicians to diagnose sepsis at the earliest possible time point and to apply a bundle of therapy strategies resulted in a relevant survival benefit in spite of

the above mentioned negative follow-up studies (Barochia 2010, Levy 2010). There are two bundles which are relevant for the treatment of sepsis: the resuscitation and the management bundle. The resuscitation bundle, which should be performed as soon as possible, but at least within the first 6 hours, consists of

- lactate measurement;
- blood cultures retrieval before start of antibiotic treatment;
- administration of broad-spectrum antibiotics within 1 hour;
- in case of hypotension (MAP ≤ 65 mmHg) and/or Lactate > 4 mmol/L.
  - use fluid resuscitation if CVP < 8–12 mmHg;
  - use Vasopressors if MAP remains < 65 mmHg;
  - use dobutamine if $S_{CV}O_2$ < 70 %.

At present, the management bundle, which should be achieved as soon as possible, but at least within the first 24 hours, consists of

- lung protective ventilation;
- insulin therapy if blood glucose is > 180 mg/dl;
- considering low dose hydrocortisone in catecholamine refractory septic shock;
- renal replacement therapy, if needed.

Increasing the compliance with the use of these two bundles led to a reduction of the unadjusted hospital mortality from 37 % to 30.8 % over 2 years. The adjusted odds ratio for mortality improved the longer a site actively participated in this campaign, resulting in an adjusted absolute mortality drop of 0.8 % per quarter and 5.4 % over 2 years (Levy et al. 2010). It can only be speculated which part of these bundles is the most effective single measure. Since it has been shown that each hour delaying the onset of effective antibiotic treatment is associated with an increase in mortality of sepsis, the earliest possible diagnosis resulting in septic source eradication in combination with an adequate hemodynamic treatment is a prerequisite of an effective sepsis therapy.

4. Conclusion

In conclusion, researchers, politicians, and clinicians have to develop commonly agreed strategies to better explore and understand the pathophysiology of sepsis, to improve the translational research process from molecular biology to humans, to educate clinicians and to create a better understanding for the significance and burden of sepsis in our society. These strategies should be supported by additional specific funding lines for sepsis research.

References

Abraham, E., Laterre, P. F., Garg, R., Levy, H., Talwar, D., Trzaskoma, B. L., Francois, B., Guy, J. S., Bruckmann, M., Reaneto, A., Rossaint, R., Perrotin, D., Sablotzki, A., Arkins, N., Utterback, B. G., Macias, W. L., and *for the Administration of Drotrecogin Alfa (Activated) in Early Stage Severe Sepsis (ADDRESS) Study Group*: Drotrecogin alfa (activated) for adults with severe sepsis and a low risk of death. New Engl. J. Med. *353*/13, 1332–1341 (2005)

Angus, D., Linde-Zwirble, W. T., Lidicker, J., Clermont, G., Carcillo, J., and Pinsky, M. R.: Epidemiology of severe sepsis in the United States: Analysis of incidence, outcome, and associated costs of care. Crit. Care Med. *29*, 1303–1310 (2001)

Annane, D., Sebille, V., Charpentier, C., Bollaert, P. E., Francois, B., Korach, J. M., Capellier, G., Cohen, Y., Azoulay, E., Troche, G., Chaumet-Riffaut, P., and Bellissant, E.: Effect of treatment with low doses of hydrocortisone and fludrocortisone on mortality in patients with septic shock. JAMA *288*, 862–871 (2002)

The ARDS Network: Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. New Engl. J. Med. *342*, 1301–1308 (2000)

Barochia, A. V., Cui, X., Vitberg, D., Suffredini, A. F., O'Grady, N. P., Banks, S. M., Minneci, P., Kern, S. J., Danner, R. L., Natanson, C., and Eichacker, P. Q.: Bundled care for septic shock: An analysis of clinical trials. Crit. Care Med. *38*, 668–678 (2010)

Berenholtz, S. M., Pronovost, P. J., Lipsett, P. A., Hobson, D., Earsing, K., Farley, J. E., Milanovich, S., Garrett-Mayer, E., Winters, B. D., Rubin, H. R., Dorman, T., and Perlet, T. M.: Eliminating catheter-related bloodstream infections in the intensive care unit. Crit. Care Med. *32*/10, 2014–2021 (2004)

Bernard, G. R., Vincent, J. L., Laterre, P. F., Larosa, S. P., Dhainaut, J. F., Lopez-Rodriguez, A., Steingrub, J. S., Garber, G. E., Helterbrand, J. D., Ely, E. W., and Fisher, C. J.: Recombinant human protein C worldwide evaluation in severe sepsis (PROWESS) study group. Efficacy and safety of recombinant human activated protein C for severe sepsis. New Engl. J. Med. *344*, 699–709 (2001)

Brunkhorst, F. M., Engel, C., Bloos, F., Meier-Hellmann, A., Ragaller, M., Weiler, N., Moerer, O., Gruendling, M., Oppert, M., Grond, S., Oolthoff, D., Jaschinski, U., John, S., Rossaint, R., Welte, T., Schaefer, M., Kern, P., Kuhnt, E., Kiehntopf, M., Hartog, C., Natanson, C., Loeffler, M., Reinhart, K., and *German Competence Network Sepsis (SepNet)*: Intensive insulin therapy and pentastarch resuscitation in severe sepsis. New Engl. J. Med. *358*/2, 125–139 (2008)

Brunkhorst, F. M., Hagel, S., Ludewig, K., Hutagalung, R., Heublein, S., and Hartmann, M.: Direct costs related to severe sepsis in Germany: an update. Infection. *39*/Suppl. 2, 143–144 (2011)

Engel, C., Brunkhorst, F., Bone, H. G., Brunkhorst, R., Gerlach, H., Grond, S., Gruendling, M., Huhle, G., Jaschinski, U., John, S., Mayer, K., Oppert, M., Olthoff, D., Quintel, M., Ragaller, M., Rossaint, R., Stuber, F., Weiler, N., Welte, T., Bogatsch, H., Hartog, C., Loeffler, M., and Reinhart, K.: Epidemiology of sepsis in Germany: results from a national prospective multicenter study. Intensive Care Med. *33*, 606–618 (2007)

Gastmeier, P., Brunkhorst, F., Schrappe, M., Kern, W., and Geffers, C.: How many nosocomial infections are avoidable? Dt. Med. Wochenschr. *135*, 91–93 (2010)

Iwashyna, T. J., Ely, E. W., Smith, D. M., and Langa, K. M.: Long-term cognitive impairment and functional disability among survivors of severe sepsis. JAMA *304*/16, 1787–1794 (2010)

Macheboeuf, P., Buffalo, C., Fu, C. Y., Zinkernagel, A. S., Cole, J. N., Johnson, J. E., Nizet, V., and Ghosh, P.: Streptococcal M1 protein constructs a pathological host fibrinogen network. Nature *472*/7341, 64–68 (2011)

Levy, M. M., Dellinger, R. P., Townsend, S. R., Linde-Zwirble, W. T., Marshall, J. C., Bion, J., Schorr, C., Artigas, A., Ramsay, G., Beale, R., Parker, M. M., Gerlach, H., Reinhart, K., Silva, E., Harvey, M., Regan, S., and Angus, D. C., *on behalf of The Surviving Sepsis Campaign*: Results of an international guideline-based performance improvement program targeting severe sepsis. Crit. Care Med. *38*, 367–374 (2010)

Nadel, S., Goldstein, B., Williams, M. D., Dalton, H., Peters, M., Macias, W. L. Abd-Allah, S. A., Levy, H., Angle, R., Wang, D., Sundin, D. P., and Giroir, B.: For the researching severe sepsis and organ dysfunction in children: a global perspective (RESOLVE) study group. Drotrecogin alfa (activated) in children with severe sepsis: a multicentre phase III randomised controlled trial. Lancet *369*, 836–843 (2007)

Rivers, E., Nguyen, B., Havstad, S., Ressler, J., Muzzin, A., Knoblich, B., Peterson, E., and Tomlanovich, M.: For the early goal-directed therapy collaborative group. Early goal-directed therapy in the treatment of severe sepsis and septic shock. New Engl. J. Med. *345*/19, 1368–1377 (2001)

Sprung, C. L., Annane, D., Keh, D., Moreno, R., Singer, M., Freivogel, K., Weiss, Y. K., Benbenishty, J., Kalenka, A., Forst, H., Laterre, P. F., Reinhart, K., Cuthbertson, B. H., Payen, D., and Briegel, J., *for the Corticus Study Group*: Hydrocortisone therapy for patients with septic shock. New Engl. J. Med. *358*/2, 111–124 (2008)

Van den Berghe, G., Wouters, P., Weekers, F., Verwaest, C., Bruyninckx, F., Schetz, M., Vlasselaers, D., Ferdinande, P., Lauwers, P., and Bouillon, R.: Intensive insulin therapy in critically ill patients. New Engl. J. Med. *345*/19, 1359–1367 (2001)

Van den Berghe, G., Wilmer, A., Hermans, G., Meersseman, W., Wouters, P. J., Wilants, I., Bobbaers, H., and Bouillon, R.: Intensive insulin. Therapy in the medical ICU. New Engl. J. Med *354*/5, 449–461 (2006)

Villar, J., Blanco, J., Anón, J. M., Santos-Bouza, A., Blanch, L., Ambrós, A., Gandía, F., Carriedo, D., Mosteiro, F., Basaldúa, S., Fernández, R. L., and Kacmarek, R. M., *for the ALIEN Network*: The ALIEN study: incidence and outcome of acute respiratory distress syndrome in the era of lung protective ventilation. Intensive Care Med. *37*/12, 2380–2384 (2011)

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Basic Mechanisms – New Insights in an Old Problem

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Pathogenesis of Sepsis and Multiple Organ Dysfunction Syndrome

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Abstract

Sepsis is a severe illness caused by overwhelming infection of the bloodstream, affecting millions of individuals around the world each year, killing one in four, and increasing in incidence. This contribution deals with the initiation of inflammatory response, the amplification of inflammation, the sepsis-induced immunosuppression, the organ failures, the role of platelets and neutrophils and innate immunity in sepsis.

Zusammenfassung

Sepsis ist eine schwere Erkrankung, die durch eine in den Blutkreislauf übertretende Infektion verursacht wird, Millionen Menschen jedes Jahr befällt, einen von vier Betroffenen tötet und eine steigende Inzidenz aufweist. Der Beitrag beschäftigt sich mit der Entzündungsreaktion, der Ausbreitung der Entzündung, der sepsisinduzierten Immunsuppression, dem Organversagen und der Rolle von Plättchen und Neutrophilen im Rahmen der Sepsis.

1. Introduction

Sepsis is a severe illness caused by overwhelming infection of the bloodstream, affecting millions of individuals around the world each year, killing one in four, and increasing in incidence (Dellinger et al. 2008). The American College of Chest Physicians/Society of Critical Care Medicine (*ACCP/SCCM*) defines sepsis as systemic inflammatory response syndrome (SIRS) caused by an infection (bacterial, viral, parasitic or fungal) (Bone et al. 1992). Complications of sepsis can lead to severe sepsis, septic shock, and multiple organ dysfunction syndrome (*MODS*), and this often results in death. Severe sepsis is a major cause of death in the intensive care unit, causing 215,000 deaths in the US every year (Angus et al. 2001). The immune system combats microbial infections but the inappropriate activation and positioning of neutrophils within the microvasculature contributes to the pathological manifestations of multiple organ failure. Sepsis is a complex disease involving many biological mediators and cell types. There is growing evidence that both cells of the immune system (e.g. neutrophils) and platelets are involved in the pathogenesis of sepsis, and together they cooperate to contribute to the inflammatory response.

Bacteria or LPS provoke uncontrolled release of pro-inflammatory cytokines (e.g. tumor necrosis factor-α, interleukin-1,-6, and -8) from immune cells, including monocytes and macrophages. In response to the cytokine storm and bacterial shedding of lipopolysaccharide (*LPS*) during the initial phase of sepsis, neutrophils become inappropriately activated and get stuck primarily in the capillaries of the lungs and the sinusoids of the liver (Welbourn and Young 1992). Neutrophils are ideally suited for the elimination of pathogenic bacteria because of their large stores of proteolytic enzymes and rapid production of reactive oxygen species to degrade internalised pathogens (Nathan 2006). If these lytic factors or pro-inflammatory cytokines (Nathan 2006) are released extracellularly from tissue-infiltrating neutrophils, local damage will ensue (Holman and Saba 1988, Smith 1994, Grimminger et al. 1990, Chosay et al. 1990). Indeed, neutrophil-induced tissue injury occurs at sites of localised bacterial infection, which, in its extreme form, leads to abscess formation, although any generalised tissue infiltration or organ damage in this situation is rare. By contrast, in severe sepsis, local infection is accompanied by systemic neutrophil activation.

The mechanism by which inappropriately activated neutrophils induce injury is indicated by *in vitro* studies showing that endotoxin and other pro-inflammatory mediators directly and potently activate neutrophils to produce elastase (Smedly et al. 1986) and superoxide ions (Holman and Maier 1988). *In vitro* and *in vivo* data indicate that activated neutrophils are capable of inflicting considerable endothelial injury *via* combined action of these agents, probably acting synergistically (Weiss 1989). Neutrophils incubated with endotoxin are capable of causing acute lung injury when re-injected into animals (Welsh et al. 1989). Furthermore, endotoxin primes neutrophils to produce an enhanced respiratory burst in response to a second activating stimulus (Forehand et al. 1989). This priming effect is shown by the ability of trace amounts of endotoxin to act synergistically in producing lung injury in animals whose neutrophils have been exposed to small amounts of a chemotactic peptide (Worthen et al. 1987). Injection of either agent alone does not cause lung injury in this model, demonstrating that sublethal doses of endotoxin can still provoke significant injury in the presence of other predisposing factors.

The release of the pro-inflammatory mediators including TNF-α and IL-1 during the early phase of sepsis makes the endothelium and the underlying tissue more susceptible to neutrophil-mediated killing (Varani et al. 1988), presumably because TNF-α and IL-1 increase neutrophil adhesiveness and recruitment.

2. Initiation of Inflammatory Response

Microbes express motifs that are called pathogen-associated molecular patterns (*PAMPs*) (Mackey and McFall 2006). PAMPs are recognised by a family of transmembrane or intracytoplasmic receptors, named pathogen recognition receptors (*PRRs*), that are expressed on immune cells (Akira et al. 2006). The engagement of PRR induces the activation of immune cells characterised by initiation of microbe-killing systems, production and secretion of pro-inflammatory cytokines and chemokines (Cohen 2002). Normally, the immune response is tightly regulated by different mechanisms that are triggered by PRRs themselves to control the process. These mechanisms include production of anti-inflammatory cytokines, inhibition of the TLR-related intracellular signalling, and neutralisation of extracellular pro-inflammatory cytokines (Fernandez-Botran 1991, Foster et al. 2007). In some patients with sepsis, a

markedly imbalanced cytokine response occurs that converts beneficial responses into damaging inflammation.

3. Amplification of Inflammation

Bacteria activate different cascades that finally lead to harmful effects. Released tissue factor (*TF*) produces activated thrombin that triggers the formation of microthrombi leading to disseminated intravascular coagulation and booster pro-inflammatory pathways (RITTIRSCH et al. 2008b, AMARA et al. 2010). Furthermore, damaged cells release cell parts called alarmins that can booster immune response through PRRs engagement (ZHANG et al. 2010). In addition to this, the alarmins can also sensitize immune cells to PAMPs, amplifying the inflammation (PATERSON et al. 2003). The generation of large amounts of C5, an end-product of the complement system, in sepsis is associated with poor prognosis (GERARD 2003). C5a induces the activation of the coagulation cascade and the release of proinflammatory cytokines (RITTIRSCH et al. 2008a). The early phase of sepsis is characterised by sympathetic nervous system activation and release of catecholamines, which amplify inflammatory response through immune cells activation (MIKSA et al. 2009). Furthermore, the gut is considered as an immunologically active organ and a cornerstone in the burden of infection induced systemic inflammation. The sepsis-induced loss of intestinal mucosal integrity facilitates translocation of viable bacteria and bacterial products into gut-associated lymphatic tissue and mesenteric lymph nodes amplifying the deleterious inflammatory response and the development of organ failures (COOPERSMITH et al. 2002, LEAPHART and TEPAS 2007).

4. Sepsis-induced Immunosuppression

If patients survive the initial phase of the sepsis, which is characterised by a 'cytokine storm', they often have features of immunosuppression, called 'compensatory anti-inflammatory response syndrome', and are vulnerable to develop nosocomial infections (HOTCHKISS and KARL 2003, ADIB-CONQUY and CAVAILLON 2009). Immune dysfunction during sepsis is mediated by apoptosis of immune cells (WHEELER 2009). It has been shown that anergy of lymphocyte correlates with mortality in sepsis (HEIDECKE et al. 1999). Monocytes from septic patients show decreased human leukocyte antigen (*HLA*)-DR expression generally predicting higher risk of secondary infection (WARD et al. 2008). Moreover, in the later phase of sepsis a shift to anti-inflammatory cytokines can be observed, which correlates with a higher mortality (GOGOS et al. 2000).

5. Organ Failures

Multiple organ failure is often the cause of death in patients with sepsis (VINCENT et al. 2000). However, the pathogenesis of organ dysfunction is still unknown (GUSTOT et al. 2009, LEVI and TEN CATE 1999, LANDRY and OLIVER 2001, IGNARRO et al. 1987, PITTNER et al. 2003, DE BACKER et al. 2002, PIAGNERELLI et al. 2003, BREALEY et al. 2002). Tissue hypoxia and hypoperfusion play an important role. The mechanisms involve decreased microperfusion sec-

ondary to thrombi formation, reduced red blood cells deformability, blood maldistribution, tissue edema caused by increased capillary permeability, and decrease in perfusion pressure due to nitric oxide-related vasoplegia (DE BACKER et al. 2002, REGGIORI et al. 2009). In addition, cells may be unable to properly utilise available oxygen due to nitric oxide-induced impairment in mitochondrial respiration. Finally, cellular infiltrates, in particular neutrophils, damage tissue directly by releasing lysosomal enzymes and superoxide derived free radicals.

6. Platelets and Innate Immunity

Platelets play an important role in hemostasis, but it is well established that they also participate in the induction of inflammation and defence against infection (VON HUNDELSHAUSEN and WEBER 2007, KLINGER and JELKMANN 2002). Platelets rapidly localise to sites of infection and injury (WEYRICH and ZIMMERMAN 2004). The cells store different cytokines and mediators within their different compartments that are rapidly released upon activation and contribute to inflammation and immune progression (WEYRICH and ZIMMERMAN 2004). Furthermore, platelets can interact directly with and act on other cells through direct cell-cell contact mediated by adhesion molecules or by the release of various mediators (ZARBOCK et al. 2007). Platelets also have the ability to bind and internalise bacteria and viruses (YOUSSEFIAN et al. 2002).

7. Platelet-Neutrophil Interaction in Sepsis

During septicemia, activated neutrophils get stuck primarily, but not exclusively, in the sinusoids of the liver and the capillaries of the lung leading to liver and lung dysfunction (WELBOURN and YOUNG 1992). It is suspected that this is the effect of inappropriate neutrophil activation due to endotoxemia or bacteremia (WELBOURN and YOUNG 1992). Neutrophils activated by LPS are more rigid and this altered biomechanical properties leads to the trapping of neutrophils in the small capillaries of the lung (MCCLENAHAN et al. 2000). Similar to neutrophils, platelets also migrate into the liver and lung (ANDONEGUI et al. 2005). Neutrophils are essential for the platelet recruitment, because depleting neutrophils before inducing endotoxemia reduces the migration of platelets to liver and lung (ANDONEGUI et al. 2005). Platelets can either directly interact with neutrophils or bind at sites made pro-adhesive by neutrophils. Several studies demonstrated that platelet-neutrophil aggregates occur *in vitro* and *in vivo* after stimulation (LARSEN et al. 1989, ZARBOCK et al. 2006). Activated platelets are more spherical with arm-like protrusions that foster binding to leukocytes and endothelial cells (ZARBOCK et al. 2007).

Clinical trials including patients with sepsis have shown an increased platelet activation and adhesion to endothelial cells and neutrophils (MAVROMMATIS et al. 2000, GAWAZ et al. 1997). A similar observation was made in mice with acid-induced acute lung injury (ZARBOCK et al. 2006) and endotoxemic mice (ANDONEGUI et al. 2005). By visualising the microcirculation of the liver, it was demonstrated that platelets directly adhere to neutrophils (CLARK et al. 2007). Similar to this *in vivo* observation, it was demonstrated *in vitro* that LPS stimulated platelets bound more likely to immobilised neutrophils compared to endothelial cells (CLARK et al. 2007). Plasma from septic patients induces the formation of platelet-neutrophil aggregates

(CLARK et al. 2007), suggesting that it contains mediators that are required for the formation of this cell-cell interaction. By using a TLR4 antagonist it was demonstrated that platelets can be activated by TLR4 ligands as well as other mediators and/or bacterial products (CLARK et al. 2007). These data suggest the possibility that there exists an interplay between neutrophils and platelets in the fight against infection.

8. Platelets Activate Neutrophils to Trap Bacteria

Neutrophils as well as platelets are able to trap microbial pathogens independently of each other (NATHAN 2006, YOUSSEFIAN et al. 2002). The interaction of platelets with neutrophils induces transcellular synthesis and hyperactivation of neutrophils to produce increased pro-inflammatory cytokines (MARCUS et al. 1984). In a recently published paper, CLARK et al. (2007) identified a new mechanism of how the interaction between platelets and neutrophils improves the trapping of bacteria. During endotoxemia and sepsis, activated platelets adhere to neutrophils and activate them. Subsequently, the activated neutrophils release their DNA, which in turn contribute to the trapping of bacteria (CLARK et al. 2007). These structures were similar to neutrophil extracellular traps (*NETs*) that were originally identified by BRINKMAN et al. (2004). Plasma from septic patients can also induce the formation of platelet-neutrophil aggregates and NET formation. Importantly, the treatment of NETs with DNase resulted in degradation of these NETs (WARTHA et al. 2007), and also significantly reduced trapping of bacteria (CLARK et al. 2007). It was demonstrated that NETs were also produced *in vivo*.

