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Molecular mediators of polymicrobial sepsis

Sandra Wintersteller[1], Jens Hahnhaussen[2], Barbara Kofler[1], Klaus Emmanuel[2]

[1]Department of Pediatrics, Laura Bassi Centre of Expertise, THERAPEP, Research Program for Receptor Biochemistry and Tumor Metabolism, Paracelsus Medical University, A-5020 Salzburg, AUSTRIA, [2]Department of Surgery, Paracelsus Medical University, A-5020 Salzburg, AUSTRIA

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1. ABSTRACT

Sepsis is still a major cause of postoperative morbidity and mortality. Numerous biochemical indicators have been evaluated regarding their potential in predicting prognosis in sepsis. Generally, one must differentiate between indicators: those for preoperative risk of lethal sepsis, those for early prediction of lethal outcome and those for evaluating effectiveness of therapy. In the past, immunomodulatory therapies developed in various animal studies failed to be successful in humans. It has been proposed that present models have to be reevaluated, and new, clinically more relevant models should be evolved. This article will give a short overview on the most common animal models and a comprehensive overview on markers for sepsis in animal models and clinical studies. The focus will be on abdominal sepsis with a mortality rate up to 80% after major surgery. Two animal models designed to closely mimic the clinical course of intra-abdominal sepsis, will be compared. Furthermore, relevant clinical parameters for predicting prognosis before and after major visceral surgery are illustrated.

2. INTRODUCTION

Despite more than 20 years of extensive research and development of numerous therapeutic approaches used in clinical settings, the incidence of sepsis and, most notably, the number of sepsis-related deaths are rising (1, 2). Despite the existence of general criteria, there is still no consensus about the definition of sepsis onset and the classification of sepsis severity in different hospitals. This is one of the problems in attempting to compare the results of different clinical studies of sepsis. In addition, a current major problem is the heterogeneity of sepsis patients enrolled in clinical studies. Studies have frequently compared septic complications resulting from conditions as diverse as pneumonia, peritonitis, soft tissue infection, burn injury. However, the pathogenesis of sepsis may differ markedly among these groups owing to differences in the cause of the infection, the pathogen spectrum (mono- or poly-microbial), underlying co-morbidity, or other patient characteristics. It therefore appears important to reduce the heterogeneity of sepsis patients in clinical investigations

and to focus on the immune and clinical conditions of a more homogeneous patient group to gain a thorough understanding of each particular type of sepsis. Only then we can be in a position to implement the most appropriate and efficacious therapy. New developments emerging from animal models that reflect the various etiologies of sepsis may point the way to future clinical therapeutic strategies (3).

The septic response is an exceedingly complex series of events involving inflammatory and anti-inflammatory processes, circulatory abnormalities, and cellular and humoral reactions (3-5). The diagnosis of sepsis and evaluation of its severity are complicated by the highly variable and non-specific signs and symptoms of sepsis (6). However, early diagnosis and classification of the severity of sepsis are very important because they increase the likelihood of a prompt and specific treatment (3, 7, 8).

Several animal models of sepsis are available, but frequently they are not translatable to clinical practice in humans. We will focus on abdominal sepsis, where good models are available and seem, at least in part, to be applicable to humans.

3. ANIMAL MODELS OF SEPSIS

The animal models used to investigate sepsis are very well established and, depending on the model, different types of septic conditions can be addressed (1, 9-19). First, we will give a short overview on the most common models and then a comprehensive overview on markers for sepsis. In addition, we compare data from the literature with our own data on a range of markers in the colon ascendens stent peritonitis (CASP) model of polymicrobial sepsis. We have chosen this model because it best resembles the conditions of human abdominal sepsis.

3.1. Inhalation of endotoxins

Bacterial lipopolysaccharide (LPS), the main biologically active component of endotoxin, causes several sepsis-like symptoms, but it represents only a single component of a bacterial stimulus. Moreover, there are major differences in the response to LPS, depending on the route of administration. Inhalation of LPS induces lung injury and respiratory distress syndrome, which are major parts of endotoxic sepsis syndrome and Gram-negative pneumonia, both marked by massive tissue infiltration of neutrophils and activation of alveolar macrophages. Although this model may not serve as an animal model for generalized sepsis, it is a good model to investigate lung injury, which is a major side complication during sepsis (20-24).

3.2. Injection of endotoxins

Injection/infusion of endotoxin or other bacterial cell-wall products is one of the most popular animal models of sepsis, especially administration of LPS. Depending on the site of injection, there are several differences in the response to the stimulus. For example, intraperitoneal (i.p.) injection of LPS causes a hyperdynamic cardiovascular response, whereas intravenous (i.v.) injection does not. Both modes of administration increase the concentration of several proinflammatory cytokines in the serum. Some of these are markers of disease severity and are also found in human patients, but the cytokine profile in the LPS model also shows several differences compared to humans. In particular, the peaks of cytokine release appear earlier in LPS-challenged animals compared to other animal models or human sepsis (1, 12, 25).

The effects of certain cytokines, including tumor necrosis factor alpha (TNF-alpha) and interleukin 1 (IL-1), on the outcome of sepsis were tested in LPS animal models. For example, in the LPS murine model it was possible to show a beneficial effect on sepsis by blocking TNF-alpha or IL-1 receptor with specific antibodies (26, 27). After these promising results, several clinical trials were conducted using TNF-alpha antibodies and IL-1 antagonists. However, TNF-alpha attenuation using a soluble receptor worked in just a small subgroup of the more seriously ill septic patients, although some patients showed some improvement from these treatments (28). IL-1 antagonists similarly failed to significantly reduce the mortality of sepsis, and the only beneficial effects were observed in patients with the highest risk of death (28-30). Failure of the clinical trials may be due to the fact that, in human septic patients and in the cecal ligation and puncture (CLP) animal model of sepsis (below), cytokines peak much later than in the LPS model, and it was shown that administration of TNF-alpha antibodies actually lowered the survival of CLP mice (31-33).

One major difference between animal models and humans is the response to LPS. Very low doses administered to human volunteers induced pathophysiological signs and symptoms similar to those of septic patients (34). In contrast, mice require a very high dose of LPS to develop septic shock (11, 34). LPS treatment is appropriate to characterize the response to a single bacterial component and to evaluate certain disease patterns, such as lung injury, but it fails to mimic the systemic effects of polymicrobial infections.

3.3. Bacteremia

Because LPS is the active component of endotoxin and not the whole live bacterium, several studies were performed using bolus infusion of aerobic bacterial species, mainly Escherichia coli (35). Bacterial infusion is able to introduce a reproducible infection caused by a single pathogen (13). Clinical relevance remains unproven, because high amounts of infused bacteria do not colonize and replicate *in vivo* in contrast to the clinical course of sepsis with its ongoing release of organisms from the septic focus (35). This animal model may not be appropriate for studying septic conditions, but it serves as a tool for investigating mechanisms of the immune response to pathogens (12, 34, 36).

3.4. Surgical models

Two murine models that mimic more closely human abdominal sepsis associated with polymicrobial infection and the systemic inflammatory response

syndrome (SIRS) are the cecal ligation and puncture (CLP) and the colon ascendens stent peritonitis (CASP) models (1, 12, 14, 15, 37-39).

3.4.1. Cecal ligation and puncture

The CLP model is one of the most widely used animal models of sepsis and was first described by Chaudry and colleagues in 1980 (36). The principle of this model is to induce a septic focus by disruption of the intestinal barrier via ligation of the cecum below the ileocecal valve followed by multiple punctures with a needle of defined diameter. Severity of sepsis and survival depend on the number of punctures and the size of the needle used. Clinical symptoms develop within 12-24 hours after surgery and the peaks of proinflammatory cytokines appear later than in the LPS model (14, 15, 37, 40, 41). Inspection of the abdominal situs 24 hours after CLP reveals clear signs of inflammation and a kind of small-bowel adherent loop, which seems to cover the lesions at the ligated cecum spontaneously. The remaining intestine appears to be quite unaffected. Additionally, the bacterial load in the liver, lungs and peritoneal lavage fluid never reaches the high colony forming units (CFU) counts observed in animals that undergo CLP with one puncture in the ligated cecum. Using two punctures leads to a steadily increasing bacterial load between 6 and 18 hours (15). These observations represent a major difference to the CASP model described below. In standard CLP models, antibiotic and fluid treatment are not continued, and only a single dose of antibiotics and a small amount of fluid is given immediately after surgery (13).

