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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