phase of sepsis may counteract the cytokine storm. Furthermore, stabilization of Pellino3 by inhibition of autophagy in the hypoinflammatory phase of sepsis may improve immunity. In consideration of these two conflictive sepsis phases, modulation of Pellino3 may provide a new strategy for the development of a therapy approach in sepsis.

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Attenuated NOX2 expression impairs ROS production during the hypoinflammatory phase of sepsis

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Background: The multicomponent phagocytic NADPH oxidase produces reactive oxygen species (ROS) after activation by microorganisms or inflammatory mediators [1]. In the hypoinflammatory phase of sepsis, macrophages are alternatively activated by contact with apoptotic cells or their secretion products. This inhibits NADPH oxidase and leads to attenuated ROS production [2] and furthermore contributes among others to a hyporeactive host defense. Due to this immune paralysis, sepsis patients suffer from recurrent and secondary infections [3]. We focused on the catalytic subunit of NADPH oxidase, the transmembrane protein NOX2 [4]. We assume that after induction of sepsis the expression of NOX2 is reduced and hence ROS production is decreased.
Methods: We induced polymicrobial sepsis in mice by cecal ligation and puncture. The ability of peritoneal macrophages (PMs) to produce ROS was determined by FACS via hydroethidine assay. NOX2 expression of PMs was determined by western blot and qPCR. To elucidate the mechanism causing mRNA destabilization, we performed *in vitro* experiments using J774 macrophages. To obtain an alternatively activated phenotype, macrophages were stimulated with conditioned medium from apoptotic T cells (CM). By luciferase assays we figured out a 3'UTR-dependent regulation of NOX2 mRNA stability. Assuming that a protein is involved in the mRNA degradation, we performed a RNA pulldown with biotinylated NOX2-3'UTR constructs followed by mass spectrometry. We verified the role of SYNCRIP by siRNA approach. Additionally, we overexpressed NOX2 in J774 cells and analyzed the ROS production (w/wo CM treatment) by FACS.
Results: We found an impaired expression of NOX2 at RNA and protein level along with decreased ROS production after induction of sepsis in mice as well as stimulating J774 macrophages with CM of apoptotic T cells. This is due to a time-dependent NOX2 mRNA degradation depending on SYNCRIP, a RNA-binding protein, which stabilizes NOX2 mRNA through binding to its 3'UTR under normal conditions. In line, knockdown of SYNCRIP also decreases NOX2 mRNA expression. We assume that a CM-dependent modification or degradation of SYNCRIP prevents its stabilizing function. As the overexpression of NOX2 restores ROS production of CM-treated J774 cells, we assume that NOX2 expression is crucial for maintaining NADPH activity during the hypoinflammatory phase of sepsis.
Conclusion: Our data imply a regulatory impact of SYNCRIP on NOX2 stability during the late phase of sepsis. Therefore, further understanding of the regulation of NADPH oxidase could lead to the design of a therapy to reconstitute NADPH oxidase function, finally improving immune function in sepsis patients.

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Kinetic characterization of selective peroxisome-proliferator-activated receptor gamma modulators *in vitro*