3.4.2. Colon ascendens stent peritonitis

CASP surgery mimics very closely the clinical course of diffuse peritonitis with different states of the disease, as occurs, for example, after major abdominal surgery in humans. Under general anesthesia the abdominal wall is opened through a midline incision, followed by exposure of the ascending colon. Afterwards, a venous catheter with a notch for fixing the stent is stitched through the antimesenteric wall into the lumen of the ascending colon. The inner needle of the stent is removed and the rest of the stent is cut. To ensure the right position, stool is milked from the cecum until a small drop appears. Afterwards the abdominal wall is closed by suturing (39). Sepsis is induced by placement of a stent in the colon ascendens leading to bacterial invasion of the peritoneal cavity and diffuse fecal peritonitis followed by multiple organ failure, septic shock and death. Similar to human septic patients, lung injury and renal failure are reported after CASP. The mortality rate after CASP surgery can be set to 100% by using a 14-gauge stent, and to 50% with an 18-gauge stent, depending on the aims of the study (15, 19, 38, 39, 42). In contrast to CLP surgery, macroscopic investigation of the abdominal cavity shows massive edema formation, vasodilation of intestinal vessels and paralysis of the intestine. The presence of a persistent septic focus after CASP surgery is indicated by a steadily increasing bacterial load in peritoneal lavage fluid, lungs, liver, kidneys and spleen. At 18 hours the CFU counts in these organs are more than 10-fold higher in the CASP model than in the CLP model. At 12 hours, the CFU counts in the lungs were found to be 200-fold higher in CASP as compared to CLP (15).

Comparing these two surgical models of sepsis, Maier *et al.* concluded that CLP serves as a model for intra-abdominal abscess formation, whereas CASP leads to diffuse peritonitis (15). Serum cytokines and bacterial cultures measured at different time points (6, 12, 18 hours) after CASP surgery indicate a steadily increasing polymicrobial systemic inflammation, whereas the levels are prominently increased in the CLP model (15). Therefore, we decided to perform an extensive analysis of known and potential novel markers of sepsis, including not only cytokines but also chemokines, chemokine receptors and neuropeptides.

4. MOLECULAR MEDIATORS IN DIFFERENT ANIMAL MODELS OF SEPSIS

In the literature, studies using animals typically measure protein levels in the plasma, whereas studies using tissue samples report changes in mRNA expression levels and/or protein concentrations.

In this review we give an extensive overview of a wide panel of cytokines, chemokines and neuropeptides (tables 2-5). We focus on the quantitative changes of these mediators in plasma and in lung tissue, because plasma levels are an important indicator of a systemic response and the lungs have a major role in sepsis-induced multiple organ failure. Data from the literature are compared to those of our present study, in which we analyzed the expression of chemokines, cytokines and a specific panel of neuropeptides 12 hours after the onset of polymicrobial sepsis induced by CASP surgery (tables 2-5, figures 1-3).

5. METHODS

5.1. CASP surgery

Female C57BL/6 mice, purchased from Charles River (Sulzfeld, Germany), were used at 8-12 weeks of age and a body weight of 20-25 g. Animals were allowed to recover for 7 days after arrival in a conventional animal facility with free access to food and water and a 12-hour light/dark cycle. All animal procedures were authorized by the Federal Government Department for Science and Research and performed in accordance with the Austrian animal protection law BGBL. I Nr. 162/2005. For the surgical procedure, animals were anesthetized by i.p. administration of ketamine (Ketasol 100 mg/ml, aniMedica GmbH Senden-Bosensell, Germany, 1.2 mg / 20 g bodyweight) and xylazine (Xylasol 20 mg/ml, Dr. E. Graeub AG, Bern, Swiss, 0.28 mg / 20 g bodyweight). For analgesia, 0.08 mg/kg buprenorphin (0.3 mg/ml, Temgesic, Essex Pharma, Munich, Germany) was administered subcutaneously (s.c.).

The CASP surgery was performed as described by Zantl *et al.* (39). Briefly, a notch was created 2 mm from the orifice of a 14 gauge venous catheter (BD Venflon, Helsingborg, Sweden). After laparotomy, the cecum, the terminal ileum and the ascending colon were exposed. At

Table 1. RT-Primers used for mRNA expression analysis of neuropeptides

Gene		Primer sequence	Product size
mRPL4	sense	5'- GTATGGCACTTGGCGGAAGG-3'	124bp
	antisense	5'- TGCTCGGAGGGCTCTTTGG-3'	
mHPRT	sense	5'- GTCCCAGCGTCGTGATTAGC-3'	138bp
	antisense	5'- GAGCAAGTCTTTCAGTCCTGTCC-3'	
mGalanin	sense	5'- ATGGCCAGGGGCAGCGTTAT-3'	264bp
	antisense	5'- AGAAACTCCATTATCGTGCG-3'	
mNPY	sense	5'- TCCGCTCTGCGACACTACATC-3'	152bp
	antisense	5'- TCCCATCACCACATGGAAGG-3'	
mSP	sense	5'- TTCCACTCAACTGTTTGCACAGG-3'	140bp
	antisense	5'- TGGGTCTTCGGGCGATTCTC-3'	

10-15 mm distal to the ileocecal valve the wall of the ascending colon was pierced with a 7/0 Ethilon suture (Johnson & Johnson Intl., Belgium) and the venous catheter was inserted carefully into the colon, avoiding perforation of the bowel and its penetrating blood vessels. The stent was fixed with the 7/0 surgical thread at the prepared notch followed by removal of the inner needle. The extra-luminal part of the venous catheter was cut to a length of 1-2 mm.

To verify the correct positioning of the stent, stool was milked carefully from the cecum into the ascending colon until a small drop of feces appeared on top of the stent. After repositioning the colon into the abdominal cavity, fluid resuscitation by administration of 0.5 ml sterile saline solution was performed. The abdominal layers were closed separately with a 5/0 continuous suture for the peritoneal wall and a 5/0 singular suture for the skin. The control group (sham) underwent the same surgical procedure except for insertion of the stent.

5.2. RNA isolation and cDNA synthesis

Twelve hours after CASP surgery, the mice were sacrificed. Half of a lung was transferred to Tri-Reagent (Molecular Research Center Inc., Cincinnati, OH), and RNA was isolated according to manufacturer's instructions. Two micrograms of total RNA were used for cDNA synthesis after DNAse (Ambion, Texas, USA) treatment. For cDNA synthesis, RNA, oligo (dT) 15 primer (500 µg/ml, PromegaTM, Madison, WI) and dNTPs (deoxynucleotidetriphosphate-mix, 10 mM, Fermentas, St. Leon-Rot, Germany) were mixed, heated for 5 min at 65°C and immediately cooled on ice. After addition of 5x First Strand Buffer and Super Skript II reverse transcriptase (200 U/µl, Invitrogen, Carlsbad, CA) samples were kept at 42°C for 50 minutes followed by heat inactivation at 70°C for 15 minutes.

5.3. Cytokines and Receptors RT2 Profiler

We used the Mouse Inflammatory Cytokines and Receptors RT2 Profiler PCR Array System (SABiosciences, Frederick, MD) according to the manufacturer's instructions to determine the expression of 84 key genes involved in the inflammatory response. cDNA (100 ng) was mixed with RT2 SYBR Green / Fluorescein qPCR Master Mix and loaded into a 96-well plate. Real-time PCR (RT-PCR) was performed with a two-step cycling program on a Bio-Rad iCyclerR: 10 minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C and 1 minute at 65°C.

For each gene the dCt = CtGOI – Ct AVG HKG value was calculated (GOI, gene of interest; AVG, average; HKG, housekeeping genes). To compare groups (sham vs. CASP) the ddCt was calculated: ddCt = dCt (group 1) – dCt (group 2). The fold-change for each gene was calculated as $2^{(-ddCt)}$.

5.4. Protein expression levels in lungs of septic mice

For investigation of the protein expression levels of cytokines and chemokines the Proteome Profiler mouse cytokine array kit (R&D Systems, Abingdon, UK) was used according to the manufacturer's instructions. Lung tissues (50-80 mg) were homogenized in 1 ml phosphate buffered saline (PBS) with proteinase inhibitors. After addition of Triton X-100 (1% final concentration) and a freeze/thaw cycle, lungs were centrifuged to remove cellular debris. The protein concentration was quantified by using a BCA protein assay (Thermo Fisher Scientific, Waltham, MA). The samples (200 µg) were mixed with biotinylated detection antibodies and incubated with the Mouse Cytokine Array membrane. After washing, streptavidin-HRP and chemiluminescent detection reagents were added sequentially to the membrane. The membranes were then exposed to X-ray film for 5 minutes.