References

ADIB-CONQUY, M., and CAVAILLON, J. M.: Compensatory anti-inflammatory response syndrome. Thromb. Haemost. *101*, 36–47 (2009)

AKIRA, S., UEMATSU, S., and TAKEUCHI, O.: Pathogen recognition and innate immunity. Cell *124*, 783–801 (2006)

AMARA, U., FLIERL, M. A., RITTIRSCH, D., KLOS, A., CHEN, H., ACKER, B., BRUCKNER, U. B., NILSSON, B., GEBHARD, F., LAMBRIS, J. D., and HUBER-LANG, M.: Molecular intercommunication between the complement and coagulation systems. J. Immunol. *185*, 5628–5636 (2010)

ANDONEGUI, G., KERFOOT, S. M., MCNAGNY, K., EBBERT, K. V., PATEL, K. D., and KUBES, P.: Platelets express functional Toll-like receptor-4. Blood *106*, 2417–2423 (2005)

ANGUS, D. C., LINDE-ZWIRBLE, W. T., LIDICKER, J., CLERMONT, G., CARCILLO, J., and PINSKY, M. R.: Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit. Care Med. *29*, 1303–1310 (2001)

BACKER, D. DE, CRETEUR, J., PREISER, J. C., DUBOIS M. J., and VINCENT, J. L.: Microvascular blood flow is altered in patients with sepsis. Amer. J. Respir. Crit. Care Med. *166*, 99–104 (2002)

BONE, R. C., BALK, R. A., CERRA, F. B., DELLINGER, R. P., FEIN, A. M., KNAUS, W. A., SCHEIN, R. M., and SIBBALD, W. J.: Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest *101*, 1644–1655 (1992)

BREALEY, D., BRAND, M., HARGREAVES, I., HEALES, S., LAND, J., SMOLENSKI, R., DAVIES, N. A., COOPER, C. E., and SINGER, M.: Association between mitochondrial dysfunction and severity and outcome of septic shock. Lancet *360*, 219–223 (2002)

BRINKMANN, V., REICHARD, U., GOOSMANN, C., FAULER, B., UHLEMANN, Y., WEISS, D. S., WEINRAUCH, Y., and ZYCHLINSKY, A.: Neutrophil extracellular traps kill bacteria. Science *303*, 1532–1535 (2004)

CHOSAY, J. G., ESSANI, N. A., DUNN, C. J., and JAESCHKE, H.: Neutrophil margination and extravasation in sinusoids and venules of liver during endotoxin-induced injury. Amer. J. Physiol. *272*, G1195–G1200 (1997)

Clark, S. R., Ma, A. C., Tavener, S. A., McDonald, B., Goodarzi, Z., Kelly, M. M., Patel, K. D., Chakrabarti, S., McAvoy, E., Sinclair, G. D., Keys, E. M., Allen-Vercoe, E., Devinney, R., Doig, C. J., Green, F. H., and Kubes, P.: Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. Nature Med. *13*, 463–469 (2007)

Cohen, J.: The immunopathogenesis of sepsis. Nature *420*, 885–891 (2002)

Coopersmith, C. M., Stromberg, P. E., Dunne, W. M., Davis, C. G., Amiot, D. M. 2nd, Buchman, T. G., Karl, I. E., and Hotchkiss, R. S.: Inhibition of intestinal epithelial apoptosis and survival in a murine model of pneumonia-induced sepsis. JAMA *287*, 1716–1721 (2002)

Dellinger, R. P., Levy, M. M., Carlet, J. M., Bion, J., Parker, M. M., Jaeschke, R., Reinhart, K., Angus, D. C., Brun-Buisson, C., Beale, R., Calandra, T., Dhainaut, J. F., Gerlach, H., Harvey, M., Marini, J. J., Marshall, J., Ranieri, M., Ramsay, G., Sevransky, J., Thompson, B. T., Townsend, S., Vender, J. S., Zimmerman, J. L., and Vincent, J. L.: Surviving Sepsis Campaign: International guidelines for management of severe sepsis and septic shock: 2008. Crit. Care Med. *36*, 296–327 (2008)

Fernandez-Botran, R.: Soluble cytokine receptors: their role in immunoregulation. FASEB J. *5*, 2567–2574 (1991)

Forehand, J. R., Pabst, M. J., Phillips, W. A., and Johnston, R. B. Jr.: Lipopolysaccharide priming of human neutrophils for an enhanced respiratory burst. Role of intracellular free calcium. J. Clin. Invest. *83*, 74–83 (1989)

Foster, S. L., Hargreaves, D. C., and Medzhitov, R.: Gene-specific control of inflammation by TLR-induced chromatin modifications. Nature *447*, 972–978 (2007)

Gawaz, M., Dickfeldm, T., Bognerm, C., Fateh-Moghadamm, S., and Neumann, F. J.: Platelet function in septic multiple organ dysfunction syndrome. Intensive Care Med. *23*, 379–385 (1997)

Gerard, C.: Complement C5a in the sepsis syndrome – too much of a good thing? New Engl. J. Med. *348*, 167–169 (2003)

Gogos, C. A., Drosou, E., Bassaris, H. P., and Skoutelis, A.: Pro- versus anti-inflammatory cytokine profile in patients with severe sepsis: a marker for prognosis and future therapeutic options. J. Infect. Dis. *181*, 176–180 (2000)

Grimminger, F., Kreusler, B., Schneider, U., Becker, G., and Seeger, W.: Influence of microvascular adherence on neutrophil leukotriene generation. Evidence for cooperative eicosanoid synthesis. J. Immunol. *144*, 1866–1872 (1990)

Gustot, T., Durand, F., Lebrec, D., Vincent, J. L., and Moreau, R.: Severe sepsis in cirrhosis. Hepatology *50*, 2022–2033 (2009)

Heidecke, C. D., Hensler, T., Weighardt, H., Zantl, N., Wagner, H., Siewert, J. R., and Holzmann, B.: Selective defects of T lymphocyte function in patients with lethal intraabdominal infection. Amer. J. Surg. *178*, 288–292 (1999)

Holman, R. G., and Maier, R. V.: Superoxide production by neutrophils in a model of adult respiratory distress syndrome. Arch. Surg. *123*, 1491–1495 (1988)

Holman, J. M. Jr., and Saba, T. M.: Hepatocyte injury during post-operative sepsis: activated neutrophils as potential mediators. J. Leukoc. Biol. *43*, 193–203 (1988)

Hotchkiss, R. S., and Karl, I. E.: The pathophysiology and treatment of sepsis. New Engl. J. Med. *348*, 138–150 (2003)

Hundelshausen, P. von, and Weber, C.: Platelets as immune cells: bridging inflammation and cardiovascular disease. Circ. Res. *100*, 27–40 (2007)

Ignarro, L. J., Byrns, R. E., and Wood, K. S.: Endothelium-dependent modulation of cGMP levels and intrinsic smooth muscle tone in isolated bovine intrapulmonary artery and vein. Circ. Res. *60*, 82–92 (1987)

Klinger, M. H., and Jelkmann, W.: Role of blood platelets in infection and inflammation. J. Interferon Cytokine Res. *22*, 913–922 (2002)

Landry, D. W., and Oliver, J. A.: The pathogenesis of vasodilatory shock. New Engl. J. Med. *345*, 588–595 (2001)

Larsen, E., Celi, A., Gilbert, G. E., Furie, B. C., Erban, J. K., Bonfanti, R., Wagner, D. D., and Furie, B.: PADGEM protein: a receptor that mediates the interaction of activated platelets with neutrophils and monocytes. Cell *59*, 305–312 (1989)

Leaphart, C. L., and Tepas, J. J. 3rd.: The gut is a motor of organ system dysfunction. Surgery *141*, 563–569 (2007)

Levi, M., and Ten Cate, H.: Disseminated intravascular coagulation. New Engl. J. Med. *341*, 586–592 (1999)

Mackey, D., and McFall, A. J.: MAMPs and MIMPs: proposed classifications for inducers of innate immunity. Mol. Microbiol. *61*, 1365–1371 (2006)

Marcus, A. J., Safier, L. B., Ullman, H. L., Broekman, M. J., Islam, N., Oglesby, T. D., and Gorman, R. R.: 12S,20-dihydroxyicosatetraenoic acid: a new icosanoid synthesized by neutrophils from 12S-hydroxyicosatetraenoic acid produced by thrombin- or collagen-stimulated platelets. Proc. Natl. Acad. Sci. USA *81*, 903–907 (1984)

Mavrommatis, A. C., Theodoridis, T., Orfanidou, A., Roussos, C., Christopoulou-Kokkinou, V., and Zakynthinos, S.: Coagulation system and platelets are fully activated in uncomplicated sepsis. Crit. Care Med. *28*, 451–457 (2000)

McClenahan, D. J., Evanson, O. A., Walcheck, B. K., and Weiss, D. J.: Association among filamentous actin content, CD11b expression, and membrane deformability in stimulated and unstimulated bovine neutrophils. Amer. J. Vet. Res. *61*, 380–386 (2000)

Miksa, M., Das, P., Zhou, M., Wu, R., Dong, W., Ji, Y., Goyert, S. M., Ravikumar, T. S., and Wang, P.: Pivotal role of the alpha(2A)-adrenoceptor in producing inflammation and organ injury in a rat model of sepsis. PLoS One *4*, e5504 (2009)

Nathan, C.: Neutrophils and immunity: challenges and opportunities. Nature Rev. Immunol. *6*, 173–182 (2006)

Paterson, H. M., Murphy, T. J., Purcell, E. J., Shelley, O., Kriynovich, S. J., Lien, E., Mannick, J. A., and Lederer, J. A.: Injury primes the innate immune system for enhanced Toll-like receptor reactivity. J. Immunol. *171*, 1473–1483 (2003)

Piagnerelli, M., Boudjeltia, K. Z., Brohee, D., Piro, P., Carlier, E., Vincent, J. L., Lejeune, P., and Vanhaeverbeek, M.: Alterations of red blood cell shape and sialic acid membrane content in septic patients. Crit. Care Med. *31*, 2156–2162 (2003)

Pittner, A., Nalos, M., Asfar, P., Yang, Y., Ince, C., Georgieff, M., Bruckner, U. B., Radermacher, P., and Froba, G.: Mechanisms of inducible nitric oxide synthase (iNOS) inhibition-related improvement of gut mucosal acidosis during hyperdynamic porcine endotoxemia. Intensive Care Med. *29*, 312–316 (2003)

Reggiori, G., Occhipinti, G., Gasperi, A. de, Vincent, J. L., and Piagnerelli, M.: Early alterations of red blood cell rheology in critically ill patients. Crit. Care Med. *37*, 3041–3046 (2009)

Rittirsch, D., Flierl, M. A., Nadeau, B. A., Day, D. E., Huber-Lang, M., Mackay, C. R., Zetoune, F. S., Gerard, N. P., Cianflone, K., Kohl, J., Gerard, C., Sarma, J. V., and Ward, P. A.: Functional roles for C5a receptors in sepsis. Nature Med. *14*, 551–557 (2008a)

Rittirsch, D., Flierl, M. A., and Ward, P. A.: Harmful molecular mechanisms in sepsis. Nature Rev. Immunol. *8*, 776–787 (2008b)

Smedly, L. A., Tonnesen, M. G., Sandhaus, R. A., Haslett, C., Guthrie, L. A., Johnston, R. B. Jr., Henson, P. M., and Worthen G. S.: Neutrophil-mediated injury to endothelial cells. Enhancement by endotoxin and essential role of neutrophil elastase. J. Clin. Invest. *77*, 1233–1243 (1986)

Smith, J. A.: Neutrophils, host defense, and inflammation: a double-edged sword. J. Leukoc. Biol. *56*, 672–686 (1994)

Varani, J., Bendelow, M. J., Sealey, D. E., Kunkel, S. L., Gannon, D. E., Ryan, U. S., and Ward, P. A.: Tumor necrosis factor enhances susceptibility of vascular endothelial cells to neutrophil-mediated killing. Lab. Invest. *59*, 292–295 (1988)

Vincent, J. L., Ferreira, F., and Moreno, R.: Scoring systems for assessing organ dysfunction and survival. Crit. Care Clin. *16*, 353–366 (2000)

Ward, N. S., Casserly, B., and Ayala, A.: The compensatory anti-inflammatory response syndrome (CARS) in critically ill patients. Clin. Chest Med. *29*, 617–625, viii (2008)

Wartha, F., Beiter, K., Normark, S., and Henriques-Normark, B.: Neutrophil extracellular traps: casting the NET over pathogenesis. Curr. Opin. Microbiol. *10*, 52–56 (2007)

Weiss, S. J.: Tissue destruction by neutrophils. New Engl. J. Med. *320*, 365–376 (1989)

Welbourn, C. R., and Young, Y.: Endotoxin, septic shock and acute lung injury: neutrophils, macrophages and inflammatory mediators. Br. J. Surg. *79*, 998–1003 (1992)

Welsh, C. H., Lien, D. C., Worthen, G. S., Henson, P. M., and Weil J. V.: Endotoxin-pretreated neutrophils increase pulmonary vascular permeability in dogs. J. Appl. Physiol. *66*, 112–119 (1989)

Weyrich, A. S., and Zimmerman, G. A.: Platelets: signaling cells in the immune continuum. Trends Immunol. *25*, 489–495 (2004)

Wheeler, D. S.: Death to sepsis: targeting apoptosis pathways in sepsis. Crit. Care *13*, 1010 (2009)

Worthen, G. S., Haslett, C., Rees, A. J., Gumbay, R. S., Henson, J. E., and Henson, P. M.: Neutrophil-mediated pulmonary vascular injury. Synergistic effect of trace amounts of lipopolysaccharide and neutrophil stimuli on vascular permeability and neutrophil sequestration in the lung. Amer. Rev. Respir. Dis. *136*, 19–28 (1987)

Youssefian, T., Drouin, A., Masse, J. M., Guichard, J., and Cramer, E. M.: Host defense role of platelets: engulfment of HIV and *Staphylococcus aureus* occurs in a specific subcellular compartment and is enhanced by platelet activation. Blood *99*, 4021–4029 (2002)

Zarbock, A., Polanowska-Grabowska, R. K., and Ley, K.: Platelet-neutrophil-interactions: linking hemostasis and inflammation. Blood Rev. *21*, 99–111 (2007)

Zarbock, A., Singbartl, K., and Ley, K.: Complete reversal of acid-induced acute lung injury by blocking of platelet-neutrophil aggregation. J. Clin. Invest. *116*, 3211–3219 (2006)

Zhang, Q., Raoof, M., Chen, Y., Sumi, Y., Sursal, T., Junger, W., Brohi, K., Itagaki, K., and Hauser, C. J.: Circulating mitochondrial DAMPs cause inflammatory responses to injury. Nature *464*, 104–107 (2010)

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Mechanisms of Neutrophil Activation during Inflammation

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Abstract

Neutrophil rolling under high shear stress is facilitated by whole cell deformation and flattening, by microvillus extension into tethers, and by integrin engagement through a signalling pathway that starts with P-selectin glycoprotein ligand-1.

Zusammenfassung

Das Neutophilenrollen unter hohem Scheerstress wird gefördert durch vollständige Zelldeformation und Abflachung, durch Mikrovillierweiterung zu Ketten und durch Integrinbeteiligung im Signalweg, der mit P-Selektin-Glycoprotein-Ligand 1 startet.

Neutrophils are the first responders in bacterial, fungal and many viral infections. Acute inflammation is characterised by dilated vessels with high blood flow, resulting in elevated wall shear stresses (up to 10 dyn/cm^2 and higher). In spite of these unfavourable conditions, neutrophils are very successful at adhering to the inflamed vessel wall through a series of molecular interactions known as the adhesion cascade (LEY et al. 2007). Relevant molecular pairs are endothelial P-selectin and E-selectin, which bind to P-*s*electin *g*lycoprotein *l*igand-1 (*PSGL-1*) on neutrophils, and neutrophil LFA-1 ($\alpha_L\beta_2$ integrin), which binds endothelial *i*nter*c*ellular *a*dhesion *m*olecule-1 (*ICAM-1*) after stimulation by inflammatory chemokines. In acute inflammation, the extraction rate of neutrophils can reach 95 %, meaning that 95 % of the neutrophils contained in the blood entering the inflamed organ do not leave through the draining vein but are recruited into the tissue (JUNG et al. 1998). In traditional flow chamber experiments, neutrophil adhesion is limited to low shear stresses (1–2 dyn/cm^2), most likely due to neutrophil activation associated with pertinent isolation procedures. We recently developed a series of microfluidic systems (SMITH et al. 2004, CHESNUTT et al. 2006, SUNDD et al. 2010, KUWANO et al. 2010) that allow the investigation of mouse or human neutrophils in their native whole blood, thus recapitulating adhesion under high shear stress *in vitro*. In these systems, the molecular nature of the adhesive substrate can be manipulated, controlled and quantified. Investigations into neutrophil adhesion under high shear have yielded a number of key insights.

1. Neutrophil Microvilli do not Collapse during Rolling

Resting neutrophils are spheres studded with hundreds of microvilli and ridges that are about 350 nm high (Bruehl et al. 1996). PSGL-1 is concentrated on the tips of microvilli. Quantitative dynamic footprinting (*qDF*) microscopy (Sundd et al. 2010) reveals that the microvillus structure on the surface touching the molecular substrate is preserved and the microvilli do not flatten when neutrophils roll on P-selectin. As the first microvillus at the leading edge of the rolling neutrophil comes within 75 nm of the substrate, PSGL-1 binds P-selectin. As the cell continues to roll, this bond goes into compression and bears no load, because the distance between microvillus tips and the substrate is only about 35 nm under the centre of the cell, so the bond is slack (Sundd et al. 2010, Pospieszalska et al. 2009). At the trailing edge, the bonds are stretched to 140 nm, almost twice their resting length.

2. Neutrophils Flatten against the Surface on which They Roll

Neutrophils have long been known to deform into teardrop-like shapes while rolling (Firrell and Lipowsky 1989, Damiano et al. 1996). qDF shows that this results in an at least fourfold increase in contact area (Sundd et al. 2010) compared to a non-deformable neutrophil (Pospieszalska et al. 2009). This means that more microvilli are close to the substrate and more P-selectin-PSGL-1 bonds are available to support rolling.

3. Neutrophil Microvilli Extend to Form Long Tethers

Force applied to the PSGL-1 molecules on the tips of the microvilli at the trailing edge of the rolling neutrophil not only stretch the PSGL-1 and P-selectin molecules, but also the cytoskeleton inside the microvilli (Pospieszalska and Ley 2009). For small deformations, this is a viscoelastic process, but the spring component systematically decreases as these tethers are extended further (Pospieszalska et al. 2011). qDF shows that each neutrophil rolling at 6 dyn/cm^2 has 3–4 tethers that are 10–15 μm long. Like the tethering cables of a hot air balloon, these long tethers support rolling by preserving PSGL-1-P-selectin bonds longer, by distributing the force and by balancing the torque.

4. PSGL-1 as a Signalling Molecule

When neutrophils roll on P- or E-selectin, PSGL-1 engagement triggers a signalling cascade that results in phosphorylation of spleen tyrosine kinase (*Syk*), which is required for LFA-1 engagement with its ligand ICAM-1 (Zarbock et al. 2007). This signalling requires at least one of two ITAM adaptor molecules, DAP-12 or Fc receptor γ chain, and the Src family kinase Fgr (Zarbock et al. 2008). Downstream of Syk, Bruton's tyrosine kinase (*Btk*) becomes phosphorylated and activates phospholipase Cγ (*PLCγ2*) and PI3 kinase γ. Both pathways contribute to LFA-1 engagement (Mueller et al. 2010). Downstream of PLCγ2, the small GTPase Rap-1 is activated, requiring the nucleotide exchange factor CalDAG-GEFI (Stadtmann et al. 2011).

5. Slow Neutrophil Rolling is Mediated by Extended LFA-1

This signalling pathway activates LFA-1. LFA-1 exists in least three conformations: low affinity with bent ectodomain, intermediate affinity with extended ectodomain and closed headpiece, and high affinity with extended ectodomain and open headpiece (SHIMAOKA et al. 2003). The extended conformation of LFA-1 binds the reporter antibodies KIM127 and NKI-L16, whereas the high affinity conformation also binds the reporter antibody mAb 24. Using these reporter antibodies in a homogeneous binding assay and in an arrest assay in microfluifdic flow chambers, we showed that PSGL-1 engagement induces extended, but not high affinity LFA-1, whereas chemokine triggers the high affinity conformation (KUWANO et al. 2010).

In conclusion, neutrophil rolling under high shear stress is facilitated by whole cell deformation and flattening, by microvillus extension into tethers, and by integrin engagement through a signalling pathway that starts with PSGL-1.

References

BRUEHL, R. E., SPRINGER, T. A., and BAINTON, D. F.: Quantitation of L-selectin distribution on human leukocyte microvilli by immunogold labeling and electron microscopy. J. Histochem. Cytochem. *44*, 835–844 (1996)

CHESNUTT, B. C., SMITH, D. F., RAFFLER, N. A., SMITH, M. L., WHITE, E. J., and LEY, K.: Induction of LFA-1-dependent neutrophil rolling on ICAM-1 by engagement of E-selectin. Microcirculation *13*, 99–109 (2006)

DAMIANO, E. R., WESTHEIDER, J., TOZEREN, A., and LEY, K.: Variation in the velocity, deformation, and adhesion energy density of leukocytes rolling within venules. Circ. Res. *79*, 1122–1130 (1996)

FIRRELL, J. C., and LIPOWSKY, H. H.: Leukocyte margination and deformation in mesenteric venules of rat. Amer. J. Physiol. *256*, H1667–H1674 (1989)

JUNG, U., NORMAN, K. E., RAMOS, C. L., SCHARFFETTER-KOCHANEK, K., BEAUDET, A. L., and LEY, K.: Transit time of leukocytes rolling through venules controls cytokine-induced inflammatory cell recruitment in vivo. J. Clin. Invest. *102*, 1526–1533 (1998)

KUWANO, Y., SPELTEN, O., ZHANG, H., LEY, K., and ZARBOCK, A.: Rolling on E- or P-selectin induces the extended but not high-affinity conformation of LFA-1 in neutrophils. Blood *116*, 617–624 (2010)

LEY, K., LAUDANNA, C., CYBULSKY, M. I., and NOURSHARGH, S.: Getting to the site of inflammation: the leukocyte adhesion cascade updated. Nature Rev. Immunol. *7*, 678–689 (2007)

MUELLER, H., STADTMANN, A., VAN AKEN, H., HIRSCH, E., WANG, D., LEY, K., and ZARBOCK, A.: Tyrosine kinase Btk regulates E-selectin-mediated integrin activation and neutrophil recruitment by controlling phospholipase C (PLC)g2 and PI3Kg pathways. Blood *115*, 3118–3127 (2010)

POSPIESZALSKA, M. K., LASIECKA, I., and LEY, K.: Cell protrusions and tethers: A unified approach. Biophys. J. *100*, 1697–1707 (2011)

POSPIESZALSKA, M. K., and LEY, K.: Dynamics of microvillus extension and tether formation in rolling leukocytes. Cellular and Molecular Bioengineering *2*, 207–217 (2009)

POSPIESZALSKA, M. K., ZARBOCK, A., PICKARD, J. E., and LEY, K.: Event tracking model of adhesion identifies load-bearing bonds in rolling leukocytes. Microcirculation *16*, 115–130 (2009)

SHIMAOKA, M., XIAO, T., LIU, J. H., YANG, Y., DONG, Y., JUN, C. D., MCCORMACK, A., ZHANG, R., JOACHIMIAK, A., TAKAGI, J., WANG, J. H., and SPRINGER, T. A.: Structures of the alphaL I domain and its complex with ICAM-1 reveal a shape-shifting pathway for integrin regulation. Cell *112*, 99–111 (2003)

SMITH, M. L., SPERANDIO, M., GALKINA, E. V., and LEY, K.: Autoperfused mouse flow chamber reveals synergistic neutrophil accumulation through P-selectin and E-selectin. J. Leukoc. Biol. *76*, 985–993 (2004)

STADTMANN, A., BRINKHAUS, L., MUELLER, H., ROSSAINT, J., BOLOMINI-VITTORI, M., BERGMEIER, W., VAN AKEN, H., WAGNER, D. D., LAUDANNA, C., LEY, K., and ZARBOCK, A.: Rap1a activation by CalDAG-GEFI and p38 MAPK is involved in E-selectin-dependent slow leukocyte rolling. Eur. J. Immunol. *41*, 2074–2085 (2011)

SUNDD, P., GUTIERREZ, E., POSPIESZALSKA, M. K., ZHANG, H., GROISMAN, A., and LEY, K.: Dynamic footprinting reveals mechanisms of leukocyte rolling. Nature Meth. *7*, 821–824 (2010)

ZARBOCK, A., ABRAM, C. L., HUNDT, M., ALTMAN, A., LOWELL, C. A., and LEY, K.: PSGL-1 engagement by E-selectin signals through Src kinase Fgr and ITAM adapters DAP12 and FcR gamma to induce slow leukocyte rolling. J. Exp. Med. *205*, 2339–2347 (2008)

ZARBOCK, A., LOWELL, C. A., and LEY, K.: Spleen tyrosine kinase Syk is necessary for E-selectin-induced aLb2 integrin mediated rolling on intercellular adhesion molecule-1. Immunity *26*, 773–783 (2007)

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Adhesion and Signalling Molecules Controlling Leukocyte Transmigration

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Abstract

This presentation summarises two recent studies that advanced our understanding of two central regulatory steps in the process of leukocyte extravasation. By generating genetically modified mice with highly stabilised endothelial junctions it was possible to show that endothelial junctions need to be opened for leukocyte extravasation in various tissues, demonstrating that the junctional pathway is the major diapedesis route for leukocytes *in vivo*. In another study it was possible to identify a novel anti-inflammatory mechanism that acts *via* counteracting the activation of leukocyte integrins. This mechanism is triggered by the anti-inflammatory cytokine GDF15, which was shown to block leukocyte extravasation of neutrophils and monocytes in different inflamed tissues.

Zusammenfassung

Der Beitrag fasst zwei aktuelle Studien zusammen, die unser Verständnis von zwei zentralen Regulationsschritten im Prozess der Leukozytenextravasation erweiterten. Durch die Erzeugung genetisch modifizierter Mäuse mit hoch stabilisierten Endothelzellkontakten war es möglich zu zeigen, dass die Endothelkontakte für den Übertritt der Leukozyten in verschiedene Gewebe geöffnet werden müssen. Dadurch wurde gezeigt, dass Leukozyten durch diesen Weg auswandern. In einer anderen Studie gelang es, einen neuen Antientzündungsmechanismus aufzufinden, der der Aktivierung der Leukozytenintegrine entgegenwirkt. Dieser Mechanismus wird vom entzündungshemmenden Zytokin GDF15 veranlasst, das die Leukozytenextravasation von Neutophilen und Monozyten in verschiedene entzündete Gewebe blockiert.

1. Introduction

Leukocyte extravasation and entry into tissue is essential for the inflammatory process as well as for immune surveillance by lymphocytes. Recruitment of leukocytes is initiated by signalling factors such as cytokines and chemokines that act in concert with selectins, integrins and members of the Ig-superfamily, which in combination mediate capturing, rolling, adhesion and eventually migration of leukocytes at the luminal surface of the endothelium of postcapillary venules (LEY et al. 2007). This complex process is the prelude for diapedesis, the transmigration of leukocytes through the barrier of the blood vessel wall formed by endothelial cells, the basement membrane and perivascular cells. Leukocytes can principally use two different routes to overcome the endothelium: They can move through the junctions between adjacent endothelial cells, or they can move directly through the body of a single endothelial cell. The molecular and cellular mechanisms of both processes are still largely unknown (VESTWEBER 2007).

