

expression was only slightly reduced. To analyze a more specific TLR-3 target, we measured IP-10 mRNA expression, which was also significantly reduced in siNinj1-transfected cells (Figure 1E).

Conclusion: Our *in vitro* data strongly indicated that Ninj1 is involved in regulation of TLR signaling and therewith contributes to inflammation. *In vivo* experiments will clarify its impact on systemic inflammation.

References

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P45

Effect of *Calotropis procera* latex extracts on the hypothalamic TNF α and PGE $_2$ levels in the rat model of yeast-induced pyrexia

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Critical Care 2012, **16**(Suppl 3):P45

Background: Sepsis, a common cause of morbidity and mortality in critically ill patients, is associated with systemic inflammatory response syndrome due to upregulation of cyclooxygenase-2 and increase in the levels of PGE $_2$. It is also associated with increase in the levels of proinflammatory cytokines like TNF α and IL-1 β . *Calotropis procera* is a plant that grows in the wild producing latex. The aqueous and methanolic extracts of dried latex of this plant (AqDL and MeDL) and proteins isolated from the fresh latex (LP) have shown anti-inflammatory and anti-arthritis properties. AqDL and MeDL are orally effective, LP is effective parenterally. The current study was designed to evaluate the efficacy of these extracts against yeast-induced pyrexia and the levels of TNF α and PGE $_2$ in the hypothalamus of rats.

Methods: Pyrexia was induced in rats by subcutaneous injection of yeast in the nape of the neck and the rectal temperature was measured at 0 hours (basal temperature), 3 hours and 6 hours. Rats were divided into groups ($n = 6$) and were treated with AqDL and MeDL given orally and LP given intravenously at 6 hours. Group I: NC (normal control); Group II: YC (yeast control); Group III: AqDL (200 mg/kg); Group IV: AqDL (400 mg/kg); Group V: MeDL (100 mg/kg); Group VI: MeDL (250 mg/kg); Group VII: LP (5 mg/kg); Group VIII: LP (25 mg/kg); Group IX: paracetamol (PCM 100 mg/kg). Rectal temperature was measured hourly until 9 hours. The levels of TNF α and PGE $_2$ were measured in the excised hypothalamus region of the brain using ELISA kits.

Results: Subcutaneous injection of yeast produced a marked increase in rectal temperature of rats with a maximum effect at 6 hours (101.17°C). Like paracetamol, treatment of rats with AqDL and MeDL produced a significant decrease in body temperature from 101.17°C at 6 hours to 97.9°C and 98.2°C at 9 hours respectively at higher doses and their effect was dose dependent while LP was found to be ineffective. The present study shows that treatment with yeast increased the tissue levels of TNF α (23.78 pg/mg) and PGE $_2$ (66.48 pg/mg) as compared with the NC group (16.31 and 41.35 respectively). All of the fractions lowered the hypothalamic TNF α level while a marked reduction in PGE $_2$ levels was observed with orally effective fractions, namely AqDL and MeDL.

Conclusion: Our results demonstrate that the orally administered fractions of latex of *C. procera* are effective in attenuating yeast-induced pyrexia and this effect is mediated through reduction in the levels of PGE $_2$.

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Regulation of sepsis-induced IFN γ upon natural killer cell or natural killer T cell depletion *in vivo*

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Critical Care 2012, **16**(Suppl 3):P46

Background: Natural killer (NK) and natural killer T (NKT) cells play a key role in bacterial infection and sepsis since they contribute to the bridging of innate and acquired immune responses. We have previously shown that *in vivo* depletion of these cell populations in a murine pneumococcal pneumonia sepsis model affected mortality.

Methods: Four groups of C57BL/6 mice ($n = 5$ to 15 mice/group) were infected intratracheally with 5×10^5 CFU *Streptococcus pneumoniae*. Twenty-four hours prior to bacterial inoculation, NK cell depletion was achieved by intravenous (i.v.) administration of anti-asialoGM1 rabbit polyclonal antibody in one group (NK^{DEPL}), or anti-CD1d monoclonal antibody, clone 1B1 was given for NKT cell depletion in a second group (NKT^{DEPL}). The control group received equal volume of isotype antibody control i.v. (C) and a fourth group received sham intratracheal installation of normal saline (S). All animals were euthanized 48 hours post infection. Serum and tissue samples were analyzed for bacterial colony counts, cytokine levels, splenocyte apoptosis rates and cell population analysis by flow cytometry. In parallel, specific miRNA expression analyses in splenocytes and lung histologic examination were also performed. Comparisons of numeric data between groups were made using the one-way ANOVA test for multiple groups.