5.5. Relative expression levels of neuropeptide mRNA in the lungs of septic mice

Primers used for quantitative RT-PCR of two housekeeping genes (mouse HPRT, mouse RPL4) and the genes encoding the neuropeptides galanin, NPY and SP are listed in table 1. One microgram of cDNA (1 µg/µl) and 5 µl B-R SYBR Green SuperMix for iQ (Quanta BioSciences Inc., Gaithersburg, MD) and 4 µl primers (400 nM, Eurofins MWG Operon, Huntsville, AL) were used per reaction. The PCR was performed with the following cycling conditions: 3 minutes at 95°C; 10 cycles with 15 seconds at 95°C, 2 minutes at 64°C and 10 seconds at 72°C; followed by 35 cycles with 15 seconds at 95°C, 30 seconds at 64°C and 10 seconds at 72°C. Relative expression was analyzed as described above.

6. CYTOKINES AND CHEMOKINES

In the following we review the literature concerning the most important markers of sepsis and, where appropriate, we compare the literature results to data for the lungs of CASP mice generated in the present study (figures 1 and 2, tables 2-5). Septic mice were compared to sham-operated mice, which served as controls. We compared sham and fit mice, which had no surgery or

Table 2. Cytokine profile in serum and lungs in animal models of sepsis

Symbol	LPS		CLP		CASP				References
	serum	lung	serum	lung	serum	lung	lung mRNA 12h[5]	lung protein 12 h[5]	
ICAM1						ns 3h, 6h, 12h[1]	nd	+++	(42)
IFN-g	ns 6h	+ 1h	+++ 1h,5h[2]		ns 18h[3]	ns 18h + 3h[1]	+	ns	(21, 39, 43, 48, 50)
IL-1 Ra				++ 8h [4]			nd	ns	(52)
IL-1a	ns 6h		ns 1-8h [2]				+	ns	(43, 50)
IL-1beta	++ 6h	++ 1h	+ 6h + 1h ++ 5h[2]	++ 8h [4] +++ 8h	++ 6h +++ 12h,18h		+++	ns	(15, 21, 50-52, 116)
IL-1f6							+	nd	
IL-1f8							+	nd	
IL-4		+ 1h					+	ns	(21)
IL-5			ns 1-8h[2]				nd	ns	(50)
IL-6	++ 3h +++ 4h	++ 2h	+ 5h +++ 8h[2] ++ 8h	+ 8h	+++ 18h[3]	+++ 18h	nd	++	(1, 21, 41, 43, 44, 48, 50, 51)
IL-10	+++ 2h		+++ 5h, 8h[2] + 6h		++ 3h +++ 12h +++ 18h[3] +++ 6h +++ 18h	ns 18h + 3h[1] ++ 12h +++ 12h	+++	ns	(15, 39, 45, 47-50)
IL-12	ns 1-6h			ns 8h[4]	ns 18h + 3h ++ 12h + 12h	++ 3h[1] - 3h -- 12h	nd	ns	(39, 43, 45, 47, 49, 52)
IL-13			+++ 5h[2]				+	ns	(50)
IL-15							-	nd	
IL-17b							+	ns	
IL-18					+++ 18h	ns 3h, 6h, 12h	ns	nd	(45, 49)
TNF-alpha	++ 1h +++ 1,5h +++ 2h		ns 6h + 5h[2]	+ 8h	+ 3h + 6h +++ 12h +++ 18h [3]	+++ 3h[1] ++ 12h +++ 12h ++ 18h	+++	ns	(1, 12, 15, 39, 41, 43-51)

Abbreviations: nd: not determined, ns: not significant (fold induction below 2). Increase (+) or decrease (-) indicated for protein levels mRNA expression[1], multiplex immunoassay[2], cytometric bead array[3] or microarray analysis from lung mRNA[4]. Data from present study[5]. Fold induction 2-5 (+), fold induction 5-20 (++), fold induction higher than 20 (+++). h: hours, CAM:intracellular adhesion molecule, IL: interleukin, IFN-g: interferon gamma, TGF: transforming growth factor, TNF: tumor necrosis factor.

treatment, and found no difference in expression levels of the investigated mediators (data not shown). This indicates that the observed effects were due to polymicrobial abdominal sepsis as a result of CASP surgery and not to the surgery per se.

The cytokine and chemokine profiles show several differences among animal models and time points (1, 41). TNF-alpha, IL-1beta, IL-6 and IL-8 are the most frequently altered cytokines in the context of sepsis (10).

One of the best investigated cytokines is TNF-alpha, which is highly increased in the serum of mice already 2 hours after LPS administration (1, 12, 25, 41, 43-45). Similar results were obtained with the CASP model, where serum levels were increased about 2- to 5-fold 3 hours after surgery, reaching a greater than 20-fold induction at 12 hours post surgery. In lung tissue of septic animals, the concentration of TNF-alpha became massively elevated quite early, at 3 hours after surgery, and was sustained until 18 hours post CASP (15, 39, 45-49). A similar up-regulation (47-fold) was also observed for TNF-alpha mRNA expression in our own study; however, in contrast to other studies, we did not detect up-regulation of TNF-alpha protein 12 hours after CASP (table 2). Differences in mRNA expression in the serum of septic mice were observerd among CLP, CASP and LPS, with several studies reporting no significant or very low levels of TNF-alpha expression in the CLP model (1, 15, 41, 50).

An important marker that is used to predict survival in humans is IL-10 (table 2). This marker is dramatically increased in murine serum in the LPS model (20-fold and higher at 2 hours), the CLP model (over 20-fold at 5 hours) and the CASP model (5- to 20-fold at 3 hours, above 20-fold after 6 hours) (15, 45, 47-50). In the latter, the RNA and protein levels of IL-10 collected from septic mice after CASP surgery steadily increased over time, from 2- to 5-fold at 3 hours to over 20-fold 12 hours after surgery. Our RT2 profiler data are in agreement with these results, showing a 64-fold increase in IL-10 expression after 12 hours. In contrast, the proteome profiler did not show a significant difference between sham and septic lungs (table 2, figures 1 and 2). Differences between mRNA expression and protein concentration in a tissue might reflect different time points when the peaks are observed; protein levels might peak 3 to 10 hours later than mRNA levels.

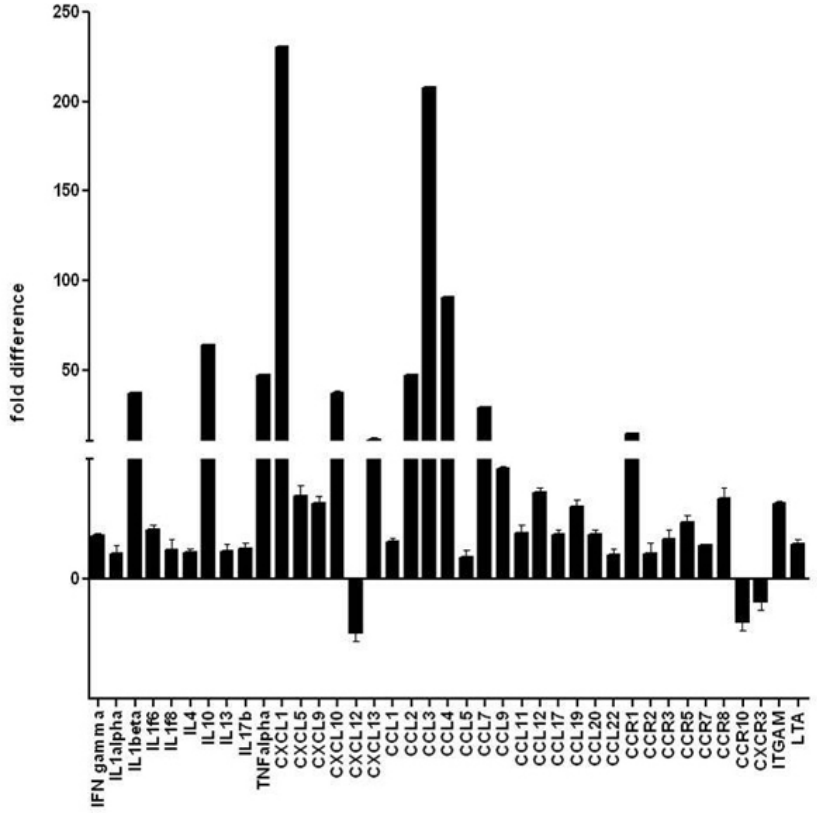

Figure 1. Significant changes in mRNA expression of different inflammatory markers in lungs of septic mice compared to sham-operated mice 12 hours after surgery. In this figure, only markers with a fold induction greater than 2 and a p-values below 0.5 are shown; n = 4 mice per group. Data were analyzed by unpaired t-test.