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Background: The ligand-activated transcription factor, peroxisome-proliferator-activated receptor gamma (PPARγ), has been shown to play an essential role in immunosuppression during sepsis. PPARγ is upregulated in T cells of septic patients, sensitizing these cells to PPARγ-dependent apoptosis and thus contributing to T-cell depletion [1,2]. In the polymicrobial cecum ligation and puncture (CLP) sepsis model in mice, both T-cell-specific gene knockout (Lck-Cre PPARγ$^{fl/fl}$) and systemic pharmacological PPARγ antagonism by GW9662 improved survival [3]. Because GW9662 was only effective when applied 3 hours after CLP, we were interested to extend this time frame. For this reason we characterized the kinetics of SPPARγMs when administered before or in combination with the agonist thiazolidinedione, rosiglitazone.
Methods: A PPARγ-dependent transactivation assay was used in HEK293T cells. It is based on the vector pFA-PPARγ-LBD-GAL4-DBD encoding the hybrid protein PPARγ-LBD-GAL4-DBD and the reporter vector pFR-Luc, carrying a GAL4-responsive element in front of the *Firefly* luciferase gene. These two vectors were co-transfected, in combination with a control vector encoding *Renilla* luciferase (pRL-CMV) to normalize *Firefly* luciferase activity for transfection efficiency. Following transfection, cells were incubated with the SPPARγMs F-MOC and MCC-555 and the PPARγ antagonist GW9662 for different times (2 to 48 hours) and at increasing doses (0.01 to 10 μM), with or without rosiglitazone (0.01 to 10 μM). Transactivation was analyzed using a 96-well plate format.
Results: Rosiglitazone transactivated PPARγ in a time-dependent and dose-dependent manner, the response gradually increasing to a maximum at 48 hours with 10 μM. Low concentrations (0.01 to 0.1 μM) of SPPARγMs F-MOC and MCC-555 and the PPARγ antagonist GW9662 all exerted dose-independent antagonistic effects at an early incubation time point (2 hours). From 10 hours onwards, MCC-555 and GW9662, given alone, both exerted PPARγ agonistic effects, MCC-555 in parallel to responses to rosiglitazone, but GW9662 with characteristics of partial antagonism. F-MOC showed no dose-dependent effect at any concentration at later time points. Only GW9662 (1 to 10 μM) was able to inhibit rosiglitazone (0.1 to 1 μM)-induced PPARγ transactivation after 10 hours.
Conclusion: Our kinetic analysis reveals clear differences in the modulatory characteristics of PPARγ inhibitors, with previously unreported early inhibitory effects and late agonistic or partial agonistic activity. New SPPARγMs with extended inhibitory activity may prove useful in the therapy of sepsis.

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IL-6 and IFNγ play a role in fatal cases of 5N1 influenza in children

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Background: Fatal human critical cases associated with influenza A subtype H5N1 have been documented in Bandung, Indonesia. Of four children, three died. We determined the level of cytokines and chemokines in those patients.
Methods: The Luminex method was used to look for the profile of cytokine and chemokine gene expression induced by H5N1 influenza virus from patient's serum.
Results: We found that H5N1 influenza virus in the dead children was a more potent inducer of IL-6, the level being higher (17.00, 74.31, 85.75) than in the one child who survived (4.78). The IFNγ level of the fatal causes was also higher (21.43, 31.75, 384.38) than in the one child who recovered (5.51). This suggested that a cytokine storm may play a role in the pathogenesis of fatal H5N1 cases.
Conclusion: The H5N1 influenza A virus is a potent inducer of proinflammatory cytokines and chemokines. This hyperinduction of cytokines may be relevant to mortality of children with H5N1 infection.

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Involvement of thrombopoietin in the development of organ injury in a mouse model of cecal ligation and puncture-induced sepsis

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Background: Sepsis-induced organ damage is a leading cause of death in critically ill patients. Thrombopoietin (TPO) is a humoral growth factor mainly involved in regulation of platelet number and function. High circulating levels of TPO are detectable in septic adults and children and are related to sepsis severity. We have previously shown a correlation between TPO levels and platelet activation in septic burned patients, where circulating activated platelets may cause microthrombotic events that lead to organ damage. Our aim was to evaluate the contribution of TPO in organ injury in a murine model of polymicrobial sepsis. To this end, we synthesized and used a chimeric fusion protein, named mouse TPO Receptor-Maltose Binding Protein (mTPOR-MBP), able to inhibit TPO biological activity.
Methods: Male C57BL/6 mice were randomized to cecal ligation and puncture-induced sepsis (CLP) or to laparotomy (sham) surgery. CLP mice received 40 μg mTPO-MBP or sterile saline 10 minutes after surgery. Immediately after and 6 hours after surgery all animals received 0.08 mg/kg buprenorfin in 1.5 ml sterile saline subcutaneously. After 18 hours blood was collected from the cava vein and used for cell count, flow cytometric analysis of leukocyte-platelet interaction and to obtain plasma. Plasma TPO levels were determined by ELISA. The lung and liver were excised and fixed in 4% paraformaldehyde solution. An expert pathologist blinded to experimental groups quantified organ damage.
Results: Plasma TPO levels were significantly higher in septic mice (nine mice in each group). Leukocyte and platelet counts did not significantly differ in the CLP group treated with mTPOR-MBP compared with the CLP control group. In contrast, the percentage of monocyte-platelet aggregates, a marker of platelet activation, was significantly reduced after treatment with mTPOR-MBP. Moreover, TPO blockade by mTPOR-MBP administration induced a significant reduction of histological alterations in the lung, as evaluated by neutrophil infiltration and thickening of the alveolar-capillary membrane, and liver tissue samples, as evaluated by the number of microabscesses.
Conclusion: Increased circulating levels of TPO during experimental sepsis may have a role in the development of organ damage. Inhibition of TPO biological activity may represent a novel promising therapeutic approach to prevent organ failure in severe sepsis.