2. Stabilizing Endothelial Cell Junctions Inhibits Leukocyte Extravasation into Inflamed Tissues

It is not very well understood how leukocytes migrate through the blood vessel wall in order to enter tissues. *In vitro* studies have shown that leukocytes can migrate through the junctions between endothelial cells and can also transcytose through the body of an endothelial cell. Quantification of this process revealed that the vast majority of lymphocytes, monocytes, and neutrophils diapedesed through the junctional route whereas only 5–10 % used the transcellular route, if human umbilical vein endothelial cells (*HUVEC*) were used in transendothelial migration assays (Carman and Springer 2004). Using endothelial cells of microvascular origin in such assays changed the situation, and about 30 % of all transmigrating leukocytes used the transcellular route (Carman et al. 2007). In contrast to *in vitro* transmigration assays it is much more difficult to determine whether a leukocyte traverses the vessel wall *via* the junctional route or the transcellular route. Despite decades of research on this question using electron microscopy, reports are still contradictory and either claim that transmigration occurs exclusively only through the transcellular or only through the junctional route. The difficulty of the analysis is to a large extent based on the fact that only a large stack of serial sections allows to clearly define whether a leukocyte goes trans-cellular or trans-junctional (Schoefl 1972). Only very few studies have undertaken such a thorough analysis and even those are still contradictory. Electron microscopy simply does not lend itself for a statistically reliable analysis of such a question. Recently, this question was analysed in the inflamed cremaster by intravital confocal microscopy, revealing that about 80–90 % of all neutrophils extravasated through the trans-junctional route (Woodfin et al. 2011). Although a larger number of neutrophils could be analysed in this study than in most electron microscopy studies, also this technique does not allow to determine the extravasation route for all transmigrating cells, due to various technical difficulties.

We have recently designed a genetic approach that allows us to analyse the relevance of the trans-junctional pathway in leukocyte extravasation principally in any tissue and under any inflammatory or non-inflammatory situation. This approach was based on the genetic modification of mice with the aim to generate animals with highly stabilised or even "locked" endothelial junctions. The hypothesis behind this approach was that such mice should be strongly inhibited in leukocyte extravasation in those tissues and under those situations where the trans-junctional extravasation route would be of major importance.

This approach was based on the prominent role of VE-cadherin as the major adhesion mechanism that is essential for the stability of endothelial cell contacts. It is known that adhesion blocking antibodies, when injected intravenously, can induce vascular leaks and enhance leukocyte extravasation (Corada et al. 1999, Gotsch et al. 1997). Thus, modifying VE-cadherin in a way that would prevent down-regulation of its adhesive activity should stabilise endothelial cell contacts. VE-cadherin associates with its cytoplasmic domain with β-catenin that in turn binds to α-catenin that links the cadherin-catenin complex to the actin cytoskeleton (Kemler 1993). This interaction is thought to be essential to stabilise the adhesive function of VE-cadherin. In agreement with this, an E-cadherin-α-catenin fusion protein supported cell adhesion much more stable than E-cadherin when expressed ectopically in cells devoid of endogenous cadherins (Nagafuchi et al. 1994, Ozawa and Kemler 1998).

We hypothesised that a covalent fusion between VE-cadherin and α-catenin should stabilise endothelial cell contacts. Based on this idea we generated knock in mice by replacing VE-cadherin by the VE-cadherin-α-catenin fusion construct (Schulte et al. 2011). About 50 % of the

resulting homozygous mutant mice were embryonic lethal, but the rest survived and were healthy and fertile. Challenging the skin of these mice with vascular permeability-inducing agents such as histamine or VEGF revealed that these mice were completely resistant and did not respond by enhanced leakiness for an intravascular dye. Thus, endothelial junctions in the skin were indeed strongly stabilised. IL-1β-induced recruitment into the cremaster and LPS-stimulated neutrophil infiltration into the lung were strongly reduced (75 % and 65 %, respectively) in these mice. Recruitment of activated T cells into inflamed skin was reduced by 57 %. Thus, in these tissues and inflammation models the trans-junctional pathway is indeed of major importance (SCHULTE et al. 2011). Surprisingly, homing of naïve lymphocytes into lymph nodes was not inhibited. Thus, whereas this approach does not allow us to determine the pathway of leukocyte extravasation in the latter case, in the other tissues the junctional pathway is of major importance, and ways to stabilise endothelial junctions may be useful to block leukocyte invasion in these cases.

3. The Cytokine GDF15 Counteracts Chemokine-driven Activation of Leukocyte Integrins

GDF15 (*G*rowth *D*ifferentiation *F*actor *15*) is a cytokine that is a member of the TGF-β superfamily, although its sequence is rather distantly related to TGF-β (MIMEAULT and BATRA 2010). It controls several processes in embryonic and hematopoietic development, including embryo implantation, cartilage, and bone formation. In addition, GDF15 has various functions in the adult such as the control of adipose tissue function, regulation of cellular stress and immune responses, and it can suppress inflammation through the inhibition of macrophage activation (hence its alternative name macrophage inhibitory cytokine (*MIC-1*). GDF15 can inhibit the proliferation of primitive hematopoietic progenitors, and participate in the repair of the brain, bone, heart, liver, lung, kidney, and other tissues after severe injuries.

A group of cardiologist (Dr. Tibor KEMPF and Dr. Kai WOLLERT) at the MHH found recently that GDF15 is dramatically up-regulated in the myocardium upon infarction triggered by coronary artery ligation in the mouse. Analysing GDF15–/– mice, they found that gene ablation for GDF15 rendered these mice much more vulnerable to coronary artery ligation, leading to the death of about 80 % of the treated mice compared to only 20–30 % of wild type mice. At the same time, recruitment of neutrophils and macrophages to ischemic myocardium was strongly enhanced in GDF15–/– mice. Investigating the mechanism behind this enhanced lethality and leukocyte recruitment, we could show that GDF15 did not act like other classical anti-inflammatory cytokines that usually repress the expression of pro-inflammatory cytokines. Instead, GDF15 acted more directly by inhibiting transmigration of neutrophils through endothelial cell layers (KEMPF et al. 2011). We found that it was the adhesion of neutrophils to ICAM-1 that was inhibited by GDF15. Since the expression levels of integrins on the surface of neutrophils was unaffected and since GDF15 exerted its inhibitory effects within minutes, we assumed that GDF15 would directly affect the function of leukocyte integrins. Indeed, we could show that chemokine-driven activation of β2-integrins, as recorded by an activation monitoring mAb that recognises a conformational activation epitope on β2-integrins (*mAb 24*), was inhibited by GDF15. Importantly, also the activation of the β1-integrin α4β1 (*VLA-4*) on monocytes was inhibited by GDF15. Investigating the mechanism for these effects, we found that GDF15 inhibited the activation of the small GTPase Rap1 that is an essential key regulator for the activation of leukocyte integrins. Looking for additional steps within this inhibitory signalling

mechanism, we found that GDF15 activated the GTPase Cdc42 which, in turn, de-activated Rap1. Thus, GDF15 is an anti-inflammatory cytokine that acts *via* a novel hitherto unknown mechanism: the blocking of leukocyte integrin activation (Kempf et al. 2011). Importantly, GDF15 is also up-regulated in other tissues under inflammatory conditions.

We could show that IL-1β-induced inflammation of the cremaster stimulated a much stronger recruitment of neutrophils into GDF15–/– than in wt mice. This suggests that GDF15 might be an anti-inflammatory cytokine that is of more general importance for restricting and thereby balancing the recruitment of leukocytes to inflamed tissues (Kempf et al. 2011).

References

Carman, C. V., Sage, P. T., Sciuto, T. E., de la Fuente, M. A., Geha, R. S., Ochs, H. D., Dvorak, H. F., Dvorak, A. M., and Springer, T. A.: Transcellular diapedesis is initiated by invasive podosomes. Immunity *26*, 784–797 (2007)

Carman, C. V., and Springer, T. A.: A transmigratory cup in leukocyte diapedesis both through individual vascular endothelial cells and between them. J. Cell Biol. *167*, 377–388 (2004)

Corada, M., Mariotti, M., Thurston, G., Smith, K., Kunkel, R., Brockhaus, M., Lampugnani, M. G., Martin-Padura, I., Stoppacciaro, A., Ruco, L., McDonald, D. M., Ward, P. A., and Dejana, E.: Vascular endothelial-cadherin is an important determinant of microvascular integrity in vivo. Proc. Natl. Acad. Sci. USA *96*, 9815–9820 (1999)

Gotsch, U., Borges, E., Bosse, R., Böggemeyer, E., Simon, M., Mossmann, H., and Vestweber, D.: VE-cadherin antibody accelerates neutrophil recruiment in vivo. J. Cell Sci. *110*, 583–588 (1997)

Kemler, R.: From cadherins to catenins: cytoplasmic protein interactions and regulation of cell adhesion. Trends Genet. *9*, 317–321 (1993)

Kempf, T., Zarbock, A., Widera, C., Butz, S., Stadtmann, A., Rossaint, J., Bolomini-Vittori, J., Korf-Klingebiel, M., Napp, L. C., Hansen, B., Kanwischer, A., Bavendiek, U., Beutel, G., Hapke, M., Sauer, M. G., Laudanna, C., Hogg, N., Vestweber, D., and Wollert, K. C.: GDF-15 is an inhibitor of leukocyte integrin activation required for survival after myocardial infarction in mice. Nature Med. *17*, 581–588 (2011)

Ley, K., Laudanna, C., Cybulsky, M. I., and Nourshargh, S.: Getting to the site of inflammation: the leukocyte adhesion cascade updated. Nature Rev. Immunol. *7*, 678–689 (2007)

Mimeault, M., and Batra, S. K.: Divergent molecular mechanisms underlying the pleiotropic functions of macrophage inhibitory cytokine-1 in cancer. J. Cell Physiol. *224*, 626–635 (2010)

Nagafuchi, A., Ishihara, S., and Tsukita, S.: The roles of catenins in the cadherin-mediated cell adhesion: functional analysis of E-cadherin-alpha catenin fusion molecules. J. Cell Biol. *127*, 235–245 (1994)

Ozawa, M., and Kemler, R.: Altered cell adhesion activity by pervanadate due to the dissociation of alpha-catenin from the E-cadherin.catenin complex. J. Biol. Chem. *273*, 6166–6170 (1998)

Schoefl, G. I.: The migration of lymphocytes across the vascular endothelium in lymphoid tissue. A reexamination. J. Exp. Med. *136*, 568–588 (1972)

Schulte, D., Küppers, V., Dartsch, N., Broermann, A., Li, H., Zarbock, A., Kamenyeva, O., Kiefer, F., Khandoga, A., Massberg, S., and Vestweber, D.: Stabilizing the VE-cadherin-catenin complex blocks leukocyte extravasation and vascular permeability. EMBO J. *30*, 4157–4170 (2011)

Vestweber, D.: Adhesion and signaling molecules controlling the transmigration of leukocytes through endothelium. Immunol. Rev. *218*, 178–196 (2007)

Woodfin, A., Voisin, M. B., Beyrau, M., Colom, B., Caille, D., Diapouli, F. M., Nash, G. B., Chavakis, T., Albelda, S. M., Rainger, G. E., Meda, P., Imhof, B. A., and Nourshargh, S.: The junctional adhesion molecule JAM-C regulates polarized transendothelial migration of neutrophils in vivo. Nature Immunol. *12*, 761–769 (2011)

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Toll-Like Receptors

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With 1 Table

Abstract

Toll-like receptors (TLRs) enable the innate immune system to detect microorganisms and their fragments. Furthermore, endogenous mediators, released during non-infectious insults (e. g. trauma, major surgery, etc.), act as TLR agonists. TLR activation is followed by the release of various mediators, leading to inflammation. If the latter becomes more general, systemic inflammatory response syndrome (*SIRS*; endogenous mediators) or sepsis (microorganisms) occurs. Both conditions are critical, and associated with a mortality rate of 20 % (SIRS) up to 80 % (septic shock). Systemic inflammation affects all organ systems and is e. g. cross-linked to the endocrine system. Recent research revealed a crucial role for TLRs in the immunoendocrine stress response. With specific therapeutic approaches for systemic inflammation still being very limited, this might help understanding the complex pathophysiology of systemic inflammation, leading to new therapeutic options in the future.

Zusammenfassung

Toll-like-Rezeptoren (TLRs) ermöglichen dem angeborenen Immunsystem die Erkennung von Mikroorgansimen und deren Bestandteilen. Darüber hinaus detektieren sie endogene Mediatoren, wie sie im Rahmen nicht-infektiöser Traumata (z. B. Polytrauma, Operationen usw.) freigesetzt werden. Der Aktivierung von TLRs folgt die Freisetzung verschiedener inflammatorischer Mediatoren. Erfolgt dies systemisch, resultieren die Krankheitsbilder *Systemic Inflammatory Response Syndrome* (SIRS; endogene Mediatoren) oder Sepsis (Mikroorganismen). Diese kritischen Erkrankungen sind mit einer Letalität von 20 % (SIRS) bis zu 80 % (Septischer Schock) behaftet. Eine systemische Inflammation betrifft alle Organsysteme, u. a. das endokrine System. Es mehren sich Hinweise, dass TLRs eine zentrale Rolle bei der immuno-endokrinen Stressantwort spielen. Bei den derzeit sehr eingeschränkten Möglichkeiten einer spezifischen Therapie bei systemischer Inflammation könnten diese Erkenntnisse die Entwicklung zukünftiger Therapieansätze unterstützen.

1. SIRS, Sepsis and Innate Immunity

Depending on the source, the main cause of death on non-coronary intensive care units is stated as sepsis or multi organ failure (*MOF*) (ANGUS et al. 2001, BRABER and VAN ZANTEN 2010, MAYR et al. 2006). With the latter being caused mostly by systemic inflammation (e. g. sepsis), sepsis seems to be the leading cause of death on non-coronary intensive care units. Sepsis is diagnosed by the presence of the systemic inflammatory response syndrome (*SIRS*) plus infection (proven or strongly suspected) (BONE et al. 1992). Severe sepsis is defined as sepsis with organ dysfunction, and septic shock as severe sepsis with refractory, sepsis induced

hypotension (systolic pressure < 90 mm Hg or mean arterial pressure < 60 mm Hg) despite adequate fluid resuscitation (Bone et al. 1992). SIRS is diagnosed by the presence of two or more of the following four criteria (patient not physically or psychologically stressed):

– Heart rate > 90/min;
– Respiratory rate > 20/min or $PaCO_2$ < 32 mm Hg;
– White blood cell count above 12,000/mm^3 or below 4000/mm^3, or > 10 % immature forms;
– Body temperature > 38 °C or < 36 °C.

SIRS develops following major surgery or trauma, ischemia/reperfusion (I/R), pancreatitis or burns. As stated above, if SIRS is caused/accompanied by a proven or suspected infection, sepsis is diagnosed. Clinically, SIRS and sepsis are often difficult to discriminate. One explanation for the similarities of these two diseases can be found when looking at the innate immune system. The latter includes toll-like receptors (*TLRs*) which detect bacterial, viral, fungal (sepsis) and also endogenous mediators (*SIRS*).

TLRs were first investigated and described in the context of the embryonic development of the fruit fly *Drosophila melanogaster* in the early 1980s (Anderson et al. 1985). 1997 the first human TLR was cloned and characterised (Medzhitov et al. 1997). The following decades revealed the key role of TLRs in innate immunity. TLRs belong to the family of pattern recognition receptors (*PRRs*), detecting pathogen-associated patterns (*PAMPs*) (Kawai and Akira 2010, Van der Poll et al. 2008).

2. Toll-Like Receptors and Agonists

To date, 13 different TLRs have been described in humans and mice. Humans express 10 different TLRs on the cell surface (TLR1, TLR2, TLR4, TLR5, TLR6 and TLR10) or intracellularly (TLR3, TLR7, TLR8 and TLR9).

TLR4 is probably the best investigated and the first being fully characterised. It detects lipopolysaccharide (*LPS*; endotoxin), a cell wall fragment of Gram-negative bacteria. Indirectly, the proof of the essential role of TLR4 in LPS detection dates back to 1968, when mice of the C3H/HeJ strain were found to survive much higher doses of endotoxin compared to other strains (Sultzer 1968). Decades later, the explanation for that phenomenon could be described as a mutation in the TLR4 gene in the C3H/HeJ mice (Poltrak et al. 1998). TLR4 associates with MD2 (lymphocyte antigen 96), resulting in a TLR4/MD2 complex, a homodimer (consisting both of one TLR4 and one MD2), which is considered as the actual LPS receptor (Akashi-Takamura et al. 2008, Miyake 2003). Two further accessory molecules are involved in TLR4 sensitivity: CD14 and LPS-binding Protein (*LBP*). CD14 is expressed on the cell surface and enhances LPS responsiveness by binding LPS and supporting LPS transfer to TLR4/MD2 (Latz et al. 2002). LBP seems to play a crucial role in splitting LPS multimers (which form as soon LPS is released to the blood stream) to monomers, which is the only LPS constitution CD14 and TLR4/MD2 are able to detect (Schumann 2011).

TLR2 is best known as the receptor for Gram-positive cell wall fragments, such as lipoteichoic acid (*LTA*) and peptidoglycan. In addition TLR2 detects a wide range of other PAMPs including components of the Gram-negative bacterial cell wall, fungi, viruses and parasites (Akira et al. 2006). The reason for that comparable broad spectrum lies in the fact, that TLR2 forms heterodimers with TLR1 or TLR6. The TLR2-TLR1 heterodimer detects lipopeptides

from Gram-negative bacteria and mycoplasma. With the finding, that the TLR2-TLR1 heterodimer recognises lipopeptides originating from Gram-negative cell walls, LPS extraction and purification became more challenging. LPS is experimentally commonly used as a TLR4 specific agonist and obtained from Gram-negative cell walls. During the extraction and purification process of LPS which originates from Gram-negative call walls, the removal of lipopeptides became an essential quality challenge, as it could be demonstrated, that commercial LPS often was contaminated with lipopeptides, i. e. TLR2 agonists (HIRSCHFELD et al. 2000, LEE et al. 2002). The heterodimer TLR2-TLR6 detects the lipopeptides of Gram-positive bacteria and is also involved in mycoplasma recognition. PAMPs include bacterial cell wall fragments, such as LPS (endotoxin), LTA, peptidoglycan, lipopeptides derived from Gram-negative and/or Gram-positive bacteria, bacterial DNA, viral RNA and fungal components.

TLR3 detects double-stranded RNA (*dsRNA*) of viruses or virus infected cells. Viruses detected by TLR3 include, but are not limited to, cytomegalovirus, Herpes simplex virus type 1, respiratory syncytial virus, encephalomyocarditis virus (XAGORARI et al. 2008). In full blood, TLR3 are quite exclusively expressed in dentric cells (*DCs*) (GAUZZI et al. 2010, SCHLATZER et al. 2012). Compared to patients with SIRS, patients with sepsis have significant lower DC counts (D'ARPA et al. 2009). *In vitro* whole blood stimulation with TLR3 agonists therefore could become an interesting diagnostic tool.

TLR5 recognises flagellin protein which is a component of bacterial flagella (AKIRA et al. 2006). This receptor is particularly expressed in DCs of the small intestine, where the TLR5 mediated activation of DCs promotes the differentiation of helper T cells and B cells to immunoglobulin A-producing plasma cells (UEMATSU et al. 2008). Therefore, TLR5 is crucially involved in protecting the host from many different microbes (nearly all flagellated bacteria) entering or colonising the huge contact face to the outer world (VIJAY-KUMAR et al. 2009).

TLR7 detects single-stranded RNA (*ssRNA*) of viruses, such as vesicular stomatitis virus, HIV, influenza A virus, etc. (AKIRA et al. 2006). TLR7 is highly expressed on plasmacytoid DCs (*pDCs*) which produce high levels of type I interferon. TLR7 is essential for cytokine production during viral infection and seems to play an important role as first line sensor for ssRNA infections (AKIRA et al. 2006, KAWAI and AKIRA 2006).

TLR8 recognises – like TLR7 – ssRNA and is also phylogenetically very similar to TLR7.

TLR9 detects unmethylated 2^{l}-deoxyribo(cytidine-phosphate-guanosine) (*CpG*) DNA motifs of bacteria and viruses, such as *Mycobacterium tuberculosis* (in cooperation with TLR2) (BAFICA et al. 2005), *Brucella* (in cooperation with TLR4) (COPIN et al. 2007), *Streptococcus pneumoniae* (in cooperation with TLR2) (LEE et al. 2007), *Helicobacter pylori* (ANDERSON et al. 2007) and DNA viruses (e. g. herpes simplex virus type 1 and type 2, adenovirus) (KAWAI and AKIRA 2006).

TLR10 belongs to the TLR1 subfamily (TLR1, 2, 6, and 10), forms homo- and heterodimers with TLR1 and 2, and possibly is activated by the synthetic TLR agonists tri-acylated (Pam_3CSK_4) and di-acylated lipopeptide (Pam_2CSK_4). However, no natural ligand has been identified yet (GOVINDARAJ et al. 2010).

The agonists of TLRs can be summarised as danger-associated molecular patterns (*DAMPs*). DAMPs represent a wide range of mediators/components, which are agonists for PRRs like TLRs, but also mannose receptor (*MR*), nucleotide oligomerisation domain (NOD)-like receptors, etc. DAMPs are subdivided into PAMPs and alarmins. Whereas PAMPs originate from microbes, alarmins are host cell-derived ligands. Alarmins are released when tissue damage, physical stress, and trauma lead to cell necrosis, resulting in the liberation of nuclear

and cytoplasmic proteins (Bianchi 2007). Heat-shock proteins (*HSPs*), fibrinogen, hyaluronan and high mobility group box 1 (*HMGB1*) represent the to date best investigated alarmins (Yang et al. 2009). Therefore, following the concept of PAMPs and alarmins, PAMPs would be involved in the pathogenesis of sepsis, whereas alarmins triggered SIRS.

3. Toll-Like Receptor Expression and Signalling

TLRs are expressed in a variety of immune and non-immune cell types (Tab. 1). All immune competent cells of the peripheral blood express TLRs. Depending on the subtype, different distribution patterns can be observed; e. g. macrophages and myeloid DCs (*mDCs*) preferentially express TLR2, TLR3, TLR4 and TLR8, pDCs relatively distinct express TLR7 and TLR9, and neutrophiles express all TLRs (except TLR3) (Hornung et al. 2002, Parker et al. 2005). With the exception of TLR7 and TLR8, all TLRs can also be found on/in endothelial cells, underlining their active involvement in innate immunity (Opitz et al. 2009). TLRs have

Tab. 1 Human toll-like receptors, damage-associated molecular patterns (DAMPs) and their origin

TLR	DAMPs	Origin	Major cell types*
TLR1/TLR2	Triacyl lipopeptides	Bacteria and mycobacteria	TLR1: T and B cells, NK cells, endothelial and epithelial cells, keratinocytes TLR2: see below
TLR2	Peptidoglycan Porins Lipoarabinomannan Phospholipomannan tGPI-mutin Hemagglutinin protein ND HMGB1	Gram-positive bacteria *Neisseria* Mycobacteria *Candida albicans* *Trypanosoma* Measles virus HCMV, HSV1 Host	T and B cells, endothelial and epithelial cells, keratinocytes, adrenocorti cal cells, CNS
TLR2 and TLR4	Glucuronoxylo-mannan	*Cryptococcus neoformans*	see above and below
TLR3	dsRNA	Viruses	T cells, NK cells, mDCs, endothelial and epithelial cells, keratinocytes, neurons
TLR4	LPS Mannan Glycoinositolphospho-lipids Envelope proteins Heat-shock protein 60, 70 Fibrinogen Hyaluronan	Gram-negative bacteria *Candida albicans* *Trypanosoma* RSV, MMTV Host	T cells, NK cells, mast cells, endothelial and epithelial cells, keratinocytes, adrenocortical cells, CNS

TLR	DAMPs	Origin	Major cell types*
TLR5	Flagellin	Flagellated bacteria	T cells, NK cells, endothelial and epithelial cells, keratinocytes
TLR6/TLR2	LTA Diacyl lipopeptides Zymosan	Group B *Streptococcus* *Mycoplasma* *Saccharomyces cerevisiae*	TLR6: T and B cells, endothelial and epithelial cells, keratinocytes TLR2: see above
TLR7 and TLR8	ssRNA	RNA viruses	TLR7: T and B cells, NK cells, pDCs, endothelial cells, keratinocytes TLR8: NK cells, endothelial cells
TLR9	CpG-DNA Hemozoin DNA	Bacteria and mycobacteria *Plasmodium* Viruses	T and B cells, NK cells, pDCs, endothelial and epithelial cells, keratinocytes, adrenocortical cells
TLR10	ND	ND	T and B cells, NK cells, endothelial and epithelial cells

(Akira et al. 2006, Bornstein et al. 2004a, Chakravarty et al. 2005, Fitzner et al. 2008, Gauzzi et al. 2010, Gilliet et al. 2008, Girart et al. 2007, Hart et al. 2005, Holm et al. 2009, Miller and Modlin 2007, Pegu et al. 2008, Phulwani et al. 2008, Schlatzer et al. 2012, Shah et al. 2009, Tran et al. 2007, modified; * myeloid cells not listed, as they express all known TLRs; ND not detected)

also been found in spleen tissue, ovaries, prostate, pancreas, placenta, testis and in adrenal glands (Bornstein et al. 2004a, Zarember et al. 2002).