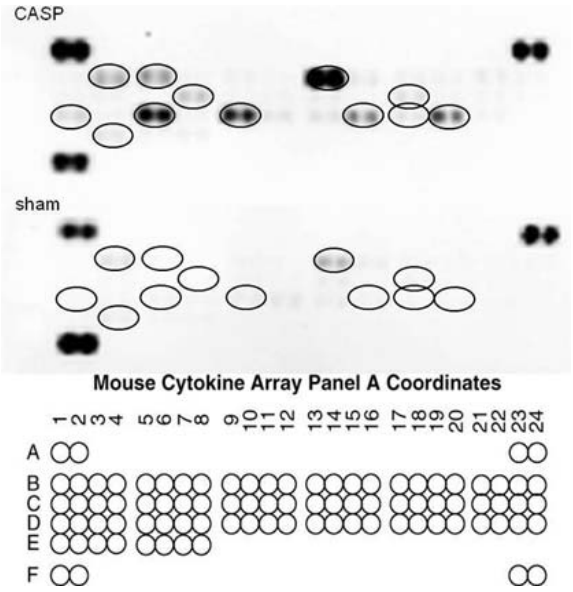

Figure 2. Mouse cytokine production in lungs of CASP and sham groups 12 hours after surgery. The protein levels of C5a (position B3, B4), G-CSF (position B5, B6), sICAM (position B13, B14), IL-1ra , IL6 (position C7, C8), IL16 (position C17, C18), IP10 (position D1, D2), KC (position D5, D6), CCL2 (position D9, D10), MIP-1alpha (position D15, D16), MIP-1beta (position D17, D18) and MIP-2 (position D19, D20) are increased in mouse lungs 12 hours after CASP surgery compared to sham controls. n = 2 mice per group.

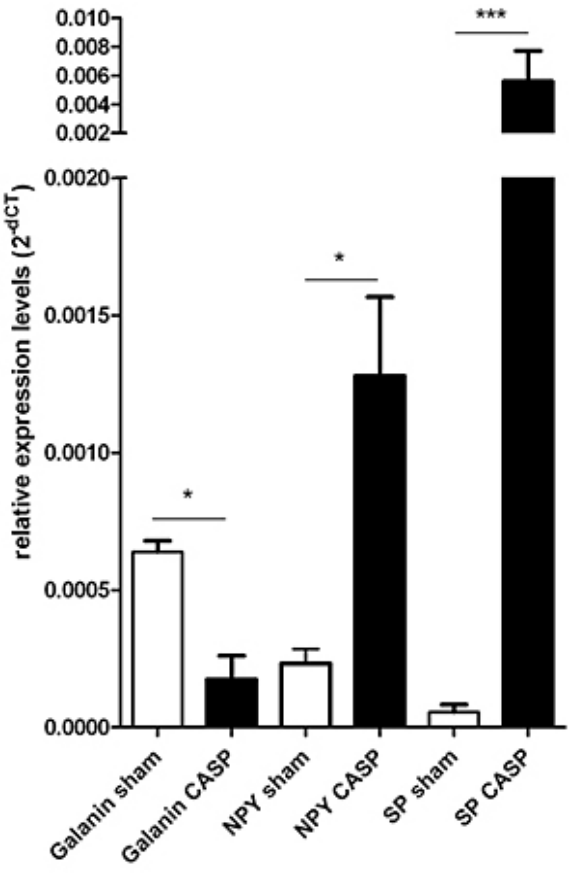

Figure 3. Comparison of the expression of neuropeptides in septic and sham mouse lungs. Expression of galanin mRNA was down-regulated (7-fold), whereas expression of NPY (8-fold) and SP (62-fold) mRNA was significantly up-regulated. Sham group n = 3, CASP n = 5. Data were analyzed by unpaired t-test.

IL1-beta was elevated in murine lungs 1 hour after LPS administration and in the sera of CLP mice 1 hour after surgery (21, 50). The highest expression level with CLP surgery was recorded at 8 hours post surgery in lung tissue (51). Greater than 20-fold induction was reported for plasma levels in the CASP model at 12 and 18 hours, which is in accordance with the 37-fold induction of IL-1beta mRNA that we observed in septic lungs in the present study (15). No difference in protein expression was detected with the proteome profiler.

IL-6 was reported to be elevated in all studies, including IL-6 protein in the present study (table 2, figure 2) (1, 21, 41, 43, 44, 48, 51, 52).

The CC-chemokines CCL2 and CCL3 were up-regulated in all studies, which is in accordance with our data (21, 42, 46-48, 50, 52-54). We detected a 47-fold increase of CCL2 and a 200-fold increase of CCL3 in septic mouse lungs compared to sham-lungs (table 3, figure 1), and we recorded a massive increase in the protein levels of these chemokines in septic lungs (table 3, figure 2).

The results of our present study regarding CXC-chemokines CXCL1 and CXCL2 (table 4, figures 1 and 2) are in agreement with the literature and show highly elevated mRNA expression and protein levels in septic versus non-septic mice (41, 42, 45-47, 50-53).

Our results concerning protein expression levels in the lungs of septic mice are displayed in figure 2 and tables 2-4. Increased protein levels in septic lungs were found for complement component 5a (C5a), granulocyte colony-stimulating factor (G-CSF), soluble inter-cellular adhesion molecule 1 (sICAM), IL-6, IL-16, CXCL10, cytokine-induced neutrophil chemoattractant (KC), CCL2, CCL3, CCL4, and macrophage inflammatory protein 2 (MIP-2). No differences in protein levels were observed for CXCL13, GM-CSF, CCL1, CCL11, interferon gamma (IFN-gamma), IL-1alpha, IL-1beta, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-7, IL-10, IL-13, IL-12p70, IL-17, IL-23, IL-27, CXCL11, macrophage colony-stimulating factor (M-CSF), CCL12, CXCL9, CCL5, CXCL12, CCL17, tissue inhibitor of metalloproteinases (TIMP-1), TNF-alpha and triggering receptor expressed on myeloid cells (TREM-1).

Twelve hours after CASP, in agreement with the study of Neumann *et al.*, we detected up-regulation of mRNA expression in the lungs of septic mice for KC, MIP-1alpha, IFN-inducible protein 10 (IP-10), monocyte chemotactic protein (MCP-1), and regulated upon activation normal T-cell expressed and secreted (RANTES) (42). ICAM-1 mRNA expression seemed to be unaffected, whereas the protein expression was massively increased in septic lungs (table 2, figure 2).

In human septic patients, elevated levels of MIP-1alpha, MCP-1, GRO-alpha, IL-18 and IL-6 have been reported in bronchoalveolar lavage (BAL) fluid, but only IL-6 was up-regulated in the sera of these patients (55).

In addition to the common, well-known pro- and anti-inflammatory cytokines and chemokines described above, the RT2-profiler revealed significant changes of CCL7, CCL20, CXCL5, CXCL12, CXCL13, CCR8 and CCR10, which to our knowledge have not been reported previously in animal models of abdominal polymicrobial sepsis. Of course, all of the chemokines and cytokines present on the profiler are linked somehow to an inflammatory response, but not all of them have been described in cases of experimental sepsis. CCL7, also known as monocyte chemotactic protein 3 (MCP-3), has activating and chemoattractant abilities for monocytes, lymphocytes, eosinophils and basophils. The production of CCL7 is enhanced in inflammatory bowel disease mucosa, in inflamed human bronchial mucosa, and during bronchial asthma (56). CCL7 protein was up-regulated in mouse brains after LPS treatment, but there is no published information about its regulation during abdominal

Table 3. CC-Chemokine profile in serum and lungs in animal models of sepsis and serum

Symbol	Synonym	LPS	CLP		CASP				References
		lung	serum	lung	serum	lung	lung mRNA 12h[5]	lung protein 12 h[5]	
CCL1	I-309, TCA-3						+	ns	
CCL2	MCP-1	++ 2h	++ 5h[2]	++ 8h[4]	+++ 3h, peak 12h	+++ 6h[1]	+++	+++	(21, 42, 46-48, 50, 52-54)
			+++ 5-20h	++ 6h	+++ 18h[3]	++ 12h			
				+++ 1-20h		+++ 18h			
CCL3	MIP-1alpha		++ 5h	++ 8h[4]		+++ 3h	+++	++	(42, 47, 50-52, 54)
				+6h		++ 12h			
CCL4	MIP -1beta		+ 5h[2]	+ 8h[4]			+++	ns / +	(50, 52)
CCL5	RANTES		+++ 1h[2]			ns 3h	ns	ns	(42, 50)
CCL6	C10, MRP-2			ns 8h[4]			ns	nd	(52)
CCL7	MARC, MCP-3						+++	nd	
CCL9	MRP-2, CCF18, MIP-1			+ 8h[4]			++	nd	(52)
CCL11	Eotaxin		++ 5h[2]				+	ns	(50)
CCL12	MCP-5				+++ 12h		++	nd	(46)
CCL17	TARC, dendrokine, ABCD-2			ns 8h[4]			+	ns	(52)
CCL19	ELC, Exodus-3, Ck beta4						++	nd	
CCL20	LARC, Exodus-1, Ck beta4						+	nd	
CCL22	MDC, DC/beta-CK			ns 8h[4]			+	nd	(52)

Abbreviations: nd: not determined, ns: not significant (fold induction below 2). Increase (+) or decrease (-) indicated for protein levels or mRNA expression[1], multiplex immunoassay[2], cytometric bead array[3] or microarray analysis from lung mRNA[4]. Data from present study[5]. Fold induction 2-5 (+), fold induction 5-20 (++), fold induction higher than 20 (+++).