Results: We found that upon NK cell depletion there was a significant increase in the spleen NKT (CD3⁺/CD1d⁺) cell population compared with NKT^{DEPL}, C and S ($P = 0.014$, $P = 0.021$ and $P = 0.033$, respectively). Interestingly, upon NKT cell depletion, spleen NK (CD3⁺/NK1.1⁺) cells increased significantly compared with NK^{DEPL}, C and S ($P < 0.0001$, $P < 0.0001$ and $P = 0.001$, respectively). NKT depletion led to decreased lymphocyte apoptosis compared with C ($P = 0.035$), higher bacterial load in the lung compared with C and NK^{DEPL} ($P = 0.014$ and $P = 0.022$ respectively) and in the liver compared with C ($P = 0.012$). In addition, serum levels of IFN γ were significantly increased and splenocytes from NKT depleted animals, incubated *ex vivo* in the presence or absence of IL-2, produced more IFN γ in comparison with all other groups. Furthermore, splenocyte miRNA analysis showed that miR-200c and miR-29a were downregulated, while miR-125a-5p was upregulated, in the NKT depleted animals compared with all other groups.

Conclusion: For the first time we have shown that NKT cell depletion resulted in an increase in spleen NK (CD3⁺/NK1.1⁺) cells and a higher IFN γ production, which were associated with specific changes in splenocyte miRNA expression.

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Pattern recognition receptors as key players in adrenal gland dysfunction during sepsis

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Critical Care 2012, **16**(Suppl 3):P47

Background: Undergoing systemic inflammation, the innate immune system releases excessive proinflammatory mediators, which finally can lead to organ failure. Pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) and NOD-like receptors (NLRs), form the interface between bacterial and viral toxins and innate immunity. During sepsis, patients with diagnosed adrenal gland insufficiency are at high risk of developing a multiorgan dysfunction syndrome, which dramatically increases the risk of mortality. To date, little is known about the mechanisms leading to adrenal dysfunction under septic conditions. Here, we investigated the sepsis-related activation of the PRRs, cell inflammation, and apoptosis within adrenal glands.

Methods: Two sepsis models were performed: the *polymicrobial sepsis model (caecal ligation and puncture (CLP))* and the LTA-induced intoxication model. All experiments received institutional approval by the Regierungspräsidium Darmstadt. CLP was performed as previously described [1], wherein one-third of the caecum was ligated and punctured with a 20-gauge needle. For LTA-induced systemic inflammation, TLR2

knockout (TLR2^{-/-}) and WT mice were injected intraperitoneally with pure LTA (pLTA; 1 mg/kg) or PBS for 2 hours. To detect potential direct adrenal dysfunction, mice were additionally injected with adrenocorticotrophic hormone (ACTH; 100 µg/kg) 1 hour after pLTA or PBS. Adrenals and plasma samples were taken. Gene expressions in the adrenals (rt-PCR), cytokine release (multiplex assay), and the apoptosis rate (TUNEL assay) within the adrenals were determined.

Results: In both models, adrenals showed increased mRNA expression of TLR2 and TLR4, various NLRs, cytokines as well as inflammasome components, NADPH oxidase subunits, and nitric oxide synthases (data not shown). In WT mice, ACTH alone had no effect on inflammation, while pLTA or pLTA/ACTH administration showed increased levels of the cytokines IL-1β, IL-6, and TNFα. TLR2^{-/-} mice indicated no response as

expected (Figure 1, left). Interestingly, surviving CLP mice showed no inflammatory adrenal response, whereas nonsurvivors had elevated cytokine levels (Figure 1, right). Additionally, we identified a marked increase in apoptosis of both chromaffin and steroid-producing cells in adrenal glands obtained from mice with sepsis as compared with their controls (Figure 2).

Conclusion: Taken together, sepsis-induced activation of the PRRs may contribute to adrenal impairment by enhancing tissue inflammation, oxidative stress and culminate in cellular apoptosis, while mortality seems to be associated with adrenal inflammation.