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Cholecystokinin protects rats against *Staphylococcus aureus*-induced sepsis

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Background: Cholecystokinin (CCK) was firstly described as a gastrointestinal hormone, but immune cells express their receptors, suggesting a possible involvement of this peptide in pathophysiological processes. Our aims were to evaluate the role of CCK on resistance against Gram-positive *Staphylococcus aureus*-induced sepsis, as well as cell influx to infectious focus. Furthermore, since nitric oxide, TNFα and IL-10 play a key role in the innate immune system controlling bacterial infection, we also evaluated the synthesis of these inflammatory mediators during this sepsis model.
Methods: Male Wistar rats (180 to 200 g) received an intraperitoneal injection of proglumide (P) (nonselective CCK receptor antagonist; 30 or 50 mg/kg) 30 minutes before bacterial *S. aureus* inoculum (0.5 to 1 × 10^{10} CFU/animal). At 4 and 24 hours after sepsis induction, blood and peritoneal lavage fluid (PLF) were collected for microbiological analysis, cytokines and nitrate quantifications and also differential cell counting. Nitrate was detected by chemiluminescence, while TNFα and IL-10 were determined by ELISA sandwich kits.
Results: The pretreatment with P at higher dose (50 mg/kg) increased bacteremia in comparison with the saline-injected group (2,052 ± 810.7 vs. 154.3 ± 47.0 CFU/ml, $P < 0.01$) at 4 hours after sepsis induction. At the same time point, the bacterial counting in PLF increased in a dose-dependent manner in the P-treated rats ($P < 0.05$). On the other hand, only the higher P dose elevated significantly the CFU/ml in the PLF at 24 hours (97.75 ± 12.77 × 10^4 vs. 35 ± 10.05 × 10^4 CFU/ml, $P < 0.05$). The plasma TNFα and nitrate concentrations were not changed by treatment or time after sepsis induction. However, the administration of CCK receptors antagonist reduced the TNFα production in comparison with the control group in PLF, at both time points. The plasma IL-10 concentration increased at 4 hours in P-treated rats, while at 24 hours it was reduced (85.83 ± 48.0 vs. 1,698 ± 265.6 pg/ml, $P < 0.001$). In PLF, the rats pretreated with P reduced the IL-10 measurements ($P < 0.05$) when compared with the control group. In agreement, the macrophage influx to peritoneal infectious focus was compromised by treatment with a high P dose at 24 hours after *S. aureus*-induced sepsis (3,947.73 ± 269.99 vs. 5,629.61 ± 786.90 cells/μl; $P < 0.05$). Moreover, the neutrophil count did not change among the experimental groups (6,860.59 ± 211.90 vs. 6,273.54 ± 798.91 cells/μl).
Conclusion: These data suggest a protective role for CCK peptide during *S. aureus*-induced sepsis, modulating the systemic and local inflammatory response, as well as increasing the macrophage influx to the infectious focus.

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Polymyxin B-direct hemoperfusion therapy contributes to oxygen delivery in septic patients

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Background: Since 1994, Polymyxin B-direct hemoperfusion (PMX-DHP) (Toraymyxin®; Toray Medical Co., Tokyo, Japan) has been approved as