Table 4. CXC-Chemokine profile in serum and lungs in animal models of sepsis and serum

Symbol	Synonym	LPS	CLP		CASP				References
		serum	serum	lung	serum	lung	lung mRNA 12h[4]	lung protein 12 h[4]	
CXCL1	Gro-alpha, GRO1, NAP-3, KC	+++ 2h	+++ 5h[2]	++ 8h[3]	+++ 6h	+++ 3h[1]	+++	+++	(41, 42, 45-47, 50, 52)
		++ 4h	++ 4h			+++ 12h			
			+++ 8h						
CXCL2	Gro-beta, MIP-2a	+++ 4h	+++ 8h	+++ 8h[3]		+++ 3h[1]	nd	++	41, 42, 47, 51-53)
				++ 8h		+++ 12h			
				+++ 1-20h					
CXCL5	ENA-78		++ 4h				++	nd	(59)
			ns 8h						
CXCL9	MIG, CRG-10		++ 24h peritoneal lavage				++	ns	(117)
CXCL10	IP-10, CRG-2			++ 8h [3]	+++ 3h	+++ 6h[1]	+++	+	(42, 45, 46, 52)
					- 6h	+++ 12h			
					+++ 12h				
CXCL12	SDF-1alpha/beta, PBSF						-	ns	
CXCL13	BLC/BCA-1						++	ns	

Abbreviations: nd: not determined, ns: not significant (fold induction below 2). Increase (+) or decrease (-) indicated for protein levels or mRNA expression[1], multiplex immunoassay[2]or microarray analysis from lung mRNA[3]. Data from present study[4]. Fold induction 2-5 (+), fold induction 5-20 (++), fold induction higher than 20 (+++).

polymicrobial sepsis (57). In our cytokine expression profile of murine septic lungs, we found CCL7 to be increased 29-fold compared to sham controls.

CCL19 (or ELC, Exodus-3, Ck beta4, MIP-3 beta) expression is mainly located in lymph nodes, thymus, colon and trachea, and very low levels of expression were reported in the lungs, kidneys, spleen and small intestine (58). Increased CCL19 protein expression was observed in brains of LPS-treated mice, similar to CCL7 (57). To date, no data are available concerning the functions of CCL19 in the lungs and plasma during sepsis. We detected a 6-fold increase in mRNA expression in lung tissue 12 hours after CASP surgery.

CXCL5 (ENA-78) was shown to be increased about 10-fold 4 hours after CLP surgery in murine serum and was back to baseline levels at 8 hours (59). Another study reported increased CXCL5 mRNA and protein levels after IL-1 beta-induced leukocyte recruitment in mesenteric tissue (60). No information about CXCL5 expression levels has been reported for CASP or lung tissue. In our study we found CXCL5 mRNA to be increased 7-fold in lung tissue 12 hours after CASP (table 4, figure 1).

No data concerning regulation of CXCL13 during polymicrobial sepsis were found in the literature, whereas we detected an 11-fold up-regulation of CXCL13 message in septic lungs. No regulation was observed for protein levels (table 4, figures 1 and 2).

Table 5. Chemokine receptor profile in lungs in animal models of sepsis and serum

Symbol	Ligands	CLP	CASP	References
		lung	lung mRNA 12h[2]	
CCR1	CCL 3,4,6,8,9,14,15,16,23	+ 8h[1]	++	(52)
CCR2	CCL 2,7,11,13,16	ns 8h[1]	+	(52)
CCR5	CCL 4,5,8,11,13,16	ns 8h[1]	+	(52)
CCR6	CCL 20	ns 8h[1]	ns	(52)
CCR8	CCL 1,16		++	
CXCR 6	CXCL 16	ns 8h[1]	nd	(52)

Abbreviations: nd: not determined, ns: not significant (fold induction below 2). Increase (+) or decrease (-) indicated for microarray analysis from lung mRNA[1]. Data from present study[2]. Fold induction 2-5 (+), fold induction 5-20 (++), fold induction higher than 20 (+++).

Matsukawa *et al.* demonstrated that CCR8 knock-out mice survived CLP in contrast to CCR8 wild-type mice. They hypothesize that this effect might be due to the increased expression of TNF-alpha, IL-12, MIP-2 and KC in LPS-treated isolated CCR8 -/- macrophages (61). We detected a 7-fold up-regulation of CCR8 mRNA expression in lung tissue 12 hours after CASP, which has not been reported anywhere else (table 5, figure 1).

B-cell leukemia/lymphoma 6 (Bcl6), caspase 1 (Casp1), ATP-binding cassette sub-family F member 1 (Abcf1), integrin beta 2 (Itgb2), small inducible cytokine subfamily E member 1 (Scye1), secreted phosphoprotein 1 (Spp1), CD 40 ligand (CD40lg), Toll interacting protein (Tollip), C-reactive protein (CRP), macrophage migration inhibitory factor (MIF) and lymphotoxin A did not show significant differences in mRNA expression levels between the sham and CASP surgery groups (data not shown). Up to 5-fold up-regulation of CD14, CD80, E-selectin, FC receptor IgE high-affinity I gamma polypeptide, interferon-induced transmembrane protein 6, matrix metallopeptidase 8 and 9, metallothionein 1, neutrophilic granule protein, P-selectin, plasminogen activator, s100 calcium binding protein A8 and A9 and serum amyloid A3 were detected by Hegde *et al.* and Neumann *et al.*, but these factors were not part of our study (42, 52).

6.1. Neuropeptides

Neuropeptides have a widespread distribution in the central (CNS) and peripheral nervous systems (PNS), but they are also produced in non-neuronal cells. Many neuropeptides control vasodilation, smooth-muscle relaxation, hyperglycemia, analgesia, hyperthermia, gastric motility, food intake, learning and many more (64).

For a long time the immune system and the neuroendocrine system were thought to be two separate networks. The main dogma was that the endocrine system responds to external stimuli like stress, pain and temperature, and that the immune system responds to viruses, bacteria and trauma. The principal control center for dealing with infection and inflammation is the brain, which generates a febrile response including reduction of food intake and induction of sleep (62). The CNS regulates the immune response to environmental stress via the autonomic nervous system or the hypothalamus-pituitary-adrenal axis. The immune and the neuroendocrine networks share several mediators such as neuropeptides, cytokines, hormones, and their receptors. With that knowledge it became more and more clear that there might be crosstalk between the two systems (63).

6.1.1. VIP, alpha-MSH, ghrelin, urocortin, adrenomedullin, cortistatin, CRH

Neuropeptides and their receptors, such as vasoactive intestinal peptide (VIP), alpha-melanocyte-stimulating hormone (alpha-MSH), urocortin, adrenomedullin, cortistatin and ghrelin, are produced by immune cells, especially under inflammatory conditions (64, 65). Gonzales-Rey *et al.* reviewed elegantly the anti-inflammatory activities of neuropeptides (66). Ghrelin, VIP, alpha-MSH, urocortin, adrenomedullin and cortistatin are able to inhibit the production of pro-inflammatory cytokines like TNF-alpha, IL-12, IL-6, IL-18 and IL-1beta, and chemokines like IL-8, RANTES, MIP-1alpha, MIP-2 and MCP-1. They do this by, for example, activation of cAMP/protein kinase A (PKA) and down-regulation of transcription factors. Furthermore, these peptides increase the production of anti-inflammatory IL-10. Neuropeptides not only affect the innate immune system, but also the adaptive immune system by regulating T-cells and dendritic cells. They can dampen both T-cell proliferation and the TH1 response (pro-inflammatory) via IL-2 and IFN-gamma production, and VIP especially is able to stimulate the TH2 response (anti-inflammatory) via IL-4 and IL-5 (64-66). Corticotropin-releasing hormone (CRH) was reported to exert both pro- and anti-inflammatory effects, depending on its site of expression: CRH has anti-inflammatory functions when released in the CNS and direct pro-inflammatory effects on immune cells when produced in peripheral tissue (64).