Following DAMP engagement, TLRs undergo conformational changes leading to homo- or heterophilic interactions of TLRs. Several intracellular adaptor proteins such as MyD88, TIRAP, TRIF, TRAM and others are recruited, and following a complex cascade involving TAK1, TRAF3, TRAF6, IKK complexes and more, this culminates in the activation of nuclear factor (NF)-κB and mitogen-activated protein (*MAP*) kinases, resulting in the induction of inflammatory cytokines (Kawai and Akira 2010). The detailed pathways depend on the type of TLR, the cell type and differ in kinetic and effect. Some examples: TLR4 activates two different signalling pathways: the MyD88- and the TRIF-dependent pathway, which differ in rate and type of cytokine release (Barton and Kagan 2009). Intracellular signalling following TLR3 is, in contrast to all other TLRs, MyD88 independent. TLR3, TLR7 and TLR9 activation results in type I interferon release, and in that differs in the cytokine patterns, released on e.g. TLR2 or TLR4 activation (Interleukin (*IL*)-1β, tumour necrosis factor (*TNF*)-α, etc.) (Kawai and Akira 2010).

The release of various cytokines following activation of TLRs is an essential part of the pathophysiology of SIRS and sepsis. The "cytokine storm" has many different effects on subsequent mediator release and enzyme activation on various cells; e. g., inflammatory cytokines induce the synthesis of inducible nitric-oxide synthase (*iNOS*) (particular in immune competent

cells, endothelium, vascular smooth muscle cells) and activate the constitutively expressed endothelial NOS (*eNOS*) (particular in endothelial cells), both resulting in the release of nitric-oxide (NO), a potent vasodilator (Mayr et al. 2006, Symeonides et al. 1999). Furthermore, as TLR2 and TLR4 are expressed on endothelial and vascular smooth muscle cells, a cytokine independent, direct stimulation by DAMPs is possible. This seems to be true especially for TLR4. In vascular smooth muscle cells, LPS alone triggers, *via* the MyD88-independent pathway, a fast and effective intracellular signalling resulting in a significant release of cytokines. In contrast, TLR2 activation with specific agonists requires TNF co-stimulation (Cartwright et al. 2007, Connelly et al. 2005, Jimenez et al. 2005, MacMicking et al. 1997). NO induced vasodilatation is accompanied/supported by the release of mediators such as prostacyclin, bradykinin and increased phospholipase C activity (Flammer and Luscher 2010). Inflammatory triggered vasodilatation goes along with a decrease in endothelial barrier function ("capillary leak"), which is caused by cytoskeleton movement and cell retraction. Vascular endothelial (*VE*) cadherin, a junctional adhesive molecule, during inflammation becomes phosphorylated and dissociates from its cytoskeletal anchor. Also vascular endothelial growth factor (*VEGF*), which is systemically released during sepsis, seems to play a role in the pathophysiology of increased endothelial permeability (Kumar et al. 2009).

4. Systemic Inflammation and Coagulation

Systemic inflammation is accompanied, or can even be caused by coagulation activation (Levi and Van der Poll 2010). SIRS and sepsis leads to the release of tissue factor (*TF*). Similar to the induction of NO release, this can be triggered indirectly by inflammatory cytokines released from e. g. immune competent cells, or directly by TLR activation expressed in TF-producing cells such as endothelial and white blood cells (particular monocytes). TF formats with factor VII to TF-factor VII complexes, which activate factor X. Factor Xa cleaves prothrombin to thrombin, and thrombin catalyses the final stages of clot formation, i.e. the conversion of fibrinogen to fibrin. The systemic activation of clot formation leads to thrombi in the microvascular system and the occlusion of small vessels, resulting in organ hypoperfusion. In addition, during SIRS and sepsis, the fibrinolytic system is depressed by dysregulations affecting the antithrombin system, the protein C system and the tissue factor pathway inhibitor (*TFPI*) (Levi 2005). As mentioned above, coagulation can also trigger/modulate inflammation, i.e. there exists a bidirectional cross-talk between the inflammatory and the coagulation system. For example, protease-activated receptors (*PARs*), which are localised on endothelial cells, smooth muscle cells, mononuclear cells, platelets and fibroblasts, detect thrombin, TF-factor VII complexes and factor Xa, resulting the production of inflammatory cytokines and growth factors (Coughlin 2000). On the other hand there is evidence for certain clotting components to have anti-inflammatory properties. Pro- and anti-coagulatory pathways are continuously trying to maintain the balance, preventing thromboembolic events or bleeding. The counter player of fibrin is plasmin, which degrades fibrin to fibrin fragments. The fibrin-derived peptide Bβ15-42 is part of one of these fragments, the E fragment. In models of myocardial IR and hemorrhagic shock, Bβ15-42 enfolds protective properties by interacting with VE cadherin (Petzelbauer et al. 2005, Roesner et al. 2009, Zacharowski 2010).

5. Systemic Inflammation and Immunoendocrine Stress Response

The complex pathophysiology of systemic inflammation is also interacting with the endocrine system. SIRS and sepsis influence the immune-adrenal crosstalk, i.e. systemic inflammation initially activates the hypothalamic-pituitary-adrenal (*HPA*) axis (BORNSTEIN and RUTKOWSKI 2002, BORNSTEIN et al. 2006, BUTLER et al. 1993). Various pro-inflammatory cytokines trigger the release of corticotropin releasing hormone (*CRH*) and adrenocorticotropic hormone (*ACTH*) (NAVARRA et al. 1991). The physiological activation sequence of the HPA axis starts with the release of CRH in the hypothalamus, which leads to the pituitary synthesis of ACTH, which then stimulates the cortex of the adrenal gland and results in the release of corticosteroids. During systemic inflammation cytokines and DAMPs interact at probably every step of this sequence. E.g., TLR4 activation leads to a cytokine independent corticosterone rise which could be linked to TLR4 detection in tissue-resident central nervous system (*CNS*) cells, leading to the activation of the HPA axis (CHAKRAVARTY et al. 2005). Furthermore, TLRs are expressed in adrenal glands, possibly allowing a direct adrenal stimulation by DAMPs. To date, TLR2, TLR4 and TLR9 expression in murine and human adrenal glands could be demonstrated (BORNSTEIN et al. 2004a, b, TRAN et al. 2007, ZACHAROWSKI et al. 2006). The evaluation of the consequences of deficiency of the above mentioned TLRs reveal a key role for TLRs in the adrenal stress response. The adrenal glands of TRL2 deficient mice are significantly larger compared to wild-type (*WT*) mice. The difference in size is due to the enlargement of the adrenal cortex, not the adrenal medulla. Electron microscopy revealed further morphological differences. Compared to WT animals, adrenocortical cells of TLR2 deficient mice contain less mitochondria, show a transformation of vesicular internal membranes to tubular structures, and cell membranes with extensive interdigitations and in-folds. The morphological changes are accompanied by functional differences. The basal corticosterone production is lower in TLR2 deficient mice, whereas ACTH plasma concentrations are higher. The induction of a systemic inflammation by the application of bacterial cell wall fragments (LPS) leads to an impaired release of corticosterone and pro-inflammatory cytokines in TLR2 deficient mice. Whereas WT animals demonstrate a significant rise of ACTH when challenged with LPS, TLR2 deficient mice release corticosterone without an accompanying/inducing rise of ACTH (BORNSTEIN et al. 2004b). Also TLR4 deficient mice show a different physiology and pathophysiology of the HPA axis compared to WT animals. As in TLR2 deficient mice, the cortex of the adrenal glands is bigger compared to WT animals. In contrast to TLR2, TLR4 deficient animals have higher basal corticosterone plasma concentrations compared to WT mice. Furthermore, TLR4 deficiency leads to higher basal levels of pro-inflammatory cytokines. Interestingly, inducing systemic inflammation in TLR4 deficient mice leads to a drop in corticosterone plasma concentrations. Comparably to TLR2, TLR4 deficient mice seem to control corticosterone release independently of systemic ACTH concentrations, the latter not being affected by LPS induced systemic inflammation (ZACHAROWSKI et al. 2006). In contrast to TLR2 and TLR4, TLR9 deficiency has no influence on size of the adrenal glands, basal corticosterone, ACTH or cytokine plasma concentrations. However, systemic application of TLR9 agonists leads to the release of corticosterone and several cytokines. Again, the corticosterone release was not accompanied by a rise in ACTH (TRAN et al. 2007). Summarised, there is good evidence, for the involvement of TLRs in the crosstalk of immune system and the HPA axis. Deficiency of TLR2, TLR4 or TLR9 leads to an ACTH independent corticosterone release.

In humans, TLRs and their function can be affected by single nucleotide polymorphisms (*SNPs*), i.e. genetic variations. Three to 9.4 % of the Caucasian population are heterozygote carriers of the Arg753Gln SNP on TLR2, and 6 to 14 % are double heterozygote carriers of the SNPs Asp299Gly and Thr399Ile SNP, both affecting TLR4 (Ferwerda et al. 2007, Lorenz et al. 2000, Schroder et al. 2003). With the above mentioned results obtained in animals and the relatively high frequency of TLR2 and TLR4 affecting SNPs in the Caucasian population, we felt motivated to translate the experimental findings into a prospective observational clinical study. Major surgery leads to the activation of the immune system (SIRS) and HPA axis. Tissue trauma, I/R, application of bone cement, cardiac surgery, particularly with cardiopulmonary bypass, is associated with the release of alarmins, i.e. TLR agonists. With cardiac surgery (inclusive cardiopulmonary bypass) inducing a – compared to sepsis – relatively homogenous systemic inflammation with defined beginning, the study was performed on cardiac surgical patients who underwent elective coronary artery bypass graft (*CABG*) and/or valve surgery (replacement and/or reconstruction) on cardiopulmonary bypass. A total of 338 patients were included, which were screened for the above mentioned SNPs on TLR2 and TLR4. Thirteen patients carried the TLR2, 51 the TLR4 SNP. ACTH, cortisol and ten different cytokines (interferon (IFN)-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, TNF-α and granulocyte macrophage-colony stimulating factor (*GM-CSF*)) were measured before (between 07:00 and 09:00) and after (on arrival to the intensive care unit) surgery and on the first postoperative day (between 07:00 and 09:00). Basal levels of ACTH and cortisol did not differ between TLR2 or TLR4 SNP carriers and non-carriers. In all patients, cortisol serum concentrations raised significantly after surgery, reflecting the perioperative immunoendocrine stress response. Interestingly, only in non-carriers this was accompanied by a significant ACTH rise. In contrast to the physiological ACTH controlled cortisol release, during surgical induced systemic inflammation this sequence seems to be deranged if TLR4 function is restricted. Furthermore, TLR4 SNP carriers showed decreased serum concentrations of IL-8, IL-10 and GM-CSF compared to non-carriers (Koch et al. 2011). The results of this clinical observation confirmed the observations made in the animal experiments, in that TLR function is crucially involved in the regulation of the immunoendocrine stress response. These findings might influence the way of interpreting previous and planning new trials investigating adrenal insufficiency and corticosteroid substitution in patients with systemic inflammation. For decades, modulation of the HPA axis has been performed for diagnostic and therapeutic reasons in patients with systemic inflammation (SIRS and sepsis). E. g. patients undergoing cardiac surgery with cardiopulmonary bypass were traditionally treated with corticosteroids to damp the release of mediators (e.g. cytokines) associated with SIRS. During the 1990s several clinical trials failed to demonstrate beneficial effects of this modulation of the immunoendocrine system. In fact, some studies revealed detrimental effects of perioperative corticosteroid treatment, such as hyperglycemias or prolonged postoperative ventilation time. Nowadays, the routine application of corticosteroids to patients undergoing cardiac surgery is not recommended (Chaney 2002). Similarly, septic patients when treated with corticosteroids show short term benefits, like decreased cytokine concentrations, improved hemodynamic stability, etc. Long term outcome, however, is not influenced by corticosteroid substitution (Sprung et al. 2008). In critically ill, adrenal insufficiency can occur, and it has been postulated that these patients might benefit from a corticosteroid therapy (Bornstein 2009). However, the diagnosis of adrenal insufficiency in these patients is difficult and a reliable diagnostic tool still has to be established (Marik et al. 2008). Therefore,

the search for diagnostic and therapeutic approaches for the modulation of the immuno-endocrine stress response continues. With the function of TLRs influencing this system, SNP screening might be included in future studies.

6. Therapeutic Approaches for Systemic Inflammation and Sepsis

After decades of research, the therapy of SIRS and sepsis remains limited to source control, administration of broad-spectrum antibiotics and hemodynamic stabilisation; (further) specific therapy (no longer) exists. With the withdraw (October 2011) of activated protein C (Xigris), to date no licensed "sepsis drug" is available any longer (MULLARD 2011). Comparable with previous efforts to influence or block certain pathways of the complex pathophysiology of systemic inflammation, following animal experiments, smaller clinical trials and/or phase I and II trials, activated protein C failed in demonstrating a positive benefit/risk ratio. Blocking LPS, cytokines (e.g. TNF-α) and other mediators improved outcome in sepsis models of animals and (often underpowered) small clinical trials, but failed in the end in adequate clinical trials (DYSON et al. 2009). Blocking iNOS lead to a hemodynamic stabilisation of septic patients, however, increased mortality (LOPEZ et al. 2004). NO is not only a detrimental mediator during sepsis. There are several hints for NO related protective properties, direct or indirect, which would explain why complete blocking is not a therapeutic option (CAUWELS et al. 2000, KOCH et al. 2007). An example for recent, comparable disappointing, efforts of finding the "magic bullet" against sepsis, is eritoran (E5564). Eritoran blocks the toxic moiety lipid A of LPS. Under experimental conditions (i.e. defined timing between LPS injection and treatment) eritoran blocks the systemic response to LPS (LYNN et al. 2003). However, the results of the worldwide phase III randomised trial failed to demonstrate an improvement in 28-day all-cause mortality in 2000 patients with severe sepsis.

Speculations about the reasons for the failure of this and comparable clinical trials include the following aspects. In contrast to patients, the animals used in the primary experiments are young and lack of comorbidities. The latter both are strong predictors of sepsis susceptibility and outcome. Furthermore, animal models for sepsis usually not include therapeutic strategies performed in a clinical scenario: Source control (e.g. rescue surgery following the initiation of sepsis by experimental gut perforation), antibiotic therapy, comparable (to patients) hemodynamic stabilisation. In animal models, the therapeutic intervention is initiated before or shortly after the experimentally induction (e.g. LPS injection) of systemic inflammation. In a clinical scenario it is (to date) an unrealistic approach to start a therapy before, or within a defined time from onset, of e.g. sepsis. Still, no sepsis marker exists, which would theoretically allow determining the beginning of sepsis. On the other hand, the pathophysiological time course of sepsis is not limited to a pro-inflammatory component. A self-amplifying pro-inflammatory response is followed by a compensatory anti-inflammatory syndrome (*CARS*) which is at least as difficult to diagnose as sepsis. During CARS, the immune system is compromised and the organism highly susceptible to (nosocomial) infections. In this state, suppressing the immune system further by blocking important immune mediators (e.g. cytokines), aggravates the immunological anergy.

Therefore, the challenging search for therapeutic options in systemic inflammation (incl. sepsis) goes on. Still, many pathophysiological details need to be explored before we can answer the question: "Who needs when and how to be treated?"

References

Akashi-Takamura, S., and Miyake, K.: TLR accessory molecules. Curr. Opin. Immunol. *20*/4, 420–425 (2008)

Akira, S., Uematsu, S., and Takeuchi, O.: Pathogen recognition and innate immunity. Cell *124*/4, 783–801 (2006)

Anderson, A. E., Worku, M. L., Khamri, W., Bamford, K. B., Walker, M. M., and Thursz, M. R.: TLR9 polymorphisms determine murine lymphocyte responses to Helicobacter: results from a genome-wide scan. Eur. J. Immunol. *37*/6, 1548–1561 (2007)

Anderson, K. V., Bokla, L., and Nüsslein-Volhard, C.: Establishment of dorsal-ventral polarity in the Drosophila embryo: the induction of polarity by the Toll gene product. Cell *42*/3, 791–798 (1985)

Angus, D. C., Linde-Zwirble, W. T., Lidicker, J., Clermont, G., Carcillo, J., and Pinsky, M. R.: Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit. Care Med. *29*/7, 1303–1310 (2001)

Bafica, A., Scanga, C. A., Feng, C. G., Leifer, C., Cheever, A., and Sher, A.: TLR9 regulates Th1 responses and cooperates with TLR2 in mediating optimal resistance to Mycobacterium tuberculosis. J. Exp. Med. *202*/12, 1715–1724 (2005)

Barton, G. M., and Kagan, J. C.: A cell biological view of Toll-like receptor function: regulation through compartmentalization. Nature Rev. Immunol. *9*/8, 535–542 (2009)

Bianchi, M. E.: DAMPs, PAMPs and alarmins: all we need to know about danger. J. Leukoc. Biol. *81*/1, 1–5 (2007)

Bone, R. C., Balk, R. A., Cerra, F. B., Dellinger, R. P., Fein, A. M., Knaus, W. A., Schein, R. M., and Sibbald, W. J.: Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest *101*/6, 1644–1655 (1992)

Bornstein, S. R.: Predisposing factors for adrenal insufficiency. New Engl. J. Med. *360*/22, 2328–2339 (2009)

Bornstein, S. R., and Rutkowski, H.: The adrenal hormone metabolism in the immune/inflammatory reaction. Endocr. Res. *28*/4, 719–728 (2002)

Bornstein, S. R., Schumann, R. R., Rettori, V., McCann, S. M., and Zacharowski, K.: Toll-like receptor 2 and Toll-like receptor 4 expression in human adrenals. Horm. Metab. Res. *36*/7, 470–473 (2004a)

Bornstein, S. R., Zacharowski, P., Schumann, R. R., Barthel, A., Tran, N., Papewalis, C., Rettori, V., McCann, S. M., Schulze-Osthoff, K., Scherbaum, W. A., Tarnow, J., and Zacharwoski, K.: Impaired adrenal stress response in Toll-like receptor 2-deficient mice. Proc. Natl. Acad. Sci. USA *101*/47, 16695–16700 (2004b)

Bornstein, S. R., Ziegler, C. G., Krug, A. W., Kanczkowski, W., Rettori, V., McCann, S. M., Wirth, M., and Zacharowski, K.: The role of toll-like receptors in the immune-adrenal crosstalk. Ann. New York Acad. Sci. *1088*, 307–318 (2006)

Braber, A., and Van Zanten, A. R.: Unravelling post-ICU mortality: predictors and causes of death. Eur. J. Anaesthesiol. *27*/5, 486–490 (2010)

Butler, J., Rocker, G. M., and Westaby, S.: Inflammatory response to cardiopulmonary bypass. Ann. Thorac. Surg. *55*/2, 552–559 (1993)

Cartwright, N., McMaster, S. K., Sorrentino, R., Paul-Clark, M., Sriskandan, S., Ryffel, B., Quesniaux, V. F. J., Evans, T. W., and Mitchell, J. A.: Elucidation of toll-like receptor and adapter protein signaling in vascular dysfunction induced by gram-positive *Staphylococcus aureus* or gram-negative *Escherichia coli*. Shock *27*/1, 40–47 (2007)

Cauwels, A., Van Molle, W., Janssen, B., Everaerdt, B., Huang, P., Fiers, W., and Brouckaert, P.: Protection against TNF-induced lethal shock by soluble guanylate cyclase inhibition requires functional inducible nitric oxide synthase. Immunity *13*/2, 223–231 (2000)

Chakravarty, S., and Herkenham, M.: Toll-like receptor 4 on nonhematopoietic cells sustains CNS inflammation during endotoxemia, independent of systemic cytokines. J. Neurosci. *25*/7, 1788–1796 (2005)

Chaney, M. A.: Corticosteroids and cardiopulmonary bypass: a review of clinical investigations. Chest *121*/3, 921–931 (2002)

Connelly, L., Madhani, M., and Hobbs, A. J.: Resistance to endotoxic shock in endothelial nitric-oxide synthase (eNOS) knock-out mice: a pro-inflammatory role for eNOS-derived no in vivo. J. Biol. Chem. *280*/11, 10040–10046 (2005)

Copin, R., Baetselier, P. De, Carlier, Y., Letesson, J.-J., and Muraille, E.: MyD88-dependent activation of $B220^{-}CD11b^{+}LY\text{-}6C^{+}$ dendritic cells during *Brucella melitensis* infection. J. Immunol. *178*/8, 5182–5191 (2007)

Coughlin, S. R.: Thrombin signalling and protease-activated receptors. Nature *407*/6801, 258–264 (2000)

D'Arpa, N., Accardo-Palumbo, A., Amato, G., D'Amelio, L., Pileri, D., Cataldo, V., Mogavero, R., Lombardo, C., Napoli, B., and Conte, F.: Circulating dendritic cells following burn. Burns *35*/4, 513–518 (2009)

Dyson, A., and Singer, M.: Animal models of sepsis: why does preclinical efficacy fail to translate to the clinical setting? Crit. Care Med. *37*/1 Suppl., S30–S37 (2009)

Ferwerda, B., McCall, M. B., Alonso, S., Giamarellos-Bourboulis, E. J., Mouktaroudi, M., Izagirre, N., Syafruddin, D., Kibiki, G., Cristea, T., Hijmans, A., Hamann, L., Israel, S., Elghazali, G., Troye-Blomberg, M., Kumpf, O., Maiga, B., Dolo, A., Doumbo, O., Hermsen, C. C., Stalenhoef, A. F., Van Crevel, R., Brunner, H. G., Oh, D. Y., Schumann, R. R., De La, R. C., Sauerwein, R., Kullberg, B. J., Van der Ven, A. J., Van der Meer, J. W., and Netea, M. G.: TLR4 polymorphisms, infectious diseases, and evolutionary pressure during migration of modern humans. Proc. Natl. Acad. Sci. USA *104*/42, 16645–16650 (2007)

Fitzner, N., Clauberg, S., Essmann, F., Liebmann, J., and Kolb-Bachofen, V.: Human skin endothelial cells can express all 10 TLR genes and respond to respective ligands. Clin. Vaccine Immunol. *15*/1, 138–146 (2008)

Flammer, A. J., and Luscher, T. F.: Human endothelial dysfunction: EDRFs. Pflügers Arch. *459*/6, 1005–1013 (2010)

Gauzzi, M. C., Del Corno, M., and Gessani, S.: Dissecting TLR3 signalling in dendritic cells. Immunobiology. *215*/9–10, 713–723 (2010)

Gilliet, M., Cao, W., and Liu, Y. J.: Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases. Nature Rev. Immunol. *8*/8, 594–606 (2008)

Girart, M. V., Fuertes, M. B., Domaica, C. I., Rossi, L. E., and Zwirner, N. W.: Engagement of TLR3, TLR7, and NKG2D regulate IFN-gamma secretion but not NKG2D-mediated cytotoxicity by human NK cells stimulated with suboptimal doses of IL-12. J. Immunol. *179*/6, 3472–3479 (2007)

Govindaraj, R. G., Manavalan, B., Lee, G., and Choi, S.: Molecular modeling-based evaluation of hTLR10 and identification of potential ligands in Toll-like receptor signaling. PLoS.One *5*/9, e12713 (2010)

Hart, O. M., Athie-Morales, V., O'Connor, G. M., and Gardiner, C. M.: TLR7/8-mediated activation of human NK cells results in accessory cell-dependent IFN-gamma production. J. Immunol. *175*/3, 1636–1642 (2005)

Hirschfeld, M., Ma, Y., Weis, J. H., Vogel, S. N., and Weis, J. J.: Cutting edge: repurification of lipopolysaccharide eliminates signaling through both human and murine toll-like receptor 2. J. Immunol. *165*/2, 618–622 (2000)

Holm, C. K., Petersen, C. C., Hvid, M., Petersen, L., Paludan, S. R., Deleuran, B., and Hokland, M.: TLR3 ligand polyinosinic:polycytidylic acid induces IL-17A and IL-21 synthesis in human Th cells. J. Immunol. *183*/7, 4422–4431 (2009)

Hornung, V., Rothenfusser, S., Britsch, S., Krug, A., Jahrsdorfer, B., Giese, T., Endres, S., and Hartmann, G.: Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. J. Immunol. *168*/9, 4531–4537 (2002)