6.1.2. Substance P and CGRP

Substance P (SP) indirectly stimulates nuclear factor (NF)-kappaB in lung epithelial cells via intracellular signaling pathways after binding to its G-protein-coupled receptor TARC1 (67). Beer *et al.* studied the systemic concentration of SP and calcitonin-gene related peptide (CGRP) in plasma of patients with sepsis after major visceral surgery and compared survivors and non-survivors.

Table 6. Serum markers of human sepsis

Sepsis marker	Clinical studies	Prognostic factor	Comment	References
IL-6	II	yes a	Distinguished between survivors and non-survivors	(118, 119)
IL-8	II	yes c	Prediction of MOF, DIC	(120, 121)
IL-10	II	yes b	Higher in septic shock than sepsis, distinguished between survivors and non-survivors	(122-124)
Macrophage migration inhibitory factor (MIF)	I	yes b	Distinguished between survivors and non-survivors	(125, 126)
Monocyte chemotactic protein (MCP)-1 and 2	II	yes a	Distinguished between survivors and non-survivors	(125, 127)
mHLA-DR (soluble)	III	yes a	Distinguished between survivors and non-survivors in patients with septic shock	(128)
RAGE (soluble)	II	yes a	Distinguished between survivors and non-survivors	(129)
Antithrombin	II	yes b	Distinguished between survivors and non-survivors	(130)
Endothelial leukocyte adhesion molecule (ELAM)-1 (cellular and soluble)	II	yes a	Distinguished between survivors and non-survivors at 28 days	(131, 132)
Neopterin	III	yes a	Distinguished between survivors and non-survivors	(133, 134)
L-Selectin (soluble)	III	yes a	Distinguished between survivors and non-survivors	(99)
Adrenomedullin and pro-adrenomedullin	II	yes a	Predicted development of septic shock	(135, 136)
Copeptin	III	yes a	Distinguished between survivors and non-survivors correlated with APACHE II score	(137)
Atrial natriuretic peptide (ANP)	III	yes a	Distinguished between survivors and non-survivors	(138, 139)
Brain natriuretic peptide (BNP)	II	yes b	Distinguished between survivors and non-survivors at 28 days, correlated to APACHE II score	(140-142)
C-reactive protein (CRP)	III	yes a	Predicted response to therapy	(143-145)
Procalcitonin	III	yes a	Increased in infected compared to non-infected patients	(134, 146, 147)
Ceramide	II	yes b	Predicted development of MOF	(148)
G-CSF and GM-CSF	II	yes b	Distinguished between survivors and non-survivors	(149, 150)
HDL cholesterol	III	yes b	Distinguished between survivors and non-survivors predicted polonged ICU length of stay	
HLA-G5 protein (soluble)	III	yes a	Distinguished between survivors and non-survivors	(151)
NF-kappaB (activity and expression)	II	yes b	Distinguished between survivors and non-survivors correlation with APACHE II score	(101)
TIMP-1 and 2	II	yes a	Distinguished between survivors and non-survivors at 28 days	(152)
Protein C and S	C	yes a	Distinguished between survivors and non-survivors	(153, 154)

Abbreviations: yes a: sensitivity and specificity of less than 90%; yes b: sensitivity of more than 90% but specificity of less than 90%; yes c: sensitivity and specificity more than 90%; I, Clinical study with less than 20 patients; II, Clinical study with 20 to 50 patients; III, Clinical study with more than 50 patients; DIC: disseminated intravascular coagulopathy; MOF: multiple organ failure; SOFA: sequential organ failure assessment

They reported a significant 2- to 3-fold increase in CGRP concentration at all time points (1 day to 14 days) in sera of non-survivors compared to survivors. These results implicate CGRP as an early predictor of lethal outcome of postoperative sepsis. Systemic SP levels were significantly elevated in all sepsis patients, but in contrast to CGRP, the levels did not differ in the early phase of sepsis (day 1 to day 3) between survivors and non-survivors. Significant differences between the two groups of patients (survivors and non-survivors) were reported in the late phase of sepsis, leading to the theory that a continuous increase of SP in the late phase correlates with a lethal outcome of postoperative sepsis (68). In accordance with the human data, we found a massive up-regulation (62-fold) of SP in murine lungs 12 hours after CASP surgery. Zhang *et al.* showed a 2- to 3-fold increase in SP protein levels 8 hours after CLP compared to sham (51). Puneet *et al.* found a 2-fold increase in SP protein level in murine lungs 1 hour after CLP, no significant increase at 5 and 10 hours, and a significant up-regulation again at 16 and 20 hours after CLP compared to fit and sham controls (53).

Hegde *et al.* published two studies in 2010 using the CLP model and focusing on the preprotachykinin-A (PPTA) gene and its product, SP. In their first article they investigated plasma cytokine profiles in preprotachykinin-A knock-out mice (PPTA-/-) using a bead array for 18 mouse cytokines (50). In a further study they focused on protein levels in the lungs of wild-type (WT) and PPTA-/- mice 8 hours after CLP surgery using a high-throughput GeneChip Mouse Genome 430 2.0 array and ELISA (52). Their main results concerning protein levels in WT mice after CLP are shown in tables 2-5. PPTA-/- mice showed a greater increase in sepsis markers 8 hours after CLP compared to sham-operated mice (52).

6.1.3. NPY

Neuropeptide Y (NPY) is involved in the regulation of several physiological and psychological processes, for example feeding, neuroendocrine secretion and anxiety, and in modifying vasoconstriction (69, 70). NPY and its five G-protein-coupled receptors were also found on cells of the immune system. NPY is able to increase the release of reactive oxygen radicals in murine peritoneal macrophages, a mechanism also used by polymorphonuclear neutrophils (PMN) in response to microorganisms (69, 70).

Various effects of NPY on natural killer (NK) cells, neutrophils, monocytes, peripheral mononuclear cells (PBMCs), T-cells and B-cells were reviewed by Bedoui et al. in 2003 (69). NPY is able to increase IL-1 beta, IL-6, TNF-alpha and IL-4 released from PMN and PBMCs. Furthermore, NPY impairs neutrophil proliferation (69).

Table 7. Predictive indicators of sepsis outcome in human patients

Preoperative Parameter	**Type of analysis**
Monocyte IL-12 production	prospective
TNF-beta/ gene polymorphism	prospective
IL-1 gene polymorphism	prospective
Sepsis onset	
APACHE II/SAPS II	retrospective
IL-18	prospective
NF-kappaB	prospective
PCT/APACHE II	prospective

Abbreviations: APACHE: acute physiology and chronic health evaluation; SAPS: simplified acute physiology score; NF-kappaB: nuclear factor-kappaB; PCT: procalcitonin. Not included are studies showing differences between survivors and non-survivors, which may correlate with sepsis severity, without analysis of the prediction of outcome.

In our study we found an 8-fold increase in NPY mRNA expression levels in septic mice 12 hours after CASP surgery compared to sham-operated mice (figure 3).

6.1.4. Galanin

It has been demonstrated that the neuropeptide galanin, in a dose-dependent manner, is able to abolish inflammatory edema in the skin induced by co-injection of SP and CGRP, leading to the conclusion that galanin might have an anti-inflammatory function in neurogenic inflammation (71). Therefore, we investigated the expression level of galanin in the CASP model to see whether galanin is regulated during an ongoing systemic inflammation. As shown in figure 3, galanin is significantly down-regulated in the lungs of septic mice 12 hours after CASP surgery. This does not necessarily contradict the results of other studies, which reported anti-edematous or anti-inflammatory potency of this neuropeptide. It has been shown that galanin reduces plasma-extravasation and blood flow in inflamed skin (71, 72). The major difference between our study and the other studies is the site of infection: they induced inflammation locally by administration of SP and CGRP or c-fiber stimulation in contrast to our induction of polymicrobial sepsis affecting the whole body.

The observed down-regulation of galanin 12 hours after the CASP procedure could be explained as an initial immune response in favor of pro-inflammatory mediators to start anti-microbial mechanisms to protect the host against pathogens. As the disease progresses further, galanin levels may be up-regulated steadily to counter the effects of SP and thus avoid an overshooting inflammatory response accompanied by extreme vasodilation, neutrophil accumulation, hypotension and organ dysfunction.

7. BIOMARKERS IN HUMAN SEPSIS

A complex network of biological mediators underlies the clinical syndrome of sepsis. Biomarkers promise to transform sepsis from a physiologic syndrome into a group of distinct biochemical disorders (73). Potential uses of biomarkers include roles in prognostication, guiding antibiotic therapy, evaluating the response to therapy and recovery from sepsis, differentiating Gram-positive from Gram-negative microorganisms as the cause of sepsis, predicting sepsis complications and the development of multi-organ dysfunction. Further, biomarkers can differentiate viral from bacterial and fungal infection, and local infection from systemic sepsis. However, the exact role of biomarkers in the management of septic patients remains undefined (74).

More than 3000 studies in recent years have evaluated nearly 200 biomarkers in the course of sepsis. Most of the biomarkers were assessed in clinical studies. Pierrakos *et al.* assessed much of that work in their substantial review, and 24 of the best evaluated sepsis biomarkers are listed in table 6 (17). Only nine of them have a sensitivity of more than 90% or a sensitivity and specificity of more than 90%. All of them were evaluated as prognostic factors of different endpoints; however, regrettably, the studies involved different causes of infection and pathogen spectra. Sepsis biomarkers should be evaluated in a homogenous cohort of patients.

In the remainder of this article we focus on postoperative sepsis after major surgery. Severe sepsis is still a major cause of postoperative morbidity and mortality after abdominal surgery despite recent progress in understanding the immune conditions of abdominal sepsis. The postoperative incidence of sepsis after major visceral surgery is 9-12%, with a mortality rate of 42-80%. Early detection of a potentially lethal outcome and preoperative identification of patients with an increased risk of developing lethal postoperative sepsis are very important aims in the prevention and treatment of sepsis (3, 74).

7.1. Biomarkers with clinical relevance in abdominal sepsis

In the treatment of postoperative peritonitis there are three significant questions:

1. Is it possible to identify patients with an increased risk for postoperative sepsis?
2. Is early prediction of a septic course possible?
3. Was surgical treatment of abdominal sepsis successful?

7.2. Preoperative identification of patients with an increased risk of developing lethal postoperative sepsis

Predictive indicators of sepsis outcome are considered important for identifying high-risk patients and initiating early, individualized sepsis therapy. Currently available parameters that may allow prediction of sepsis outcome before surgery or at sepsis onset are summarized in table 7. There is high interest in being able to identify, either before or soon after elective major abdominal surgery, those patients who are at increased risk of developing a lethal course of postoperative sepsis. Several

studies have designed protocols for perioperative immune monitoring to address this question (75-82). The general aim of risk prediction by these approaches is to reduce the incidence and severity of infection in high-risk patients through modified clinical management, which may include less aggressive surgery (associated with fewer complications), split operations that apply second-step reconstruction procedures after the initial resection (e.g., esophagectomy), or alternative therapeutic regimens of underlying co-morbidity (3).

Various immune functions are known to be impaired as a result of major surgery or trauma. They include T-lymphocyte proliferation and cytokine secretion, delayed-type hypersensitivity skin test response, monocyte cytokine secretion, major histocompatability complex (MHC) class II expression, and neutrophil functions such as chemotaxis, phagocytosis, and oxygen radical production (83-86). It appears that the extent of surgical trauma directly correlates with the extent of postoperative immunosuppression (87-89). Remarkably, loss of monocyte HLA-DR (MHC class II cell surface receptor encoded by the human leukocyte antigen) expression and unresponsiveness to hypersensitivity skin testing have been correlated with the incidence and outcome of postoperative sepsis (90-92). These studies suggest that suppression of immune defense mechanisms after major surgery or trauma may increase the susceptibility to postoperative infection and sepsis.

Independent studies have provided evidence that genotyping of immune-response genes may be useful for sepsis risk evaluation. For example, patients homozygous for the TNF-beta allele TNFB2 (B2/B2) exhibited a higher mortality rate than patients with a B1/B2 heterozygous or a B1/B1 homozygous genotype. In addition, homozygosity for the TNFB2 allele was associated with elevated circulating TNF-alpha levels and higher multiple organ failure scores in sepsis patients. In a separate study, the presence of the IL-1 receptor antagonist allele IL-1raA2 correlated with the development of sepsis but not with outcome (76, 79, 81, 82). A study by Kahlke and coworkers investigated patients who had had major visceral surgery and showed that the heterozygous TNF-beta genotype (B1/B2) was linked to a higher risk for developing complications. If patients with the homozygous TNF-beta genotype (B2/B2) developed complications, their relative risk for severe complications was increased. Mortality was significantly elevated in the TNF-beta (B2/B2) genotype patients (77). For patients undergoing major visceral surgery, Riese et al. found that the homozygous TNFB2 genotype (B2/B2) was significantly more frequent in patients who developed postoperative complications than in those with an uneventful recovery. The development of complications was associated with a lower capacity to produce TNF-alpha after surgery. However, in patients without complications, the TNF-beta polymorphism was not related to different levels of TNF-alpha production (78).

Analysis of mononuclear phagocyte functions further supported the concept that suppression of immune defense mechanisms even before major surgery or trauma may increase the susceptibility to postoperative infection and sepsis. It was found that preoperative production of IL-12 by LPS-stimulated monocytes was markedly impaired in patients who developed postoperative sepsis and that the extent of this functional defect directly correlated with sepsis severity (80). Further investigations in 1113 patients showed that monocytes of sepsis patients exhibited impaired preoperative production of IL-10, but not of other cytokines, compared with monocytes of patients with uneventful recovery. When the study population of septic patients was classified as survivors and non-survivors, it became apparent that monocytes from sepsis non-survivors exhibited substantially reduced IL-12 production compared to the cells from sepsis survivors (75). Importantly, impaired preoperative monocyte IL-12 production was identified by multivariate analysis as an independent predictive factor for the development of lethal postoperative sepsis. Consistent with these data, host defense was found to be dependent on IL-12 and the IL-12-regulated cytokine IFN-gamma in models of polymicrobial septic peritonitis and Escherichia coli-induced peritoneal sepsis (93-95).

Collectively, these studies suggest that the TNFB2 homozygous genotype and impaired monocyte IL-12 production are risk factors for the development of severe complications and lethal sepsis after elective surgery.

7.3. Early prediction of lethal outcome during postoperative sepsis

The prognosis of abdominal sepsis depends on early diagnosis and brisk commencement of therapy. Early prediction of outcome soon after sepsis onset would therefore be of great help when treating septic patients. A large number of studies have examined immune functions during the course of sepsis, and certain alterations have even been shown to correlate with outcome (96-99). Nevertheless, only a few studies have addressed the question as to whether these alterations are present at sepsis onset and if they represent independent predictors of lethal outcome as assessed by multivariate analyses.

Correlations between immune-mediator concentrations in plasma and the outcome of sepsis are complex. Investigation of 63 patients with a follow-up of 12 months revealed that decreased serum levels of soluble L-selectin during the septic course are associated with a high mortality rate (99). Ikuta *et al.* showed that IL-18 and IL-10 were increased in the peritoneal fluid of patients with culture-positive peritonitis. Interestingly, IL-18 concentration in the peritoneal fluid seemed to reflect the severity of peritonitis (97). The immune regulatory cytokines IL-12 and IL-18 were also measured over time in 66 patients with postoperative sepsis (100). IL-12 levels were significantly reduced in sepsis patients compared with control surgical patients without sepsis but did not differ significantly between survivors and non-survivors. In contrast, IL-18 serum concentrations were significantly increased in patients with lethal sepsis compared with sepsis survivors at all time points studied, including day 1

of sepsis diagnosis. Notably, logistic regression analysis of IL-18 values measured on day 1 or 2 of sepsis revealed that high serum IL-18 represents an early predictive factor for the lethal outcome of postoperative sepsis. These studies are consistent with the concept that IL-12 contributes to protective immune reactions during sepsis, whereas IL-18 may preferentially promote organ injury and lethal shock (100).

In accordance with the detection of high concentrations of systemic inflammatory mediators during sepsis, Arnalich *et al.* demonstrated that early measurement of the activation of NF-kappaB, a transcriptional regulator of proinflammatory cytokine expression, may help predict the outcome of sepsis. That study evaluated NF-kappaB activation in peripheral blood mononuclear cells (PMBC) of 34 patients with severe sepsis. NF-kappaB activity was significantly higher in non-survivors and correlated strongly with sepsis severity as defined by the APACHE II score (101).

Identification of early predictive indicators of sepsis severity and lethal outcome is a focus of current research. Systemic neuropeptide levels were analyzed in 61 patients with sepsis after major visceral surgery and 23 control patients without sepsis (68). As noted above, postoperative sepsis was associated with a significant increase in CGRP serum levels. Systemic CGRP levels were significantly higher in non-survivors than in survivors as early as day 1 of sepsis and remained significantly elevated in non-survivors throughout the entire course of the sepsis. SP levels were also elevated in sepsis patients compared with those in controls, but significant differences between survivors and non-survivors were observed only during the final phase of sepsis. Thus, high systemic CGRP and SP levels may reflect early and late predictive indicators of sepsis lethality, respectively (68).