Jimenez, R., Belcher, E., Sriskandan, S., Lucas, R., McMaster, S., Vojnovic, I., Warner, T. D., and Mitchell, J. A.: Role of Toll-like receptors 2 and 4 in the induction of cyclooxygenase-2 in vascular smooth muscle. Proc. Natl. Acad. Sci. USA *102*/12, 4637–4642 (2005)

Kawai, T., and Akira, S.: Innate immune recognition of viral infection. Nature Immunol. *7*/2, 131–137 (2006)

Kawai, T., and Akira, S.: The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nature Immunol. *11*/5, 373–384 (2010)

Koch, A., Boehm, O., Zacharowski, P. A., Loer, S. A., Weimann, J., Rensing, H., Foster, S. J., Schmidt, R., Berkels, R., Reingruber, S., and Zacharowski, K.: Inducible nitric oxide synthase and heme oxygenase-1 in the lung during lipopolysaccharide tolerance and cross tolerance. Crit. Care Med. *35*/12, 2775–2784 (2007)

Koch, A., Hamann, L., Schott, M., Boehm, O., Grotemeyer, D., Kurt, M., Schwenke, C., Schumann, R. R., Bornstein, S. R., and Zacharowski, K.: Genetic variation of TLR4 influences immunoendocrine stress response: an observational study in cardiac surgical patients. Crit. Care *15*/2, R109 (2011)

Kumar, P., Shen, Q., Pivetti, C. D., Lee, E. S., Wu, M. H., and Yuan, S. Y.: Molecular mechanisms of endothelial hyperpermeability: implications in inflammation. Expert Rev. Mol. Med. *11*e19 (2009)

Latz, E., Visintin, A., Lien, E., Fitzgerald, K. A., Monks, B. G., Kurt-Jones, E. A., Golenbock, D. T., and Espevik, T.: Lipopolysaccharide rapidly traffics to and from the Golgi apparatus with the toll-like receptor 4-MD-2-CD14 complex in a process that is distinct from the initiation of signal transduction. J. Biol. Chem. *277*/49, 47834–47843 (2002)

Lee, H.-K., Lee, J., and Tobias, P. S.: Two lipoproteins extracted from *Escherichia coli* K-12 LCD25 lipopolysaccharide are the major components responsible for Toll-like receptor 2-mediated signaling. J. Immunol. *168*/8, 4012–4017 (2002)

Lee, K. S., Scanga, C. A., Bachelder, E. M., Chen, Q., and Snapper, C. M.: TLR2 synergizes with both TLR4 and TLR9 for induction of the MyD88-dependent splenic cytokine and chemokine response to *Streptococcus pneumoniae*. Cell Immunol. *245*/2, 103–110 (2007)

Levi, M.: Antithrombin in sepsis revisited. Crit. Care *9*/6, 624–625 (2005)

Levi, M., and Van der Poll, T.: Inflammation and coagulation. Crit. Care Med. *38*/2 Suppl, S26–S34 (2010)

Lopez, A., Lorente, J. A., Steingrub, J., Bakker, J., McLuckie, A., Willatts, S., Brockway, M., Anzueto, A., Holzapfel, L., Breen, D., Silverman, M. S., Takala, J., Donaldson, J., Arneson, C., Grove, G., Grossman, S., and Grover, R.: Multiple-center, randomized, placebo-controlled, double-blind study of the nitric oxide synthase inhibitor 546C88: effect on survival in patients with septic shock. Crit. Care Med. *32*/1, 21–30 (2004)

Lorenz, E., Mira, J. P., Cornish, K. L., Arbour, N. C., and Schwartz, D. A.: A novel polymorphism in the toll-like receptor 2 gene and its potential association with staphylococcal infection. Infect. Immun. *68*/11, 6398–6401 (2000)

Lynn, M., Rossignol, D. P., Wheeler, J. L., Kao, R. J., Perdomo, C. A., Noveck, R., Vargas, R., D'Angelo, T., Gotzkowsky, S., and McMahon, F. G.: Blocking of responses to endotoxin by E5564 in healthy volunteers with experimental endotoxemia. J. Infect. Dis. *187*/4, 631–639 (2003)

MacMicking, J., Xie, Q. W., and Nathan, C.: Nitric oxide and macrophage function. Annu. Rev. Immunol. *15*, 323–350 (1997)

Marik, P. E., Pastores, S. M., Annane, D., Meduri, G. U., Sprung, C. L., Arlt, W., Keh, D., Briegel, J., Beishuizen, A., Dimopoulou, I., Tsagarakis, S., Singer, M., Chrousos, G. P., Zaloga, G., Bokhari, F., and Vogeser, M.: Recommendations for the diagnosis and management of corticosteroid insufficiency in critically ill adult patients: consensus statements from an international task force by the American College of Critical Care Medicine. Crit. Care Med. *36*/6, 1937–1949 (2008)

Mayr, V. D., Dunser, M. W., Greil, V., Jochberger, S., Luckner, G., Ulmer, H., Friesenecker, B. E., Takala, J., and Hasibeder, W. R.: Causes of death and determinants of outcome in critically ill patients. Crit. Care *10*/6, R154 (2006)

Medzhitov, R., Preston-Hurlburt, P., and Janeway, C. A. Jr.: A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. Nature *388*/6640, 394–397 (1997)

Miller, L. S., and Modlin, R. L.: Human keratinocyte Toll-like receptors promote distinct immune responses. J. Invest. Dermatol. *127*/2, 262–263 (2007)

Miyake, K.: Innate recognition of lipopolysaccharide by CD14 and toll-like receptor 4-MD-2: unique roles for MD-2. Int. Immunopharmacol. *3*/1, 119–128 (2003)

Mullard, A.: Drug withdrawal sends critical care specialists back to basics. Lancet *378*/9805, 1769 (2011)

Navarra, P., Tsagarakis, S., Faria, M. S., Rees, L. H., Besser, G. M., and Grossman, A. B.: Interleukins-1 and -6 stimulate the release of corticotropin-releasing hormone-41 from rat hypothalamus in vitro via the eicosanoid cyclooxygenase pathway. Endocrinology *128*/1, 37–44 (1991)

Opitz, B., Eitel, J., Meixenberger, K., and Suttorp, N.: Role of Toll-like receptors, NOD-like receptors and RIG-I-like receptors in endothelial cells and systemic infections. Thromb. Haemost. *102*/6, 1103–1109 (2009)

Parker, L. C., Whyte, M. K., Dower, S. K., and Sabroe, I.: The expression and roles of Toll-like receptors in the biology of the human neutrophil. J. Leukoc. Biol. *77*/6, 886–892 (2005)

Pegu, A., Qin, S., Fallert Junecko, B. A., Nisato, R. E., Pepper, M. S., and Reinhart, T. A.: Human lymphatic endothelial cells express multiple functional TLRs. J. Immunol. *180*/5, 3399–3405 (2008)

Petzelbauer, P., Zacharowski, P. A., Miyazaki, Y., Friedl, P., Wickenhauser, G., Castellino, F. J., Groger, M., Wolff, K., and Zacharowski, K.: The fibrin-derived peptide Bbeta15-42 protects the myocardium against ischemia-reperfusion injury. Nature Med. *11*/3, 298–304 (2005)

Phulwani, N. K., Esen, N., Syed, M. M., and Kielian, T.: TLR2 expression in astrocytes is induced by TNF-alpha- and NF-kappa B-dependent pathways. J. Immunol. *181*/6, 3841–3849 (2008)

Poltorak, A., He, X., Smirnova, I., Liu, M. Y., Van Huffel, C., Du, X., Birdwell, D., Alejos, E., Silva, M., Galanos, C., Freudenberg, M., Ricciardi-Castagnolli, P., Layton, B., and Beutler, B.: Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science *282*/5396, 2085–2088 (1998)

Roesner, J. P., Petzelbauer, P., Koch, A., Tran, N., Iber, T., Vagts, D. A., Scheeren, T. W., Vollmar, B., Noldge-Schomburg, G. E., and Zacharowski, K.: Bbeta15-42 (FX06) reduces pulmonary, myocardial, liver, and small intestine damage in a pig model of hemorrhagic shock and reperfusion. Crit. Care Med. *37*/2, 598–605 (2009)

Schlatzer, D. M., Sugalski, J., Dazard, J. E., Chance, M. R., and Anthony, D. D.: A quantitative proteomic approach for detecting protein profiles of activated human myeloid dendritic cells. J. Immunol. Meth. *375*/1-2, 39–45 (2012)

Schroder, N. W., Hermann, C., Hamann, L., Gobel, U. B., Hartung, T., and Schumann, R. R.: High frequency of polymorphism Arg753Gln of the Toll-like receptor-2 gene detected by a novel allele-specific PCR. J. Mol. Med. (Berl). *81*/6, 368–372 (2003)

Schumann, R. R.: Old and new findings on lipopolysaccharide-binding protein: a soluble pattern-recognition molecule. Biochem. Soc. Trans. *39*/4, 989–993 (2011)

Shah, V. B., Williams, D. L., and Keshvara, L.: beta-Glucan attenuates. Neurosci. Lett. *458*/3, 111–115 (2009)

Sprung, C. L., Annane, D., Keh, D., Moreno, R., Singer, M., Freivogel, K., Weiss, Y. G., Benbenishty, J., Kalenka, A., Forst, H., Laterre, P. F., Reinhart, K., Cuthbertson, B. H., Payen, D., and Briegel, J.: Hydrocortisone therapy for patients with septic shock. New Engl. J. Med. *358*/2, 111–124 (2008)

Sultzer, B. M.: Genetic control of leucocyte responses to endotoxin. Nature *219*/5160, 1253–1254 (1968)

Symeonides, S., and Balk, R. A.: Nitric oxide in the pathogenesis of sepsis. Infect. Dis. Clin. North Amer. *13*/2, 449–463, x (1999)

Tran, N., Koch, A., Berkels, R., Boehm, O., Zacharowski, P. A., Baumgarten, G., Knuefermann, P., Schott, M., Kanczkowski, W., Bornstein, S. R., Lightman, S. L., and Zacharowski, K.: Toll-like receptor 9 expression in murine and human adrenal glands and possible implications during inflammation. J. Clin. Endocrinol. Metab. *92*/7, 2773–2783 (2007)

Uematsu, S., Fujimoto, K., Jang, M. H., Yang, B. G., Jung, Y. J., Nishiyama, M., Sato, S., Tsujimura, T., Yamamoto, M., Yokota, Y., Kiyono, H., Miyasaka, M., Ishii, K. J., and Akira, S.: Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5. Nature Immunol. *9*/7, 769–776 (2008)

Van der Poll, T., and Opal, S. M.: Host-pathogen interactions in sepsis. Lancet Infect. Dis. *8*/1, 32–43 (2008)

Vijay-Kumar, M., and Gewirtz, A. T.: Flagellin: key target of mucosal innate immunity. Mucosal. Immunol. *2*/3, 197–205 (2009)

Xagorari, A., and Chlichlia, K.: Toll-like receptors and viruses: induction of innate antiviral immune responses. Open Microbiol. J. *2,* 49–59 (2008)

Yang, D., and Oppenheim, J. J.: Alarmins and antimicrobial immunity. Med. Mycol. *47*, Suppl. 1, S146–S153 (2009)

Zacharowski, K.: Alternative mechanism of organ protection. Nova Acta Leopoldina NF *108*/375, 45–50 (2010)

Zacharowski, K., Zacharowski, P. A., Koch, A., Baban, A., Tran, N., Berkels, R., Papewalis, C., Schulze-Osthoff, K., Knuefermann, P., Zahringer, U., Schumann, R. R., Rettori, V., McCann, S. M., and Bornstein, S. R.: Toll-like receptor 4 plays a crucial role in the immune-adrenal response to systemic inflammatory response syndrome. Proc. Natl. Acad. Sci. USA *103*/16, 6392–6397 (2006)

Zarember, K. A., and Godowski, P. J.: Tissue expression of human Toll-like receptors and differential regulation of Toll-like receptor mRNAs in leukocytes in response to microbes, their products, and cytokines. J. Immunol. *168*/2, 554–561 (2002)

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Rolle der Wissenschaft im Globalen Wandel

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Gesellschaftliche Probleme verlangen heute sehr häufig eine Widerspiegelung im Bereich der Wissenschaften. Als Nationale Akademie der Wissenschaften ist die Leopoldina in zunehmendem Maße gefordert, auch Beratung bei Fragen zu liefern, die über Länder und Kontinentgrenzen hinausgreifen: Klimawandel, der Einsatz erneuerbarer Energien, Fragen der Gesundheitsversorgung, die Einrichtung einer effektiveren Landwirtschaft zur Bekämpfung von Hunger in Krisengebieten und die sich wandelnde Altersstruktur von Bevölkerungen in vielen Staaten sind nur einige Beispiele für entsprechende Gebiete mit dringendem Forschungsbedarf. Sie bilden Herausforderungen für die Gesellschaften, die nur in internationaler, oft globaler Zusammenarbeit zu bewältigen sein werden. Daher wählte die Leopoldina 2012 das Thema „Rolle der Wissenschaft im Globalen Wandel" für ihre Jahresversammlung. Der Band umfasst Beiträge zu den Themenkomplexen „Die Erde im Globalen Wandel", „Herausforderungen des Globalen Wandels" und „Lösungswege von Problemen des Globalen Wandels" sowie zu den gesellschaftlichen und politischen Implikationen der mit dem globalen Wandel verbundenen Prozesse.

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Intravascular Immunity to Infection and Sterile Inflammation (Extended Abstract)

Braedon McDonald and Paul Kubes (Calgary, Alberta, Canada)

During innate immune responses, the microvasculature of inflamed tissues serves as a highway for the delivery of leukocytes and effector molecules to sites of "danger". This "danger" may be posed by infectious microorganisms, or by sterile inflammation caused by tissue injury and necrotic cell death. Recent research indicates that the intravascular compartment is more than just a highway for immune cell trafficking to sites of inflammation, and is in fact home to a variety of immunological responses, collectively termed intravascular immunity (Hickey and Kubes 2009). In our presentation at the Leopoldina 2011, we presented evidence that intravascular immunity mounted by neutrophils is fundamental to the generation of sterile inflammatory responses to tissue injury, as well as protective innate immune responses against blood-borne infections such as severe bacterial sepsis.

Using confocal intravital microscopy, we have investigated the molecular guidance signals that orchestrate the recruitment of neutrophils to sites sterile (injuryinduced) inflammation in the liver (McDonald et al. 2010). Focal necrotic injury to the liver caused rapid adhesion of neutrophils within liver sinusoids, followed by chemotaxis through microvascular conduits towards sites of tissue damage, culminating in precise localisation of neutrophils within foci of injury. Intravascular chemotaxis towards foci of sterile inflammation was dependent on the generation of intralumenal gradients of chemoattractants, including CXC-chemokines (MIP-2 and KC) as well as formylatedpeptide receptor 1 (*FPR1*)-dependent danger signals released from injured cells (McDonald et al. 2010). The ability of neutrophils to chemotax through vascular channels (instead of through the tissue parenchyma) towards sites of injury may limit collateral damage to surrounding healthy tissues.

In contrast to sterile inflammation, septic liver inflammation (bacterial sepsis or endotoxemia) results in widespread neutrophil recruitment within liver sinusoids without intravascular chemotaxis. We hypothesise that neutrophils are positioned with the vasculature of the liver, where they capture bacteria from the circulation to limit hematogenous dissemination of septic infections (Clark et al. 2007). We presented new evidence that neutrophils stationed within liver sinusoids release neutrophil extracellular traps (*NETs*, extracellular webs of decondensed chromatin covered in anti-microbial proteins) that ensnare bacteria from the bloodstream in mouse models of bacterial sepsis. Blocking the production of intravascular NETs caused impaired clearance of bacteria from the bloodstream, resulting in enhanced dissemination of bacteria to distant organs. Therefore, the release of intravascular NETs during sepsis greatly improves the efficiency of bacterial trapping in the blood, revealing a novel mechanism of intravascular immunity that protects against the spread of infection during bacterial sepsis.

References

Clark, S. R., Ma, A. C., Tavener, S. A., McDonald, B., Goodarzi, Z., Kelly, M. M., Patel, K. D., Chakrabarti, S., McAvoy, E., Sinclair, G. D., Keys, E. M., Allen-Vercoe, E., Devinney, R., Doig, C. J., Green, F. H., and Kubes, P.: Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. Nature Med. *13*, 463–469 (2007)

Hickey, M. J., and Kubes, P.: Intravascular immunity: the host-pathogen encounter in blood vessels. Nature Rev. Immunol. *9*, 364–375 (2009)

McDonald, B., Pittman, K., Menezes, G. B., Hirota, S. A., Slaba, I., Waterhouse, C. C., Beck, P. L., Muruve, D. A., and Kubes, P.: Intravascular danger signals guide neutrophils to sites of sterile inflammation. Science *330*, 362–366 (2010)

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Treatment of Sepsis – Challenges and New Concepts

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Immunotherapy of Sepsis – A Review

Manfred THIEL (Mannheim)

3 Figures

Abstract

Since the start of the Surviving Sepsis Campaign by the European Society of Intensive Care Medicine, the International Sepsis Forum and the Society of Critical Care Medicine many qualitative improvements in diagnosis, management and treatment of sepsis have been made. According to resuscitation and management bundles, early administration of adequate antibiotics, fluid resuscitation by early goal directed therapy, and support of vital organ function (cardiovascular system, lung, kidney) are the mainstays of sepsis treatment besides surgical eradication of an infectious focus. Nontheless, major break-throughs in terms of improved patient survival are still lacking, and till today treatment of sepsis represents a challenge for intensivists. Alarmingly, resistance of bacteria and fungi towards anti-microbial compounds is increasing and development of new antibiotics is on average only five years ahead. Thus, if pharmaceutical industry is not able to cope with microbial resistance development, mankind runs a risk of falling back into the times of the pre-antimicrobial era. This scenario underlines the need for the development of new therapies. Reinforcement of the body's own defence systems, especially of innate and adaptive immunity, however, has been hampered for decades by the perception of sepsis to result mostly from an exaggerated systemic inflammatory response elicited by microbial products. Meanwhile, it has become clear that anti-inflammatory therapeutic approaches directed at the elimination of bacterial toxins or inflammatory mediators have failed for many reasons. In line with failure of anti-inflammatory strategies, anti-inflammatory strategies have been shown to contribute to immunosuppression by enhancement of the body's own compensatory anti-inflammatory response. To add complexity, tissue hypoxia plays an ambivalent role in the regulation of the inflammatory response of the immune system: While early acute hypoxia amplifies inflammation, hypoxia when long-lasting and profound causes down-regulation of inflammation. Against this immunophathophysiological background, the present article focuses on immunostimulatory therapeutic strategies, in particular reversal of tissue hypoxia with emphasis on early goal directed therapy known to improve perfusion-dependent oxygen delivery. In cases when chronic hypoxia cannot be reversed, application of pro-inflammatory cytokines might prove useful for restoring immune responsiveness in immunosuppressed patients.

Zusammenfassung

Seit der Initiierung der *Surviving Sepsis Campaign* durch die *European Society of Intensive Care Medicine*, das *International Sepsis Forum* und die *Society of Critical Care Medicine* sind viele Fortschritte in der Diagnostik, dem Management und der Therapie der Sepsis erzielt worden. Gemäß den „resuscitation"- und „managment"- Bündeln zählen neben der kausalen operativen Sanierung eines Infektionsherdes der frühestmögliche Beginn einer kalkulierten Antibiose, die Infusionstherapie im Sinne der „early goal directed therapy" und die supportive Therapie vitaler Organsysteme (Kardiovaskuläres System, Lunge, Niere, etc.) zu den Hauptmaßnahmen in der Therapie der Sepsis. Dennoch wurden bislang keine bahnbrechenden Behandlungserfolge erzielt, so dass die Therapie der Sepsis nach wie vor eine Herausforderung an die Intensivmedizin darstellt. Alarmierend ist hierbei, dass infektiöse Mikroorganismen (Bakterien, Pilze) zunehmend häufiger gegenüber Chemotherapeutika resistent sind und bei der Entwicklung neuer wirksamer Antibiotika der Vorsprung im Durchschnitt lediglich fünf Jahre beträgt. Gelingt es der pharmazeutische Industrie nicht, mit der Entwicklung mikrobieller Resistenzlagen Schritt zu halten, dann läuft die Medizin Gefahr, wieder in die präantibiotische Ära zurückzufallen. Vor diesem Hintergrund wird die Notwendigkeit neuer Therapie-

ansätze sehr deutlich. Therapieansätze, die auf die Stärkung körpereigener Abwehrmechanismen abzielen, wurden in ihrer Entwicklung jahrzehntelang verzögert, da das Krankheitsbild der Sepsis überwiegend auf eine überschießend ablaufende systemische Entzündung in Reaktion auf mikrobielle Produkte zurückgeführt wurde. In der Zwischenzeit hat sich jedoch herausgestellt, dass anti-inflammatorische Ansätze, die auf eine Elimination von bakteriellen Toxinen und Entzündungsmediatoren abzielen, weitgehend versagt haben. So verstärken anti-inflammatorische Ansätze eine bestehende Immunsuppression, die oftmals infolge einer kompensatorischen anti-inflammatorischen Reaktion („compensatory anti-inflammatory response syndrome, *CARS*) entstanden ist. Die Komplexität wird weiter erhöht durch die Hypoxie der Gewebe, da diese bei der Regulation immunologischer Pathomechanismen eine ambivalente Rolle spielt: Während eine akute Hypoxie inflammatorische Prozesse verstärkt, bewirkt eine lang anhaltende und stark ausgeprägte Hypoxie eine Hemmung der Inflammation mit der Folge der Immunsuppression. In diesem immunpathophysiologischen Kontext werden in der vorliegenden Übersicht immunstimulierende Therapieansätze beleuchtet und im Zusammenhang damit insbesondere die „early goal directed therapy" diskutiert, welche durch Verbesserung des perfusionsabhängigen Sauerstoffangebotes eine Gewebehypoxie beseitigen kann. Für den Fall, dass eine chronische Hypoxie der Gewebe nicht aufgehoben werden kann, erscheint der Einsatz pro-inflammatorischer Zytokine sinnvoll, um die Immunantwort beim supprimierten Patienten wieder herzustellen.

1. Introduction

Sepsis is a devastating condition accounting for 215,000 annual deaths in the United States with the cost of caring for septic patients exceeding $15 billion (Angus et al. 2001). With every third patient in the ICU (*intensive care unit*) developing sepsis and with every third sepsis patient dying thereof, the need of improving sepsis therapy is evident. Despite improvements in early antibiotic therapy, surgical eradication of the infectious focus, fluid resuscitation and supporting vital organ function (cardiovascular system, lung, kidney, etc.), mortality in sepsis has remained quite stable over the last three decades. Attempts of improving patient outcome by adjunct therapeutic measures like tight glycemic control, activated protein C administration, and low dose corticosteroid therapy have failed, these measures have not been shown to be effective in large randomised trials. Clearly, there is still a need for better treatment of the septic patient to improve his or her outcome. To this end, the immune system has been identified as a promising target since the anti-bacterial immune response plays an important role in the elimination of pathogens, while the associated inflammatory response causes collateral tissue injury and organ damage. However, previous attempts to modulate the septic inflammatory immune response have actually decreased survival in some clinical trials, mostly due to flawed concepts and failure to understand mechanisms of organ and cell injury (Suffredini and Munford 2011).

2. Why Did Immunotherapy Fail in Improving Outcome of Septic Patients?

In the 1970s till the 1980s, immunopathophysiologically, sepsis was thought to result from an exaggerated systemic inflammatory response to bacterial products. This inflammatory reaction was assumed to occur in septic patients with such intensity that it causes damage to the microcirculation, thereby compromising perfusion-dependent oxygen delivery, leading to an oxygen deficit with organ dysfunction and multiple organ failure. Based on this understanding, numerous therapeutic approaches were directed against bacterial products that could initiate such inflammatory response, like endotoxin. Elimination of endotoxin was attempted by the use of monoclonal or polyclonal antibodies (Turgeon et al. 2007), bactericidal permeability increasing proteins (Levin et al. 2000), hemoperfusion with polymyxin B coated columns

(KOJIKA et al. 2006), or high throughput hemofiltration (HOFFMANN et al. 1995). More specific therapeutic measures were directed at the level of single inflammatory mediators, i.e., removal of TNF-α or IL-1β by specific antibodies (EICHACKER et al. 2002). During the last two decades anti-inflammatory therapy with steroid hormones at low doses has come into play again, although with ambiguous results (SPRUNG et al. 2008). This list of immunotherapeutic approaches can be easily extended by numerous other examples, but there is no successful one with clear cut improvement of patients' outcome from sepsis. Simply spoken, nothing has worked so far. So the scientific and medical community may rightly ask, why did we fail in improving the outcome of septic patients with immunotherapy?

There are countless reasons for this. First of all, the immune system of a patient is highly complex, and there is large redundancy of pathogenetic mechanisms. Although it makes sense to use therapeutic compounds of high specificity to eliminate major early inflammatory mediators like TNF-α by monoclonal antibodies, it is unlikely that such a highly specific approach will work because of the presence of numerous other mediators in the body's inflammatory network. To add complexity, septic patients represent a very heterogenous population with respect to co-morbidities, age, gender, and race, all of which affect immune response. Moreover, significant variability is caused by the type and localisation of the underlying infection, the severity of illness and the time course of illness, which has to be considered when timing the application of immunotherapy. With regard to the phase of illness, many clinicians have begun to question the afore-mentioned dogma that septic death is only caused by systemic inflammation. In fact doctors and researchers have realised that besides inflammation, anti-inflammatory reactions come early into play in order to limit inflammation. It was Roger BONE who coined the term *CARS*, the Compensatory Anti-Inflammatory Response Syndrom (BONE 1996), to explain the onset of immunosuppression which weakens bacterial defence, thereby causing recurrent infections as another important cause for multiple organ failure. But what is the current clinical evidence for the existence of CARS?

3. Clinical Evidence for CARS

There is good indirect as well as direct clinical evidence for patients to develop an immunosuppressive anti-inflammatory response during the course of sepsis. Indirect evidence is provided by the finding that patients do not die from an early Systemic Inflammatory Response Syndrom (*SIRS*) but most of them die later on with recurrent infections and septic multiple organ failure. In fact, more than half of patients survive the first seven days after onset of sepsis (BERNARD et al. 2001), a time period where deaths occur mostly due to hyperinflammatory circulatory failure. However, anti-inflammatory agents applied during this phase were without any beneficial effect. By contrast, early administration of anti-inflammatory compounds is likely to further immunosuppression and trigger more infections, causing later death from multiple septic organ failure. This has been shown in histomorphological and microbiologicals studies of late septic deaths which demonstrated that at least 47 to 51 % of deceased patients were infected despite vigorous anti-biotic therapy (POOLE et al. 1993). Immunosuppression in septic patients is also supported by an increase in infections with primarily opportunistic germs like candida or less aggressive bacteria like *Acetinobacter* species (MARTIN et al. 2003), respectively. More direct evidence for immunosuppression in critically ill patients is provided by the reactivation of latent cytomegalovirus in previously immunocompetent pa-

tients, which correlates with length of stay in the intensive care unit and mortality (Limaye et al. 2008). Similarily, herpes simplex virus bronchopneumonia has been reported relatively frequently even in nonimmunocompromised patients with prolonged mechanical ventilation and is also associated with length of stay in the intensive care unit and mortality (Luyt et al. 2007). The most convincing evidence for an acquired state of immunosuppression of patients during intensive care treatment – predisposing patients to an increased risk for sepsis – is suppression of delayed type hypersensitivity reaction towards classical recall antigens (Christou et al. 1995). But what are the alterations of cellular immunity in patients subjecting them to an elevated risk for immunosuppression and subsequent infections?

4. Alterations of Cellular Immunity in ICU Patients

An effective anti-bacterial defence is mounted by a complex interaction of humoral and cellular components of the innate and adaptive part of the immune system. It would be beyond the scope of this review to address all alterations reported for these systems in septic patients. Therefore, the reader is referred to more specific literature (Cohen 2002, Hotchkiss et al. 2009). Only the major alterations with restriction to cellular immunity are briefly addressed here to give an understanding of the most relevant changes currently discussed for development of immunosuppression in critical illness and the septic state (Opal et al. 2011). With respect to innate immunity, neutrophil chemotaxis, phagocytosis and killing was reported to be significantly attenuated in septic patients. In monocytes, antigen presenting molecules like HLA-DR class II (*MHC II*) molecule expression were shown to be suppressed (Albaiceta et al. 2007), while proapoptotic molecules like the Program Death Molecule-1 (*PD-1*) were found to be up-regulated (Okazaki et al. 2006). Alterations of adaptive immunity comprise severe lymphopenia due to decreased cell proliferation and increased apoptotic death. Besides up-regulation of PD-1, a lack in trophic signals by enhanced expression of inhibitory co-receptors like the Cytotoxic T-lymphocyte Antigen 4 (*CTLA-4*) were identified (Avice et al. 2001). Immune deviation due to Th1/Th2 polarisation adds to immunosuppression by the increase of anti-inflammatory cytokine release and the reciprocal decrease in inflammatory cytokines (Heidecke et al. 1999, Manjuck et al. 2000). Moreover, the number of immunosuppressive regulatory T cells was shown to increase (Monneret et al. 2003). Thus, there is ample evidence for the development of adverse enumerative and functional alterations accounting for a state of suppression at the cellular level of the innate and adaptive part of the immune system (Giamarellos-Bourboulis 2010). Such changes are detectable already in non-infected ICU patients and become even more prominent upon stress due to infection and subsequent inflammation. But why do these functional changes of immune cells occur?

5. Mechanisms of Acquired Immunosuppression in ICU Patients

Numerous factors causing immunosuppression are still unknown and hence much more research effort has to be spent on this issue. Yet, there is already some evidence for part of cellular immunosuppressive effects being caused by activation of a psycho-neuro-endocrine stress response as sepsis is a major stressor. The sequence of events leading to the activation of the neuro-endocrine stress response starts at the cellular level. Cytokines which first are released

by immune cells locally spill over into the systemic circulation or activate vagal afferent nerve fibres to stimulate the central nervous system (TRACEY 2009), respectively. In turn, the hypothalamic-pituitary-adrenal axis becomes activated to release immunosuppressive catecholamines and steroids (PLATA-SALAMAN 1998). In addition, activation of the efferent limbs of the symphathetic and parasympthatic autonomous nervous system causes direct immunosuppression due to the intense sympathetic innervation of many secondary lympoid organs and the presence of nicotinergic receptors on immune cells (BENCHERIF 2009).

Besides control of the immune system by a psycho-neuro-endocrine stress response, there are also many metabolic factors which alter immunity. Among them, most importantly, prolonged severe hypoxia and hypoxia dependent mechanisms can strongly decrease the reactivity of the immune system (THIEL et al. 2007). However, before considering such immunosuppressive effects of prolonged hypoxia, it is worthwhile to shed some light on the pro-inflammatory effects of acute hypoxia (IMTIYAZ and SIMON 2010).

Inflammation induced by interaction of microbial Pathogen Associated Molecular Patterns (*PAMPs*) with their respective Pathogen Recognition Receptors (*PRRs*) leads to hypoxia of increasing severity (Fig. 1). This is readily explained by inflammatory damage to the microcirculation and hence compromised perfusion-dependent oxygen delivery considering an increasing metabolic demand of inflamed tissues. Hypoxia during this early phase of sepsis, however, is very likely to amplify inflammation. For instance, acute hypoxemia has been shown to enhance neutrophil inflammatory responses in humans (TAMURA et al. 2002). Stimulatory effects of acute hypoxia have also been shown in many other *in vivo* (THOMPSON et al. 2004) and *in vitro* models (CHAKRABARTI et al. 2009). One of the pro-inflammatory mechanisms very likely involved comprises hypoxic degradation of adenine nucleotides to form adenosine (ATP → ADP → AMP → Adenosine). Even at very low concentrations ($< 10^{-9}$ M) adenosine can bind to adenosine A1 receptors which are of high affinity and pro-inflammatory activity (SALMON and CRONSTEIN 1990, CRONSTEIN et al. 1992). High affinity A1 receptors are G_i-coupled receptors and hence inhibit the enzyme adenylylcyclase to decrease production of intracellular cAMP, a cyclic nucleotide with potent anti-inflammatory effects. Thus, acute mild hypoxia with only little formation of adenosine is likely to exert pro-inflammatory effects *via* binding to high affinity adenosine A1 receptors. This in turn leads to inhibition of adenylylcyclase and thereby decreases intracellular levels of anti-inflammatory cAMP. The tissue damage caused by inflammatory hypoxia ultimately leads to the release of Danger Associated Molecule Proteins (*DAMPs*) like HMG-1 and heat shock proteins, fibronectin, etc., which by interaction with PRRs are known to further amplify inflammation (CROUSER et al. 2008). As a result, acute hypoxia, in amplifying early inflammation, may end up causing a vicious cycle, which also magnifies its tissue destructive effects (SYNNESTVEDT et al. 2002, OPAL 2007). But what are the consequences of such acute and early hypoxia dependent pro-inflammatory mechanisms for sepsis treatment?

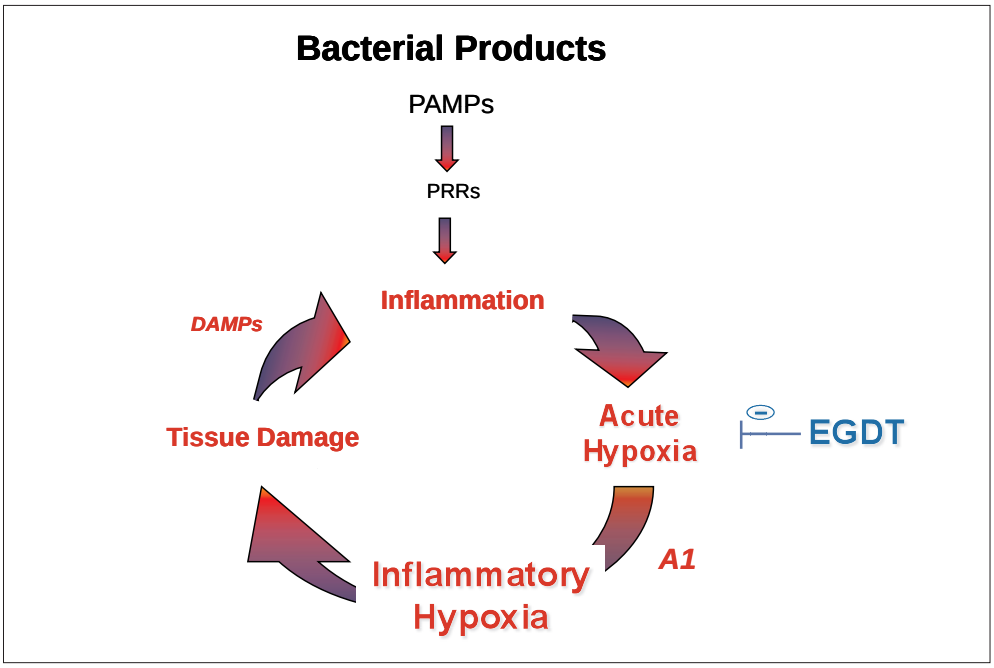

Fig. 1 Pro-inflammatory role of acute hypoxia. Pathogen-Associated Molecular Patterns (*PAMPs*) of bacteria interact with Pattern Recognition Receptors (*PRRs*) to initiate inflammation. This will increase metabolic demand of tissues leading to hypoxia. Acute hypoxia in turn potentiates the initial inflammatory response, in part by degradation of adenine nucleotides to adenosine which can bind to high affinity pro-inflammatory adenosine A1 receptors already at low concentrations ($< 10^{-9}$ M). These initial mechanisms of inflammatory hypoxia are further augmented by the release of Danger-Associated Molecular Patterns (*DAMPs*). DAMPs like HMGB1, high mobility group box 1, S100a proteins, hyaluronan, fibronectin, heat shock proteins, etc. also interact with PRRs to further magnify inflammation, ending up in a vicious cycle. Early goal directed therapy dampens inflammation by reversal of acute hypoxia thanks to an increase in perfusion-dependent oxygen delivery to inflamed tissues. For further explanation see text.

When Early Goal Directed Therapy (*EGDT*) is started according to the Resuscitation Bundle of the Surviving Sepsis Campaign at the earliest possible time after onset of sepsis, the major aim is to improve tissue perfusion. By doing so tissue hypoxia becomes reversed or at least attenuated, respectively (Fig. 1). As acute hypoxia is a strong trigger of inflammation, EGDT is expected not only to improve parameters of anaerobic tissue metabolism but also to attenuate parameters of inflammation. In fact, reversal of hypoxia by EGDT, as indicated by a decrease in lactate levels, was shown to reduce in parallel the extent of inflammation, organ dysfunction, and mortality in septic patients. These findings were described by the group around E. Rivers in a prospective observational study, in which patients with severe sepsis and septic shock received EGDT already at the emergency department (Nguyen et al. 2010). Response to EGDT was assessed by lactate clearance, defined as % change in lactate levels six hours from baseline measurement in the emergency department. Patients were monitored for quantitative changes in inflammatory biomarkers for 72 hours, organ dysfunction and mortality. Following classification of results by quartiles of lactate clearance values, it became evident that failure to respond to EGDT as indicated by a lack in metabolic clearance of lactate was associated with the highest levels of inflammation when determined within the first 24 hours. This group of patients which was unable to clear systemic lactate levels and inflammation within the first

24 hours after the start of EGDT subsequently developed multiple organ failure and died later on. Using the same treatment protocol by the same scientific group, more detailed analysis of the time course of inflammatory parameter of septic patients refractory to EGDT showed that the rise in inflammatory cytokines stopped at 24 hours (RIVERS et al. 2007). Thereafter, levels of inflammatory cytokines decreased within the next 48 hours to the range of values of patients responsive to EGDT, and this despite continuous hypoxia. In other words, in the group of septic patients with the worst lactate clearance, inflammatory cytokines first strongly increased as predicted by the stimulatory effects of acute hypoxia, but later on decreased to normal values even in the continuous presence of tissue hypoxia. What does this mean? Does this mean that hypoxia, although initially able to stimulate a pro-inflammatory response, looses its activating effect with time or with its increasing severity? Or does more long lasting and hence more profound hypoxia – especially when lasting longer than 24 hours – triggers mechanisms which are able to inhibit inflammation? What kind of factor or factors are induced by more prolonged and severe forms of hypoxia which will finally down-regulate inflammation?

6. Prolonged Hypoxia Induces Anti-inflammatory Mechanisms for Tissue Protection but also Causes Immunosuppression

In recent years, we have addressed these questions in numerous animal models of inflammatory tissue injury. Here, we summarise evidence for strong anti-inflammatory immunosuppressive effects of severe tissue hypoxia. Such mechanisms might also explain long-term immunosuppression in chronically hypoxic patients. As already discussed before with respect to the hypoxia paradox, acute hypoxia when less severe as in the beginning of an infection enhances inflammation (see Fig. 1) but will also cause strong immunosuppressive effects when hypoxia becomes more and more intense later on. In the latter situation, the adenosine formed by long-lasting and severe hypoxic inflammation will reach levels high enough to bind to adenosine $A2_A/A2_B$ receptors also expressed on immune cells (Fig. 2). In contrast to pro-inflammatory adenosine G_i-coupled A1 receptors, adenosine $A2_A$ and $A2_B$ receptors are G_s coupled receptors known to activate the adenylylcyclase. Thereby they increase intracellular levels of immunosuppressive cAMP and are able to shut down immune reactions and ongoing tissue injury (SITKOVSKY et al. 2004). In addition hypoxia will also stabilise the transcription factor HIF-1α, which was shown to inhibit T cell functions (THIEL et al. 2007). By both adenosinergic and HIF-dependent pathways, hypoxia – when profound and lasting long enough – will finally down-regulate ongoing inflammatory reactions. Therefore, high levels of adenosine and the adenosine $A2_A/A2_B$ receptor systems are part of a hypoxia triggered physiological negative feedback mechanism to protect tissue from ongoing exaggerated inflammatory tissue destructive processes. The same is true for hypoxic signalling by the hypoxia inducible factor (*HIF*)-1α dependent pathways in T cells (THIEL et al. 2007). In these negative regulatory feedback mechanisms, high levels of adenosine and severe hypoxia are reporters for tissue damage, and $A2_A/A2_B$ receptors and HIF are sensors of ongoing inflammatory tissue destruction. In other words, when inflammation has reached a degree that severe hypoxia ensues, hypoxia-dependent signalling will offer strong signals to stop inflammation. Although such mechanisms make sense to protect tissues from immediate inflammatory damage, they will also cause immunosuppression in the long run. Based on such chronically activated anti-inflammatory mechanisms in hypoxic patients, a state of acquired immunosuppression is likely to develop.

The best immunostimulatory approach in this situation hence appears to reverse tissue hypoxia to relieve immunosuppression in chronically septic patients.

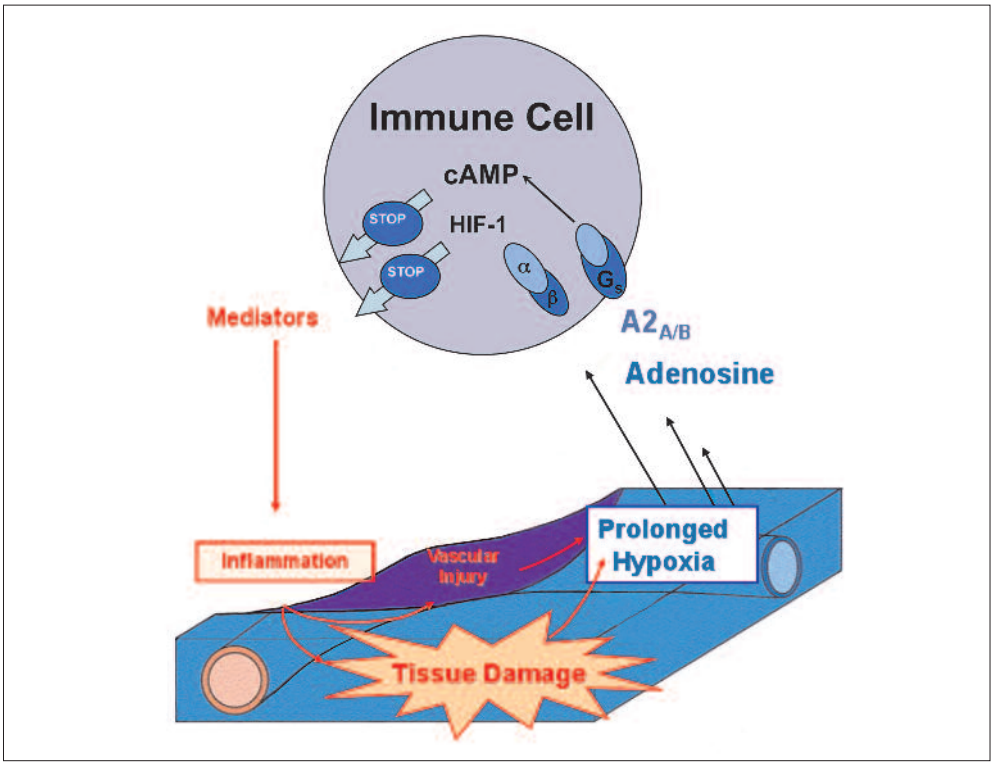

Fig. 2 Anti-inflammatory role of prolonged hypoxia. Long-lasting and severe inflammatory hypoxia triggers formation of adenosine at levels high enough ($>10^{-9}$–10^{-6} M) to interact with anti-inflammatory adenosine $A2_{A/B}$ receptors in a delayed negative feedback manner. In this model, extracellular high concentrations of adenosine serve as a "signal" and the adenosine $A2_{A/B}$ receptors as "sensors" of excessive inflammatory collateral tissue damage. Adenosine $A2_{A/B}$ receptor activation will stimulate formation of intracellular cAMP, a well known inhibitor of immune cell effector functions. In parallel, hypoxia stabilises hypoxia inducible factor (*HIF-1α*), which in T cells was shown to inhibit cellular activation. These hypoxia-dependent signalling pathways help to counteract further immune cell activation and hence limit inflammatory collateral tissue injury. Although good for tissue protection in the short term, prolonged activation of these mechanisms by severe hypoxia will lead to profound immunosuppression in the long term. Adapted from SITKOVSYK et al. (2004)

Accordingly, one might hypothesise that elimination of hypoxia-dependent anti-inflammatory, immunosuppressive mechanisms will improve outcome in bacterial sepsis. To test this hypothesis, our group used conditional knock-out mice with T-Cell-specific knock out of HIF-1α by the lck Cre lox P system in a model of abdominal sepsis (THIEL et al. 2007). Knockout (*KO*) and control mice were subjected to cecal ligation and puncture, and survival and biochemical and immonological parameters were analysed. As predicted, survival of HIF-1α KO mice was significantly better than that of wild type (*WT*) mice. The improved survival was associated with a much better capacity of mice to produce T-cell dependent cytokines, like IL-2 and IFN-γ, which play a major role for lymphocyte proliferation and anti-bacterial func-

tions. In agreement with superior anti-bacterial capacity in HIF-1α KO mice, bacterial load determined in the lung, the liver, and the spleen was much less in KO mice. Spleens of WT mice had a much higher burden of anaerobic gas forming bacteria than spleens from KO mice, in which almost no bacteria were found.

With respect to the role of the hypoxia → adenosine → $A2_A$ or $A2_B$ receptor signalling pathways, almost identical results have been reported in various models of murine abdominal infection (BELIKOFF et al. 2011a, b, NEMETH et al. 2006). These publications confirm immunosuppression and weakening of anti-bacterial defence mechanisms by adenosine → $A2_{A/B}$ receptor dependent pathways, which are mostly activated by severe hypoxia. Regarding suppression of pro-inflammatory cytokine production by such hypoxia-dependent metabolic mechanisms, it is worthwhile to remember that innate immunity is usually amplified by adaptive immunity. Upon invasion, microorganisms are identified and ingested by tissue phagocytes. The foreign material is then processed and presented to T cells. T cells in turn become activated and produce inflammatory cytokines, especially IFN-γ. IFN-γ primes and actives phagocytes for enhanced responses. Besides enhancement of phagocytic capabilities, primed phagocytes can also produce reactive oxygen metabolites with bactericidal activity much more efficiently (TENNENBERG et al. 1993). As a result anti-microbial phagocytic defence mechanisms become strongly amplified (ROILIDES et al. 1995). This sequence of events, by the way, is a good example for the cooperation and amplification of the anti-bacterial effector function of innate immunity by adaptive immunity. But what can clinicians do if cooperation between innate and adaptive immunity is permanently suppressed by hypoxia in patients still in need of catecholamines because they are unresponsive to EGDT? Are there other ways to support pro-inflammatory cytokine production that is suppressed by hypoxia? In this regard the best approach could be to substitute such pro-inflammatory cytokines with priming effects on phagocytes.

7. Immunotherapy by Pro-inflammatory Cytokines

Administration of pro-inflammatory cytokines has already been investigated in several prospective clinical studies with good results which demonstrated beneficial effects on survival. However, these studies were only observational with the exception of one study. This study is till today unique as it was not only performed in a double-blinded, randomised, placebo-controlled manner, but because it was also based on biomarker identification of patients supposed to suffer from immunosuppression (MEISEL et al. 2009). In this study degree of immunosuppression was estimated by quantitative measurement of HLA-DR II antigen expression on blood monocytes. Septic patients were enrolled only if expression of HLA-DR II was less than 8000 anti-body molecules bound per cell. The treatment group received GM-CSF with an escalating dose-regimen to increase HLA-DR II expression at least above 15,000 molecules per cell. As expected, in GM-CSF-treated patients HLA-DR II antigen expression increased. In parallel, functional capacities of blood monocytes significantly improved. For instance, when blood monocytes were activated by LPS *ex vivo*, capability of cells to produce TNFα was significantly increased. Although the study was not powered to determine the effects of restoration of monocyte functions on the outcomes, there was a trend towards a reduction in length of stay and time on ventilator in GM-CSF treated patients. GM-CSF immunotherapy also appeared to reduce the severity of illness in septic patients, which almost reached statistical significance. The findings of this study are in agreement with promising

results of previous immunotherapeutic studies that had all one goal in common, i.e., the restoration of cellular immunocompetence in suppressed patients by priming their phagocytes with pro-inflammatory cytokines (DOCKE et al. 1997, NIERHAUS et al. 2003).