There has been great interest in evaluating whether procalcitonin (PCT) might serve as a parameter for the diagnosis of sepsis or as a predictor of lethal outcome. Resch *et al.* demonstrated that PCT is a sensitive, early diagnostic parameter for sepsis in neonates (102). Wunder *et al.* investigated PCT serum levels and the APACHE III score in 33 patients with severe sepsis. Multivariate data analysis revealed a significant positive correlation between the APACHE III score, PCT levels, and sepsis outcome (98). Furthermore, in 170 patients with postoperative abdominal sepsis, PCT serum levels and the APACHE II score were measured on the first day of sepsis diagnosis. In a multivariate analysis, both PCT and the APACHE II score were identified as independent, early predictive indicators of sepsis lethality. Thus, determination of PCT levels and APACHE II scores allows a sensitive, specific prognostic evaluation as early as the time of sepsis onset (103).

7.4. Successful surgical treatment of abdominal sepsis

PCT seems to be an appropriate candidate to fulfill these requirements. PCT can be assessed quantitatively in serum using a commercially available immunoluminometric assay, which is easy to perform and can be completed within 2 hours. PCT is highly stable in collected blood samples and so can be collected with routine laboratory specimens without any need for special storage conditions (104). After an initial infectious stimulus, plasma levels of PCT increase sharply after a latency of 2 hours, reaching peak values within 12 to 24 hours and then plateau (105-107). Serum PCT has a half-life of approximately 20 to 24 hours (105, 108-110) and is not prolonged in patients with impaired kidney function (106, 109). PCT therefore offers an adequately sized diagnostic window for a routine clinical use of this parameter. Serum levels of PCT were evaluated in 104 patients with sepsis resulting from a perforation in the gastrointestinal tract undergoing operative intervention for treatment of the septic focus. Because early information about the success of the operative intervention is crucial, the study focused on serum PCT levels during the first 48 hours postoperatively. A significant decrease in PCT serum levels was observed in patients with successful operative eradication of the infectious focus with the initial laparotomy; however, in patients with a persisting infectious focus the serum PCT did not decrease. This relationship is best expressed by the PCT ratio between postoperative days 1 and 2 after the initial laparotomy, or the FI. Using classification and regression tree (CART) analysis, a cutoff value could be found for the FI that distinguished between patients with successful eradication of the septic focus and those with persisting infection with an adequate specificity (63%) and high sensitivity (95%). In contrast, the APACHE II score did not aid in the early detection of persisting infection; however, the use of APACHE II ratios did help select a patient subgroup in which use of the FI yielded better specificity (75%) and sensitivity (97.5%). Those patients, who do not show early clinical improvement after the index procedure, as quantified by the APACHE II score in this study, present the real problem in clinical practice and may profit from the use of this method.

8. CONCLUSION

Many biomarkers are proposed for sepsis, but none has sufficient specificity or sensitivity to be used routinely in clinical practice. PCT and CRP have been most widely used, but their value for distinguishing sepsis from other inflammatory conditions or to predict outcome is limited. Because of the complexity of the immune response and the heterogeneity in the etiopathology of sepsis, it is improbable that a single, ideal biomarker will ever be found.

Therefore, different animal models of sepsis representing different types of septic conditions are important for detection and evaluation of markers for each disease pattern and immunological condition that a patient may have. Secondary peritonitis can occur as a postoperative complication after visceral surgery. The aim of clinical monitoring is early identification of the complication before secondary organ failure aggravates the problem already present. Frequently, problems do not emerge from the complication itself but from inadequate acquaintance with and management of the complication;

that is, the diagnostic process is started too late, and therapy is insufficient. Well-evaluated biomarkers from specific animal models of sepsis could help to reduce mortality after major surgery.

The available animal models of sepsis are very important for investigating the different phases of the clinical course of sepsis in humans, but there are several limitations. The animals used for these studies are matched in age and sex and kept under sterile conditions, which are circumstances that will never appear with human patients who may have several additional diseases. To address these issues, Doi *et al.* and several others modified certain parameters of the standard CLP procedures (13, 111-113). For example, they used older mice (16 to 50 weeks old instead of 8 to 12). The aged mice showed a significantly higher mortality after CLP or LPS injection, and several mediators (IL-6, IL-10, IL-1beta) tended to be higher in the circulating blood in the aged animals (112). To date there is no animal model that directly resembles complex human sepsis. With all the animal models described above it is possible to take a closer look at the normally balanced sepsis inflammatory response to pathogens, including the systemic inflammatory response (SIRS) and the compensatory anti-inflammatory response (CARS), and on the imbalance of SIRS and CARS leading to septic shock and organ dysfunction (114, 115).

Our study on the mRNA expression of a wide range of cytokines, chemokines and neuropeptides known to be somehow associated with inflammation revealed some new potential markers for sepsis, including CCR8, CCL7, CCL19, CXCL5 and CXCL13. In future studies it will be important to elucidate whether the serum levels of these markers also increase in animal models and if these markers are also change in humans under septic conditions.

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Abbreviations: 14G: 14-gauge, Abcf1: ATP-binding cassette sub-family F member 1, Alpha-MSH: alpha-melanocyte stimulating hormone, ANP: atrial natriuretic

peptide, APACHE: acute physiology and chronic health evaluation, AVG: average, BAL: the bronchoalveolar lavage, BCA: bicinchoninic acid, Bcl6: B-cell leukemia/lymphoma 6, BNP: brain natriuretic peptide, C5a: complement component 5 a, CA: catecholamines, cAMP: cyclic adenosine monophosphate, CARS: compensatory anti-inflammatory reaction syndrome, CART: classification and regression tree, CASP: colon ascendens stent peritonitis, Casp1: caspase 1, CCL: CC-chemokine ligand, CCR: CC-chemokine receptor, CFU: colony forming unit, CGRP: calcitonin gene-related peptide, CLP: cecal ligation and puncture, CNS: central nervous system, CRH: corticotropin-releasing hormone, CRP: C-reactive protein, Ct: cycle threshold, CXCL: CXC-chemokine ligand, CXCR: CXC-chemokine receptor, d: delta, DIC: disseminated intravascular coagulopathy, ELAM: endothelial leukocyte adhesion molecule, ELISA: enzyme-linked immunosorbent assay, G-CSF: granulocyte colony-stimulating factor, GM-CSF: granulocyte-macrophage colony-stimulating factor, GOI: gene of interest, GRO-alpha: growth regulated oncogene, HKG: housekeeping gene, HLA: human leukocyte antigen, HPRT: hypoxanthine phosphoribosyltransferase 1, i.p.: intra-peritoneal, i.v.: intra-venous, IFITM 1: interferon-induced transmembrane protein 6, IFN: interferon, IL: interleukin, IP-10: interferon gamma-induced protein 10 kDa, Itgam: Integrin alpha M, Itgb2: Integrin beta 2, KC: cytokine induced neutrophil chemoattractant, LBP: lipopolysaccharide inhibitory factor, LPS: bacterial lipopolysaccharide, Lta / Ltb: lymphotoxin A/B, MCP: monocyte chemotactic protein, MHC: major histocompatability complex, MIF: macrophage migration inhibitory factor, MIP: macrophage inflammatory protein, MMP: matrix metallopeptidase, MOF: multiple organ failure, NF-kappaB: nuclear factor kappaB, NPY: neuropeptide Y, PBMC: peripheral blood mononuclear cells, PCT: procalcitonin, PKA: protein kinase A, PMN: polymorphonuclear neutrophils, PNS: peripheral nervous system, PPTA: preprotachykinin A, qRT-PCR: quantitative real-time polymerase chain reaction, RANTES: regulated upon activation normal T-cell expressed and secreted, RPL4: ribosomal protein 4, SAPS: simplified acute physiology score, Scye1: small inducible cytokine subfamily E member 1, Spp1: secreted phosphoprotein 1, sICAM: soluble inter-cellular adhesion molecule 1, SIRS: systemic inflammatory response syndrome, SOFA: sequential organ failure assessment, SP: substance P, TGF: transforming growth factor, Th: T helper cell, TIMP: tissue inhibitor of metalloproteinases, TNF: tumor necrosis factor, Tollip: Toll interacting protein, TREM: triggering receptor expressed on myeloid cells, VIP: vasoactive intestinal peptide

Key Words: Sepsis, Biomarkers, LPS, CLP, CASP, Cytokine, Chemokine, Neuropeptide

Send correspondence to: Klaus Emmanuel, Department of Surgery, Paracelsus Medical University, Muellner Hauptstrasse 48, A-5020 Salzburg, Austria, Tel: 43-662-4482-51099, Fax: 43-662-4482-51008, E-mail: k.emmanuel@salk.at