8. Conclusion and Perspective

Intensivists are just at the beginning of a new area of immunotherapy of sepsis. A better understanding of the dysfunction of the immune system in septic patients is needed for more successful therapy. Without exact knowledge of the status of the immune system, especially of the functional capacity of cellular immunity, any immunotherapeutic compound – either stimulatory or inhibitory – will turn out to be counter-productive when applied at the wrong time to the wrong patient. This was the case with most previous immunotherapeutic approaches

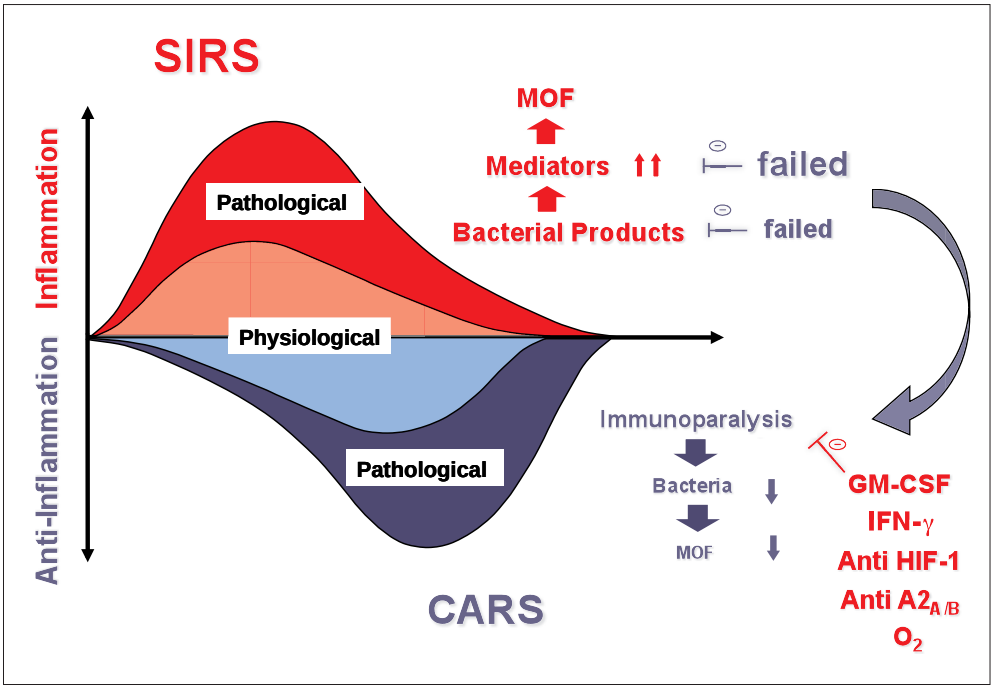

Fig. 3 Immunotherapy of sepsis. For decades immunotherapy of sepsis was based on the concept that an exaggerated systemic inflammatory response syndrome (*SIRS*) accounts for multiple organ dysfunction and failure. However, anti-inflammatory measures directed at bacterial products or secondarily released humoral mediators and effector cells failed to improve patients' outcome. By contrast, anti-inflammatory drugs are thought to augment the compensatory anti-inflammatory response syndrome (*CARS*) mounted by the body to limit SIRS and associated tissue damage. As a result, anti-inflammatory drugs exacerbate CARS related states of immunoparalysis, thereby predisposing the patient to recurrent episodes of in hospital acquired infectious complications, ultimately leading to septic multiple organ failure. In order to break this vicious cycle, stimulatory immunotherapeutics like cytokines with priming effects on phagocytes are most promising to restore immunocompetence as a prerequisite for patient recovery. Besides use of GM-CSF and IFN-γ, future immunotherapies may also target hypoxia-dependent mechanisms by using antagonists for HIF-1α in T-cells or for immunosuppressive adenosine $A2_{A/B}$ receptors, as prolonged hypoxia has been shown to exert immunosuppression by these signalling pathways. For further explanation see text.

that led to millions of dollars being spent on anti-bacterial and anti-inflammatory mediator therapies to dampen SIRS in the early phase of sepsis. None worked but rather immunosuppression of the CARS rising in parallel was further augmented (Fig. 3). Exacerbation of CARS or failure to resolve CARS clearly is an unwanted risk patients face by the uncritical use of anti-inflammatory compounds. By contrast, there is now good evidence for the beneficial therapeutic effects of pro-inflammatory compounds to restore immunocompetence in the later phase of sepsis. To this end cytokines with priming effects on phagocytes are most promising. Besides GM-CSF and IFN-γ, future immunotherapeutic approaches may target hypoxia-dependent mechanisms by HIF-1α antagonists in T-cells or anti-adenosinergic compounds.

Not to forget and perhaps most important, oxygen itself is also an immunotherapeutic, because it represents the rational therapy for hypoxia-induced immunosuppression. As hypoxia should generally be prevented in septic patients, because of its acute pro-inflammatory effects in the early phase and its anti-inflammatory immunosuppressive effects in the late phase of sepsis, simple administration of oxygen should always be considered, especially as it is one of the cheapest and highly effective immunotherapeutic measures.

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References

ALBAICETA, G. M., PEDREIRA, P. R., GARCÍA-PRIETO, E., and TABOADA, F.: Therapeutic implications of immunoparalysis in critically ill patients. Inflamm. Allergy Drug Targets *6*/4, 191–196 (2007)

ANGUS, D. C., LINDE-ZWIRBLE, W. T., LIDICKER, J., CLERMONT, G., CARCILLO, J., and PINSKY, M. R.: Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit. Care Med. *29*/7, 1303–1310 (2001)

AVICE, M. N., RUBIO, M., SERGERIE, M., DELESPESSE, G., and SARFATI, M.: Role of CD47 in the induction of human naive T cell anergy. J. Immunol. *167*/5, 2459–2468 (2001)

BELIKOFF, B., HATFIELD, S., SITKOVSKY, M., and REMICK, D. G.: Adenosine negative feedback on A2A adenosine receptors mediates hyporesponsiveness in chronically septic mice. Shock *35*/4, 382–387 (2011a)

BELIKOFF, B. G., HATFIELD, S., GEORGIEV, P., OHTA, A., LUKASHEV, D., BURAS, J. A., REMICK, D. G., and SITKOVSKY, M.: A2B adenosine receptor blockade enhances macrophage-mediated bacterial phagocytosis and improves polymicrobial sepsis survival in mice. J. Immunol. *186*/4, 2444–2453 (2011b)

BENCHERIF, M.: Neuronal nicotinic receptors as novel targets for inflammation and neuroprotection: mechanistic considerations and clinical relevance. Acta Pharmacol. Sin. *30*/6, 702–714 (2009)

BERNARD, G. R., VINCENT, J. L., LATERRE, P. F., LAROSA, S. P., DHAINAUT, J. F., LOPEZ-RODRIGUEZ, A, STEINGRUB, J. S., GARBER, G. E., HELTERBRAND, J. D., ELY, E. W., and FISHER, C. J. Jr., and *Recombinant Human Protein C Worldwide Evaluation in Severe Sepsis (PROWESS) Study Group*: Efficacy and safety of recombinant human activated protein C for severe sepsis. New Engl. J. Med. *344*/10, 699–709 (2001)

BONE, R. C.: Sir Isaac Newton, sepsis, SIRS, and CARS. Crit. Care Med *24*/7, 1125–1128 (1996)

CHAKRABARTI, S., RIZVI, M., PATHAK, D., KIRBER, M. T., and FREEDMAN, J. E.: Hypoxia influences CD40-CD40L mediated inflammation in endothelial and monocytic cells. Immunol. Lett. *122*/2, 170–184 (2009)

CHRISTOU, N. V., MEAKINS, J. L., GORDON, J., YEE, J., HASSAN-ZAHRAEE, M., NOHR, C. W., SHIZGAL, H. M., and MACLEAN, L. D.: The delayed hypersensitivity response and host resistance in surgical patients. 20 years later. Ann. Surg. *222*/4, 534–546; discussion 546–548 (1995)

COHEN, J.: The immunopathogenesis of sepsis. Nature *420*/6917, 885–891 (2002)

CRONSTEIN, B. N., LEVIN, R. I., PHILIPS, M., HIRSCHHORN, R., ABRAMSON, S. B., and WEISSMANN, G.: Neutrophil adherence to endothelium is enhanced via adenosine A1 receptors and inhibited via adenosine A2 receptors. J. Immunol. *148*/7, 2201–2206 (1992)

Crouser, E., Exline, M., Knoell, D., and Wewers, M. D.: Sepsis: links between pathogen sensing and organ damage. Curr. Pharm. Des. *14*/19, 1840–1852 (2008)

Docke, W. D., Randow, F., Syrbe, U., Krausch, D., Asadullah, K., Reinke, P., Volk, H. D., and Kox, W.: Monocyte deactivation in septic patients: restoration by IFN-gamma treatment. Nature Med. *3*/6, 678–681 (1997)

Eichacker, P. Q., Parent, C., Kalil, A., Esposito, C., Cui, X., Banks, S. M., Gerstenberger, E. P., Fitz, Y., Danner, R. L., and Natanson, C.: Risk and the efficacy of antiinflammatory agents: retrospective and confirmatory studies of sepsis. Amer. J. Respir. Crit. Care Med. *166*/9, 1197–1205 (2002)

Giamarellos-Bourboulis, E. J.: What is the pathophysiology of the septic host upon admission? Int. J. Antimicrob. Agents *36*/Suppl. 2, 2–5 (2010)

Heidecke, C. D., Hensler, T., Weighardt, H., Zantl, N., Wagner, H., Siewert, J. R., and Holzmann, B.: Selective defects of T lymphocyte function in patients with lethal intraabdominal infection. Amer. J. Surg. *178*/4, 288–292 (1999)

Hoffmann, J. N., Hartl, W. H., Deppisch, R., Faist, E., Jochum, M., and Inthorn, D.: Hemofiltration in human sepsis: evidence for elimination of immunomodulatory substances. Kidney Int. *48*/5, 1563–1570 (1995)

Hotchkiss, R. S., Coopersmith, C. M., McDunn, J. E., and Ferguson, T. A.: The sepsis seesaw: tilting toward immunosuppression. Nature Med. *15*/5, 496–497 (2009)

Imtiyaz, H. Z., and Simon, M. C.: Hypoxia-inducible factors as essential regulators of inflammation. Curr. Top. Microbiol. Immunol. *345*, 105–120 (2010)

Kojika, M., Sato, N., Yaegashi, Y., Suzuki, Y., Suzuki, K., Nakae, H., and Endo, S.: Endotoxin adsorption therapy for septic shock using polymyxin B-immobilized fibers (*PMX*): evaluation by high-sensitivity endotoxin assay and measurement of the cytokine production capacity. Ther. Apher. Dial. *10*/1, 12–18 (2006)

Levin, M., Quint, P. A., Goldstein, B., Barton, P., Bradley, J. S., Shemie, S. D., Yeh, T., Kim, S. S., Cafaro, D. P., Scannon, P. J., and Giroir, B. P.: Recombinant bactericidal/permeability-increasing protein (*rBPI21*) as adjunctive treatment for children with severe meningococcal sepsis: a randomised trial. rBPI21 Meningococcal Sepsis Study Group. Lancet *356*/9234, 961–967 (2000)

Limaye, A. P., Kirby, K. A., Rubenfeld, G. D., Leisenring, W. M., Bulger, E. M., Neff, M. J., Gibran, N. S., Huang, M. L., Santo Hayes, T. K., Corey, L., and Boeckh, M.: Cytomegalovirus reactivation in critically ill immunocompetent patients. JAMA *300*/4, 413–422 (2008)

Luyt, C. E., Combes, A., Deback, C., Aubriot-Lorton, M. H., Nieszkowska, A., Trouillet, J. L., Capron, F., Agut, H., Gibert, C., and Chastre, J.: Herpes simplex virus lung infection in patients undergoing prolonged mechanical ventilation. Amer. J. Respir. Crit. Care Med. *175*/9, 935–942 (2007)

Manjuck, J., Saha, D. C., Astiz, M., Eales, L. J., and Rackow, E. C.: Decreased response to recall antigens is associated with depressed costimulatory receptor expression in septic critically ill patients. J. Lab. Clin. Med. *135*/2, 153–160 (2000)

Martin, G. S., Mannino, D. M., Eaton, S., and Moss, M.: The epidemiology of sepsis in the United States from 1979 through 2000. New Engl. J. Med. *348*/16, 1546–1554 (2003)

Meisel, C., Schefold, J. C., Pschowski, R., Baumann, T., Hetzger, K., Gregor, J., Weber-Carstens, S., Hasper, D., Keh, D., Zuckermann, H., Reinke, P., and Volk, H. D.: Granulocyte-macrophage colony-stimulating factor to reverse sepsis-associated immunosuppression: a double-blind, randomized, placebo-controlled multicenter trial. Amer. J. Respir. Crit. Care Med. *180*/7, 640–648 (2009)

Monneret, G., Debard, A. L., Venet, F., Bohe, J., Hequet, O., Bienvenu, J., and Lepape, A.: Marked elevation of human circulating CD4+CD25+ regulatory T cells in sepsis-induced immunoparalysis. Crit. Care Med. *31*/7, 2068–2071 (2003)

Nemeth, Z. H., Csoka, B., Wilmanski, J., Xu, D., Lu, Q., Ledent, C., Deitch, E. A., Pacher, P., Spolarics, Z., and Haskó, G.: Adenosine A2A receptor inactivation increases survival in polymicrobial sepsis. J. Immunol. *176*/9, 5616–5626 (2006)

Nguyen, H. B., Loomba, M., Yang, J. J., Jacobsen, G., Shah, K., Otero, R. M., Suarez, A., Parekh, H., Jaehne, A., and Rivers, E. P.: Early lactate clearance is associated with biomarkers of inflammation, coagulation, apoptosis, organ dysfunction and mortality in severe sepsis and septic shock. J. Inflamm. (Lond.) *7*, 6 (2010)

Nierhaus, A., Montag, B., Timmler, N., Frings, D. P., Gutensohn, K., Jung, R., Schneider, C. G., Pothmann, W., Brassel, A. K., and Schulte am Esch, J.: Reversal of immunoparalysis by recombinant human granulocyte-macrophage colony-stimulating factor in patients with severe sepsis. Intensive Care Med. *29*/4, 646–651 (2003)

Okazaki, T., and Honjo, T.: The PD-1-PD-L pathway in immunological tolerance. Trends Immunol. *27*/4, 195–201 (2006)

Opal, S. M.: Immunologic alterations and the pathogenesis of organ failure in the ICU. Semin. Respir. Crit. Care Med. *32*/5, 569–580 (2011)

Opal, S. M.: The host response to endotoxin, antilipopolysaccharide strategies, and the management of severe sepsis. Int. J. Med. Microbiol. *297*/5, 365–377 (2007)

Plata-Salaman, C. R.: Brain injury and immunosuppression. Nature Med. *4*/7, 768–769 (1998)

Poole, G. V., Muakkassa, F. F., and Griswold, J. A.: The role of infection in outcome of Multiple Organ Failure. Amer. Surg. *59*/11, 727–732 (1993)

Rivers, E. P., Kruse, J. A., Jacobsen, G., Shah, K., Loomba, M., Otero, R., and Childs, E. W.: The influence of early hemodynamic optimization on biomarker patterns of severe sepsis and septic shock. Crit. Care Med. *35*/9, 2016–2024 (2007)

Roilides, E., Holmes, A., Blake, C., Pizzo, P. A., and Walsh, T. J.: Effects of granulocyte colony-stimulating factor and interferon-gamma on antifungal activity of human polymorphonuclear neutrophils against pseudohyphae of different medically important Candida species. J. Leukoc. Biol. *57*/4, 651–656 (1995)

Salmon, J. E., and Cronstein, B. N.: Fc gamma receptor-mediated functions in neutrophils are modulated by adenosine receptor occupancy. A1 receptors are stimulatory and A2 receptors are inhibitory. J. Immunol. *145*/7, 2235–2240 (1990)

Sitkovsky, M. V., Lukashev, D., Apasov, S., Kojima, H., Koshiba, M., Caldwell, C., Ohta, A., and Thiel, M.: Physiological control of immune response and inflammatory tissue damage by hypoxia-inducible factors and adenosine A2A receptors. Annu. Rev. Immunol. *22*, 657–682 (2004)

Sprung, C. L., Annane, D., Benbenishty, J., Kalenka, A., Forst, H., Laterre, P. F., Reinhart, K., Cuthbertson, B. H., Payen, D., Briegel, J., and *CORTICUS Study Group*: Hydrocortisone therapy for patients with septic shock. New Engl. J. Med. *358*/2, 111–124 (2008)

Suffredini, A. F., and Munford, R. S.: Novel therapies for septic shock over the past 4 decades. JAMA *306*/2, 194–199 (2011)

Synnestvedt, K., Furuta, G. T., Comerford, K. M., Louis, N., Karhausen, J., Eltzschig, H. K., Hansen, K. R., Thompson, L. F., and Colgan, S. P.: Ecto-5'-nucleotidase (CD73) regulation by hypoxia-inducible factor-1 mediates permeability changes in intestinal epithelia HIF-dependent induction of adenosine A2B receptor in hypoxia. J. Clin. Invest. *110*/7, 993–1002 (2002)

Tamura, D. Y., Moore, E. E., Partrick, D. A., Johnson, J. L., Offner, P. J., and Silliman, C. C.: Acute hypoxemia in humans enhances the neutrophil inflammatory response. Shock *17*/4, 269–273 (2002)

Tennenberg, S. D., Fey, D. E., and Lieser, M. J.: Oxidative priming of neutrophils by interferon-gamma. J. Leukoc. Biol. *53*/3, 301–308 (1993)

Thiel, M., Caldwell, C. C., Kreth, S., Kuboki, S., Chen, P., Smith, P., Ohta, A., Lentsch, A. B., Lukashev, D., and Sitkovsky, M. V.: Targeted deletion of HIF-1alpha gene in T cells prevents their inhibition in hypoxic inflamed tissues and improves septic mice survival. PLoS One *2*/9, e853 (2007)

Thompson, L. F., Eltzschig, H. K., Ibla, J. C., Van De Wiele, C. J., Resta, R., Morote-Garcia, J. C., and Colgan, S. P.: Crucial role for ecto-5'-nucleotidase (CD73) in vascular leakage during hypoxia. J. Exp. Med. *200*/11, 1395–1405 (2004)

Tracey, K. J.: Reflex control of immunity. Nature Rev. Immunol. *9*/6, 418–428 (2009)

Turgeon, A. F., Hutton, B., Fergusson, D. A., Mc Intyre, L., Tinmouth, A. A., Cameron, D. W., and Hébert, P. C.: Meta-analysis: intravenous immunoglobulin in critically ill adult patients with sepsis. Ann. Intern. Med. *146*/3, 193–203 (2007)

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Nachhaltigkeit in der Wissenschaft

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Im Mittelpunkt der weltweiten Überlegungen zur Bewältigung zentraler Herausforderungen des 21. Jahrhunderts steht das Konzept der Nachhaltigkeit. Damit dieses Prinzip sich in konkreten Handlungsvorschlägen widerspiegeln kann, bedarf es der Präzisierung. Der Band untersucht daher die Nachhaltigkeit in der Wissenschaft, der wichtigsten Informationsquelle der Gesellschaft. Dabei wird Nachhaltigkeit sowohl der Strukturen als auch der Aktivitäten in Forschung und Lehre betrachtet. Behandelt werden die „Erforschung von Nachhaltigkeit", die Strategien zum besseren Verständnis liefern soll, der Komplex „nachhaltig forschen", der Voraussetzungen, Verläufe und Folgen von Forschung gemäß den Kriterien der Nachhaltigkeit analysiert, und die „Nachhaltigkeit von Forschung", die Wesensprinzipien der Wissenschaft – etwa die Falsifizierbarkeit ihrer Resultate – im Lichte der Idee der Nachhaltigkeit untersucht. Schwerpunkte der Analyse bilden in allen Bereichen einerseits das Spannungsverhältnis zwischen Freiheit und Nachhaltigkeit der Wissenschaft sowie andererseits die Auswirkungen der Debatte auf die Strukturen des Wissenschaftssystems.

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Fluid Resuscitation in Sepsis – What, When and How (Much)?

Martin WESTPHAL (Münster)

Abtract

Fluid resuscitation represents the most important part of supportive therapy in patients with severe sepsis and septic shock. Despite being in the focus of research for many years, the "what, when and how (much)" of fluid therapy are still discussed controversially. The fact that not all clinical recommendations are supported by strong evidence should not be misinterpreted as "evidence of absence". In daily practice, fluid resuscitation should be "physiology-based" and "evidence-supported". This aim may be best reached by a demand-oriented therapy taking dynamic preload parameters and treatment protocols into account, whenever possible. Currently, there is no evidence that bolus administration is beneficial in septic patients.

Zusammenfassung

Die Volumenersatztherapie stellt den wichtigsten Teil der supportiven Therapie von Patienten mit schwerer Sepsis und septischem Schock dar. Obwohl sie seit Jahren im Fokus der Forschung steht, wird die Volumentherapie hinsichtlich „was, wann und wie (viel)" kontrovers diskutiert. Die Tatsache, dass nicht alle klinischen Empfehlungen Evidenz-basiert sind, sollte jedoch nicht als „Evidenz der Abwesenheit" fehlinterpretiert werden. In der täglichen Praxis sollte die Infusionstherapie „Physiologie-basiert" und „Evidenz-unterstützt" sein. Dieses Ziel kann am besten durch eine bedarfsorientierte Therapie erzielt werden, die dynamische Vorlastparameter und Behandlungsprotokolle berücksichtigt. Gegenwärtig gibt es keine Evidenz, dass Bolusgaben bei Sepsispatienten vorteilhaft sind.

1. Introduction

Severe sepsis and septic shock belong to the most common causes of death in intensive care units (LEVY et al. 2010). Therefore, early and adequate therapy is of utmost importance to optimise patients' outcome.

Since fluid resuscitation plays a pivotal role in supportive therapy of hemodynamically unstable septic patients, guidelines and algorithms on the "best therapeutic approach" are warranted. Due to the controversies concerning best practice in fluid therapy, however, there is no universal recommendation that fits all clinical conditions. This article is not exhaustive, but seeks to briefly summarise the principles of fluid therapy. In this regard, there is a special focus on the available solutions, the way of administration and the timing to optimise fluid therapy in sepsis.

2. What?

Crystalloids and colloids are the relevant infusion solutions available for fluid resuscitation. The advantage of crystalloid solutions is the absence of substance-specific side-effects, though

at the expense of the actual volume effect, which is approximately 20 % in the presence of an intact vascular barrier (ERTMER et al. 2011).

Human albumin and hydroxyethyl starches (*HES*) are the relevant representatives of the natural and synthetical colloids, respectively. While albumin may be safe in intensive care patients – except for patients with traumatic brain injury (FINFER et al. 2004) – 3rd generation starches (6 % HES 130/0.4) represent the most often used colloids in critically ill adults (FINFER et al. 2010). Colloids are characterised by a better volume effect as compared to crystalloids. However, the exact ratio of crystalloid: colloid needed for hemodynamic stabilisation in critically ill patients has been reported to being lower as compared to patients with an intact vascular barrier. The question, whether or not colloids may contribute to renal failure in septic patients is discussed controversially (BOUSSEKEY et al. 2010, DUBIN et al. 2010, BAYER et al. 2011). For a correct clinical judgment, consideration of (contra-) indications, dose-limits and physiological principles seems to be of paramount importance. Crucial information on the safety of 6 % HES 130/0.4 will soon be available from the CHEST study, evaluating the role of waxy maize-derived starch (*Voluven®*) *versus* normal saline in 7000 critically ill intensive care unit patients.

To date, the Surviving Sepsis Campaign Guidelines recommend using crystalloids or artificial/natural colloids for fluid resuscitation, because of "no evidence-based support for one type of fluid over another (*grade 1B*)" (DELLINGER et al. 2008).

3. When?

Fluid resuscitation in septic patients should be adequate and demand-oriented. Since fluid overload is associated with a poor outcome (PAYEN et al. 2008, BOYD et al. 2011), infusion therapy should be guided by protocols in order to avoid a positive fluid balance, whenever possible. Though still recommended in the Surviving Sepsis Guidelines, several studies have shown that central venous pressure (*CVP*) is not a reliable marker of the fluid status in septic patients (BRISMAN et al. 1967, BOYD et al. 2011). Therefore, a fluid therapy based on "old" parameters such as CVP and central venous oxygenation ($ScvO_2$) may potentially miss the "real" fluid demand of the patient. Dynamic preload parameters (i.e. stroke volume variation [*SSV*], pulse pressure variation [*PPV*], global end diastolic index [*GEDI*]) have proven to correlate well with the patients' fluid status, even under septic conditions (BROCH et al. 2011). However, more data are needed before a final, evidence-based conclusion can be made.

4. How (Much)?

In the daily clinical routine, fluids are often administered as a bolus infusion. This approach, however, should be questioned due to potentially harmful effects. In this context, MAITLAND et al. (2011) have recently reported a significant increased mortality in children with severe infection who received fluid boluses of saline or albumin as compared with controls having not received a bolus but just a continuous infusion. Further studies are now needed to evaluate the mechanisms, by which bolus infusions may contribute to clinically unwanted effects. In addition, it has to be clarified, if the data from the MAITLAND study can be transferred to other patient populations.

When reviewing the current literature on this topic, it appears that maintenance of fluid balance is of utmost importance. This includes a close monitoring of fluid input/output in the context of the patient's demand. Standard operating procedures and validated infusion protocols may provide a helpful orientation in this regard. However, fluid therapy should – of course – take individual needs into consideration. In view of patients with a systemic inflammatory response, an aggressive fluid therapy may be indicated, as long as a vasopressor support is needed. Afterwards, a more restrictive approach (including the use of diuretics) seems to be promising (WIEDEMANN et al. 2006). As mentioned by RIVERS "Early liberal – late conservative" may be the way to go (RIVERS 2006). Apart from this, fluid resuscitation should be re-evaluated, when the maximum dose of a specific compound has been given. Although crystalloids do not have an explicit recommended maximum dose, a close surveillance of the fluid status is necessary in order to avoid extravascular oedema (BRANDSTRUP et al. 2003).

5. Conclusion

Fluid resuscitation in patients with sepsis and septic shock still remains a challenge for the intensive care physician. Since one size does not fit all, every patient should be evaluated individually. As a guiding principle, fluid resuscitation in sepsis should be early, adequate and demand-oriented. In-house protocols and working instructions are desirable to achieve this goal. Since fluid therapy should take physiological principles into consideration and being evidence-based rather than "eminence-based", old dogmas should be challenged regularly. May a re-validation not be possible, a careful adaptation should be taken into consideration. In the absence of reliable evidence, "absence of evidence" should not be misinterpreted as "evidence of absence" (JACOB et al. 2011).

References

BAYER, O., REINHART, K., SAKR, Y., KABISCH, B., KOHL, M., RIEDEMANN, N. C., BAUER, M., SETTMACHER, U., HEKMAT, K., and HARTOG, C. S.: Renal effects of synthetic colloids and crystalloids in patients with severe sepsis: a prospective sequential comparison. Crit. Care Med. *39*/6, 1335–1342 (2011)

BOUSSEKEY, N., DARMON, R., LANGLOIS, J., ALFANDARI, S., DEVOS, P., MEYBECK, A., CHICHE, A., GEORGES, H., and LEROY, O.: Resuscitation with low volume hydroxyethylstarch 130 kDa/0.4 is not associated with acute kidney injury. Crit. Care *14*/2, R40 (2010)

BOYD, J. H., FORBES, J., NAKADA, T. A., WALLEY, K. R., and RUSSELL, J. A.: Fluid resuscitation in septic shock: a positive fluid balance and elevated central venous pressure are associated with increased mortality. Crit. Care Med. *39*/2, 259–265 (2011)

BRANDSTRUP, B., TØNNESEN, H., BEIER-HOLGERSEN, R., HJORTSØ. E., ØRDING, H., LINDORFF-LARSEN, K., RASMUSSEN, M. S., LANNG, C., WALLIN, L., IVERSEN, L. H., GRAMKOW, C. S., OKHOLM, M., BLEMMER, T., SVENDSEN, P. E., ROTTENSTEN, H. H., THAGE, B., RIIS, J., JEPPESEN, I. S., TEILUM, D., CHRISTENSEN A. M., GRAUNGAARD, B., POTT, F., and *Danish Study Group on Perioperative Fluid Therapy*: Effects of intravenous fluid restriction on postoperative complications: comparison of two perioperative fluid regimens: a randomized assessor-blinded multicenter trial. Ann. Surg. *238*/5, 641–648 (2003)

BRISMAN, R., PARKS, L. C., and BENSON, D. W.: Pitfalls in the clinical use of central venous pressure. Arch. Surg. *95*/6, 902–907 (1967)

BROCH, O., RENNER, J., GRUENEWALD, M., MEYBOHM, P., HÖCKER, J., SCHÖTTLER, J., STEINFATH, M., and BEIN, B.: Variation of left ventricular outflow tract velocity and global end-diastolic volume index reliably predict fluid responsiveness in cardiac surgery patients. J. Crit. Care *27*/3, 325e7–13 (2011)

Dellinger, R. P., Levy, M. M., Carlet, J. M., Bion, J., Parker, M. M., Jaeschke, R., Reinhart, K., Angus, D. C., Brun-Buisson, C., Beale, R., Calandra, T., Dhainaut, J. F., Gerlach, H., Harvey, M., Marini, J. J., Marshall, J., Ranieri, M., Ramsay, G., Sevransky, J., Thompson, B. T., Townsend, S., Vender, J. S., Zimmerman, J. L., Vincent, J. L., *International Surviving Sepsis Campaign Guidelines Committee*, *American Association of Critical-Care Nurses*, *American College of Chest Physicians*, *American College of Emergency Physicians*, *Canadian Critical Care Society*, *European Society of Clinical Microbiology and Infectious Diseases*, *European Society of Intensive Care Medicine*, *European Respiratory Society*, *International Sepsis Forum*, *Japanese Association for Acute Medicine*, *Japanese Society of Intensive Care Medicine*, *Society of Critical Care Medicine*, *Society of Hospital Medicine*, *Surgical Infection Society*, and *World Federation of Societies of Intensive and Critical Care Medicine*: Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. Crit. Care Med. *36*/1, 296–327 (2008)

Dubin, A., Pozo, M. O., Casabella, C. A., Murias, G., Pálizas, F. Jr., Moseinco, M. C., Kanoore Edul, V. S., Pálizas, F., Estenssoro, E., and Ince, C.: Comparison of 6 % hydroxyethyl starch 130/0.4 and saline solution for resuscitation of the microcirculation during the early goal-directed therapy of septic patients. J. Crit. Care *25*/4, 659 e651–658 (2010)

Ertmer, C., Kampmeier, T., Rehberg, S., and Lange, M.: Fluid resuscitation in multiple trauma patients. Curr. Opin. Anaesthesiol. *24*/2, 202–208 (2011)

Finfer, S., Bellomo, R., Boyce, N., French, J., Myburgh, J., Norton, R., and *SAFE Study Investigators*: A comparison of albumin and saline for fluid resuscitation in the intensive care unit. New Engl. J. Med. *350*/22, 2247–2256 (2004)

Finfer, S., Liu, B., Taylor, C., Bellomo, R., Billot, L., Cook, D., Du, B., McArthur, C., Myburgh, J., and *SAFE TRIPS Investigators*: Resuscitation fluid use in critically ill adults: an international cross-sectional study in 391 intensive care units. Crit. Care *14*/5, R185 (2010)

Jacob, M., Chappell, D., and Rehm, M.: Perioperative fluid administration: another form of "work-life balance". Anesthesiology *114*/3, 483–484 (2011)

Levy, M. M., Dellinger, R. P., Townsend, S. R., Linde-Zwirble, W. T., Marshall, J. C., Bion, J., Schorr, C., Artigas, A., Ramsay, G., Beale, R., Parker, M. M., Gerlach, H., Reinhart, K., Silva, E., Harvey, M., Regan, S., and Angus, D. C.: The Surviving Sepsis Campaign: results of an international guideline-based performance improvement program targeting severe sepsis. Intensive Care Med. *36*/2, 222–231 (2010)

Maitland, K., Kiguli, S., Opoka, R. O., Engoru, C., Olupot-Olupot, P., Akech, S. O., Nyeko, R., Mtove, G., Reyburn, H., Lang, T., Brent, B., Evans, J. A., Tibenderana, J. K., Crawley, J., Russell, E. C., Levin, M., Babiker, A. G., Gibb, D. M., and *FEAST Trial Group*: Mortality after fluid bolus in African children with severe infection. New Engl. J. Med. *364*/26, 2483–2495 (2011)

Payen, D., Pont, A. C. de, Sakr, Y., Spies, C., Reinhart, K., Vincent, J. L., and *Sepsis Occurrence in Acutely Ill Patients (SOAP) Investigators*: A positive fluid balance is associated with a worse outcome in patients with acute renal failure. Crit. Care *12*/3, R74 (2008)

Rivers, E. P.: Fluid-management strategies in acute lung injury—liberal, conservative, or both? New Engl. J. Med. *354*/24, 2598–2600 (2006)

Wiedemann, H. P., Wheeler, A. P., Bernard, G. R., Thompson, B. T., Hayden, D., Boisblanc, B. de, Connors, A. F. Jr., Hite, R. D., Harabin, A. L., and *National Heart, Lung, and Blood Institute Acute Respiratory Distress Syndrome (ARDS) Clinical Trials Network*: Comparison of two fluid-management strategies in acute lung injury. New Engl. J. Med. *354*/24, 2564–2575 (2006)

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Corticosteroids in the Treatment of Sepsis (Extended Abstract)

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The crucial role of endogenous corticosteroids in the organism's dynamic response to stress has long been recognised. Sepsis represents a specific stressful condition with the double burden of an overshooting inflammatory response due to invading microorganisms and a concomitant interference with corticosteroid synthesis, metabolism, and action at the tissue level. The frequently resulting syndrome has been called critical illness-related corticosteroid insufficiency (*CIRCI*) (Cooper and Stewart 2008, Marik et al. 2003).

First, as a general response to infection cortisol secretion is increased, mainly due to the activation of the hypothalamic-pituitary-adrenal (*HPA*) axis by cytokines as tumour necrosis factor α, interleukin 1 and interleukin 6. Moreover, in critically ill patients, the responsiveness of the HPA axis to corticotropin-releasing hormone (*CRH*) and corticotropin (*ACTH*) is amplified, and a loss of the negative feedback regulation of cortisol on the HPA axis can be assumed. The resulting hypercortisolemia, a typical feature of the acute phase of critical illness, may be further increased by a decreased hepatic clearance of cortisol. In addition, diminished binding to carrier proteins can contribute to an increase in free cortisol concentration (Vanhorebeek et al. 2006).

In the chronic phase of septic shock steroidogenesis is compromised. Several inflammatory mediators such as tumour necrosis factor, transforming growth factor β, corticostatins and other peptides have been identified to suppress cortisol production in response to ACTH in a dose-dependent manner. Also, septic shock plasma has been shown to attenuate the corticosterone production of rat adrenocortical cells in response to corticotropin stimulation. These findings may explain the progressive decline in cortisol production following endotoxin challenge in animal models, as well as a progressive dissociation of the HPA axis observed in septic patients during the course of illness (Annane et al. 2006, Arafah 2006, Koo et al. 2001). It has been recently shown by MRI imaging that reduced adrenal gland enlargement during septic shock is associated with higher mortality (Jung et al. 2011).

For the diagnosis of CIRCI, a major point of debate has been the appropriate cutoff value for serum cortisol levels and especially serum cortisol levels after corticotropin stimulation. The assignment to responders or non-responders to corticotropin depends on the time point of testing as well as on the method for cortisol determination (Briegel et al. 2009). Furthermore, interfering drugs and long-term use of corticosteroids may hinder accurate diagnosis (Mesotten et al. 2008).

In addition, it has become increasingly acknowledged that corticosteroid insufficiency does not only depend on HPA axis dysfunction, but also on numerous cellular disease mechanisms in effector tissues. Several studies reveal desensitisation of peripheral glucocorticoid receptors in septic shock. The number of cytosolic glucocorticoid receptors in leukocytes has been shown to decrease progressively during septic shock (MOLIJN et al. 1995). Similarly, in patients with septic shock, a reduction of the number of glucocorticoid receptors correlating with mean arterial blood pressure has been observed. Also, there is considerable evidence that patients with inflammatory diseases such as rheumatoid arthritis or asthma acquire glucocorticoid resistance (ADCOCK and BARNES 2007). *In vitro*, high local concentrations of cytokines are known to reduce the sensitivity to glucocorticoids. Cytokines can cause glucocorticoid resistance by activating transcription factors, which can bind with activated glucocorticoid receptors, thus reducing the number of effective glucocorticoid receptors. Desensitised target cells lead to a reduction of the anti-inflammatory effects of endogenous corticosteroids.

An important factor in alterations of the sensitivity of the glucocorticoid is the balance of the receptor subtypes α and β (GROSS et al. 2009, RHEN and CIDLOWSKI 2005). The α isoform of the receptor dimerizes after binding to glucocortiods in the cytosol, then translocates in the nucleus and is able to exert the typical glucocorticoid effects mainly by binding to glucocorticoid responsive elements in the genome. The β isoform, which is found in a relatively low concentration, is unable to bind to glucocorticoids but has a dominant negative effect on the α form (GROSS and CIDLOWSKI 2008). Consequently, mechanisms associated with alterations in the balance between the two isoforms may have significant impact on acquired glucocorticoid resistance. A focus of the work of our research group lies on deciphering underlying molecular mechanisms for this phenomenon, with special emphasis on regulation of glucocorticoid receptors by microRNA. We were able to demonstrate a crucial role of mir-124 in the pathophysiology of corticosteroid resistance in sepsis: increased expression of mir-124 in T cells, induced by steroid treatment, downregulates glucocorticoid receptor-α and hereby limits the anti-inflammatory effects of glucocorticoids (LEDDEROSE et al. 2012).

The rational in corticosteroid therapy in sepsis is twofold: First, of major significance in the first years and decades and especially in high-dose approaches, the anti-inflammatory potentials serve to counteract hyperinflammation. Second, which is increasingly recognised as an important factor especially in low-dose therapy, the administration of corticosteroids serves as a substitution in a state of diminished endogenous corticosteroid effects. Historically, high-dose glucocorticoid treatment for sepsis (e.g. methylprednisolone in a dose of 30 mg/kg body weight) failed to show overall benefit and was potentially harmful.

The term stress dose of hydrocortisone corresponds to the maximum production rate of cortisol measured in humans. This is about 10 to 20 times the production rate of cortisol in unstressed individuals and ranges between 150 and 300 mg a day. Corresponding with other inflammatory conditions as asthma, it can be hypothesised that corticosteroid in equivalent to stress doses, e. g. doses of about 40 mg prednisolone (approximately equivalent to 200 mg hydrocortisone) may be adequate to surmount the disturbances in glucocorticoid responsiveness as described above. However, large-scale clinical trials have not clearly shown an overall benefit of corticosteroid therapy in septic patients. With respect to available data from theses trials, very sick patients with septic shock unresponsive to fluid and vasopressor therapy benefit from hydrocortisone (ANNANE et al. 2002, SPRUNG et al. 2008). New methods to rapidly assess tissue steroid sensitivity may identify better the appropriate target group of patients who will benefit from steroid treatment.

References

ADCOCK, I. M., and BARNES, P. J.: Molecular mechanisms of corticosteroid resistance. Chest *134*/2, 394–401 (2007)

ANNANE, D., MAXIME, V., IBRAHIM, F., ALVAREZ, J. C., ABE, E., and BOUDOU, P.: Diagnosis of adrenal insufficiency in severe sepsis and septic shock. Amer. J. Respirat. Crit. Care Med. *174*/12, 1319–1326 (2006)

ANNANE, D., SEBILLE, V., CHARPENTIER, C., BOLLAERT, P.-E., FRANÇOIS, B., KORACH, J.-M., CAPELLIER, G., COHEN, Y., AZOULAY, E., TROCHÉ, G., CHAUMET-RIFFAUD, P., and BELLISSANT, E.: Effect of treatment with low doses of hydrocortisone and fludrocortisone on mortality in patients with septic shock. JAMA *288*/7, 862–871 (2002)

ARAFAH, B. M.: Hypothalamic pituitary adrenal function during critical illness: Limitations of current assessment methods. J. Clin. Endocrinol. Metab. *91*/10, 3725–3745 (2006)

BRIEGEL, J., SPRUNG, C. L., ANNANE, D., SINGER, M., KEH, D., MORENO, R., MÖHNLE, P., WEISS, Y., AVIDAN, A., BRUNKHORST, F. M., FIEDLER, F., VOGESER, M., and *CORTICUS Study Group*: Multicenter comparison of cortisol as measured by different methods in samples of patients with septic shock. Intensive Care Med. *35*/12, 2151–2156 (2009)

COOPER, M. S., and STEWART, P. M.: Corticosteroid insufficiency in acutely ill patients. New Engl. J. Med. *348*/8, 727–734 (2003)

GROSS, K. L., and CIDLOWSKI, J. A.: Tissue-specific glucocorticoid action: a family affair. Trends Endocrinol. Metab. *19*/9, 331–339 (2008)

GROSS, K. L., LU, N. Z., and CIDLOWSKI, J. A.: Molecular mechanisms regulating glucocorticoid sensitivity and resistance. Mol. Cell Endocrinol. *300*/1–2, 7–16 (2009)

JUNG, B., NOUGARET, S., CHANQUES, G., MERCIER, G., CISSE, M., AUFORT, S., et al.: The absence of adrenal gland enlargement during septic shock predicts mortality: a computed tomography study of 239 patients. Anesthesiology *115*/2, 334–343 (2011)

KOO, D. J., JACKMAN, D., CHAUDRY, I. H., and WANG, P.: Adrenal insufficiency during the late stage of polymicrobial sepsis. Crit. Care Med. *29*/3, 618–622 (2001)

LEDDEROSE, C., MÖHNLE, P., LIMBECK, E., SCHÜTZ, S., WEIS, F., RINK, J., BRIEGEL, J., and KRETH, S.: Corticosteroid resistance in sepsis is influenced by microRNA-124-induced downregulation of glucocorticoid recepter-α. Crit. Care Med. *40*/10, 2745–2753 (2012)

MARIK, P. E., PASTORES, S. M., ANNANE, D., MEDURI, G. U., SPRUNG, C. L., ARLT, W., KEH, D., BRIEGEL, J., BEISHUIZEN, A., DIMOPOULOU, I., TSAGARAKIS, S., SINGER, M., CHROUSOS, G. P., ZALOGA, G., BOKHARI, F., and VOGESER, M.: Recommendations for the diagnosis and management of corticosteroid insufficiency in critically ill adult patients: Consensus statements from an international task force by the American College of Critical Care Medicine. Crit. Care Med. *36*/6, 1937–1949 (2008)

MESOTTEN, D., VANHOREBEEK, I., and VAN DEN BERGHE, G.: The altered adrenal axis and treatment with glucocorticoids during critical illness. Nat. Clin. Pract. Endocrinol. Metab. *4*/9, 496–505 (2008)

MOLIJN, G. J., SPEK, J. J., VAN UFFELEN, J. C., DE JONG, F. H., BRINKMANN, A. O., BRUINING, H. A., et al.: Differential adaptation of glucocorticoid sensitivity of peripheral blood mononuclear leukocytes in patients with sepsis or septic shock. J. Clin. Endocrinol. Metab. *80*/6, 1799–1803 (1995)

RHEN, T., and CIDLOWSKI, J. A.: Antiinflammatory action of glucocorticoids – new mechanisms for old drugs. New Engl. J. Med. *353*/16, 1711–1723 (2005)

SPRUNG, C. L., ANNANE, D., KEH, D., MORENO, R., SINGER, M., FREIVOGEL, K., WEISS, Y. G., BENBENISHTY, J., KALENKA, A., FORST, H., LATERRE, P. F., REINHART, K., CUTHBERTSON, B. H., PAYEN, D., BRIEGEL, J., and *CORTICUS Study Group*: Hydrocortisone therapy for patients with septic shock. New Engl. J. Med. *358*/2, 111–124 (2008)

VANHOREBEEK I., PEETERS, R. P., VANDER PERRE, S., JANS, I., WOUTERS, P. J., SKOGSTRAND, K., et al.: Cortisol Response to Critical Illness: Effect of Intensive Insulin Therapy. J. Clin. Endocrinol. Metab. *91*/10, 3803–3813 (2006)

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Forschung und Verantwortung im Konflikt?

Ethische, rechtliche und ökonomische Aspekte der Totalsequenzierung des menschlichen Genoms

Symposium
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Die Möglichkeit, das komplette Genom von Individuen zu sequenzieren, ist seit einigen Jahren vorhanden und wird stetig weiterentwickelt. Damit werden nicht nur neue Diagnose- und Therapieformen zukünftig verfügbar, sondern es gelangen auch gesellschaftliche Probleme und neue ethische Fragen in den Fokus. Die ethischen und rechtlichen Aspekte der Totalsequenzierung des menschlichen Genoms bedürfen vor diesem Hintergrund einer wohldurchdachten interdisziplinären Auseinandersetzung. Die Beiträge des Bandes beleuchten den Konflikt zwischen Forschungsdynamik, ärztlichem Handeln und Patientenversorgung aus verschiedenen Perspektiven und wollen Orientierungspunkte für die aktuelle Debatte sein. Das Spektrum der Ansätze reicht von gesundheitsökonomischen und gesellschaftswissenschaftlichen Überlegungen über Regulierungsfragen bis zu grundlegenden ethischen Reflexionen.

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Treatment of Sepsis – Challenges and New Concepts (Extended Abstract)

Georg PETERS (Münster)

Sepsis is one of the most important infectious diseases, although not really recognised as such. It is estimated that about 60,000 deaths are caused by sepsis in Germany every year. Therefore, sepsis is one of the greatest challenges to clinicians as well as to scientists. Today it is proven by many well performed studies that time is most important: The earlier the intervention by antibiotic therapy and possibly necessary surgical procedures is started the better is the outcome. Thus, early diagnostic results leading to adequate antibiotic therapy are required. Here several measures of host responses (PMN-count, procalcitonin) as well as microbiological procedures (blood cultures, PCR based detection systems) have significantly shortened the interval between clinical suspicion and diagnosis of sepsis by final prove of the causative infectious agent and its antibiotic susceptibility. But still, much more improvement is necessary. A major general problem is also effecting efficacious treatment of sepsis: the enormous increase of antibiotic resistant bacteria. Over the last three decades one had to face the devastating increase of methicillin resistant *Staphylococcus aureus* (*MRSA*) and other staphylococci as well as the appearance of glycopeptide-resistant enterococci. While these multiresistant Gram-positive bacteria are still on a high prevalence level we are confronted with a dramatic increase of multiresistant Gram-negative bacteria: *Enterobacteriaceae* like *E. coli*, *Klebsiella* and *Enterobacter* spec., but also *Pseudomonas*, *Acinetobacter* and other non-fermenters are becoming resistant towards beta-lactams including fourth-generation-cephalosporines and carbapenemes, mainly due to the occurrence of so-called extended spectrum beta-lactamases (*ESBLs*). Very prominent here are serine-carbapenemases (*KPC*) and a brought range of metallo-beta-lactamases, for which the coding genetic material – mainly on plasmids – is spreading globally. Especially in *Pseudomonas* we are already facing an increasing number of strains which are only susceptible for polymyxins (cationic detergents), very old antibiotics with a brought and frequently occurring spectrum of possible side effects. Thus, this group of antibiotics has to be revisited, because they offer "Last Chance". And even worse, there are *Pseudomonas aeruginosa* clones which are also polymyxin-resistant, that means they show pan-resistance, no available antibiotic is working any more. Unfortunately, this is paralleled by the fact that basic research and clinical development of new antibiotics in pharmaceutical companies has steadily slowed down: There are only very few new substances to be expected in the future. This holds especially for substances active against multiresistant Gram-negative bacteria.

Furthermore, modern challenges in sepsis therapy are originating from increasing knowledge, that pharmacokinetics and – thus following pharmacodynamics – of antibiotics is very diverse in septic intensive care patients compared to normal or non-ICU patients. This concerns dosing of the antibiotic as well as duration of therapy. This may finally lead to a very individualised type of treatment which than requires a much more extensive monitoring (drug levels) which of course leads to much higher costs in sepsis treatment.

Finally, it has to be emphasised that research in microbial pathogenesis and bacteria-host interaction also leads to the requirement of a more individualised diagnosis and treatment regarding the special type of the bacterium causing sepsis in the individual patient. As an example, several strains of *S. aureus* produce a highly active toxin (PVL, *Panton-Valentine Toxin*) which in very low concentration rapidly and effectively kills human neutrophils. This may hold true for other toxins of bacteria, too. Another example in *S. aureus*: Several strains are able to invade epithelial and endothelial cells, change their phenotype inside the cell towards a non-toxic state. Inside their host cell they are protected against immune responses and most antibiotics and can thus persist for a long time. From there they can re-switch, evade and thus cause a relapse of sepsis.

Reference

Burkhardt, O., Kumar, V., Katterwe, D., Majcher-Peszynska, J., Drewelow, B., Derendorf, H., and Welte, T.: Ertapenem in critically ill patients with early-onset ventilator-associated pneumonia: pharmacokinetics with special consideration of free-drug concentration. J. Antimicrob. Chemother *59*/2, 277–284 (2007)

Kumar, A., Roberts, D., Wood, K. E., Light, B., Parrillo, J. E., Sharma, S., Suppes, R., Feinstein, D., Zanotti, S., Taiberg, L., Gurka, D., Kumar, A., and Cheang, M.: Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit. Care Med. *34*/6, 1589–1596 (2006)

Löffler, B., Hussain, M., Grundmeier, M., Brück, M., Holzinger, D., Varga, G., Roth, J., Kahl, B. C., Proctor, R. A., and Peters, G.: *Staphylococcus aureus* panton-valentine leukocidin is a very potent cytotoxic factor for human neutrophils. PLoS Pathog. *6*/1, e1000715 (2010)

Rogers, B. A., Sidibat, H. E., and Paterson, D. L.: *Escherichia coli* O25b-ST131: a pandemic, multiresistant, community-associated strain. J. Antimicrob. Chemother. *66*/1, 1–14 (2011)

Tuchscherr, L., Medina, E., Hussain, M., Völker, W., Heitmann, V., Niemann, S., Holzinger, D., Roth, J., Proctor, R. A., Becker, K., Peters, G., and Löffler, B.: *Staphylococcus aureus* phenotype switching: an effective bacterial strategy to escape host immune response and establish a chronic infection. EMBO Mol. Med. *3*/3, 129–141 (2011)

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