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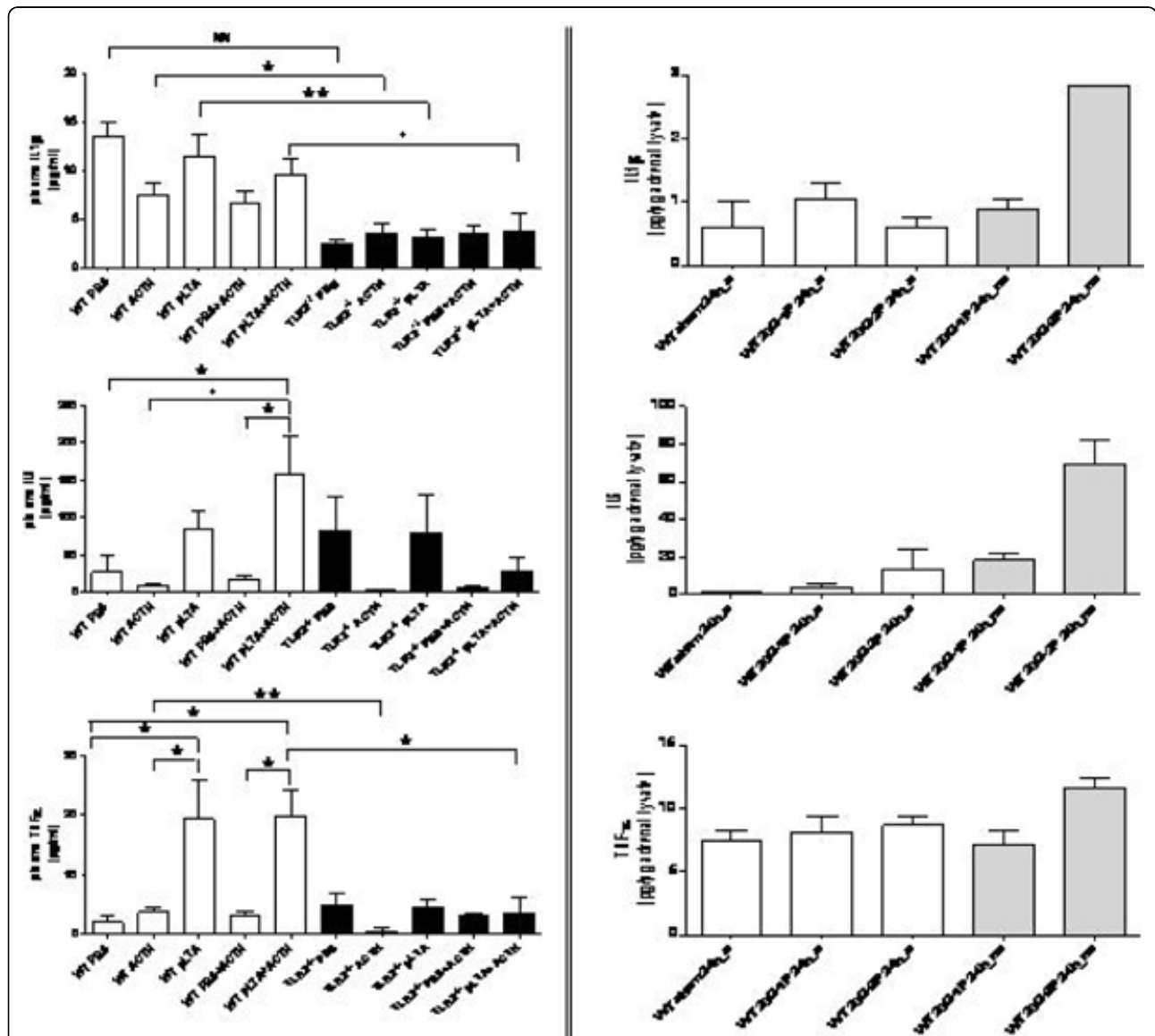


Figure 1 (abstract P47) Cytokine concentrations of IL-1β, IL-6 and TNFα in plasma and adrenal lysates. From WT and TLR2^{-/-} mice treated with PBS (2 hours), ACTH (100 µg/kg; 1 hour), pLTA (1 mg/kg; 2 hours) or PBS/ACTH and pLTA/ACTH (left) and from WT mice that underwent CLP sham operation (sham), CLP with single (CLP-1P) or double (CLP-2P) puncture (right). Animals that survived after 24-hour CLP are labelled 's' and deceased ones 'ns'. Data are presented as mean ± SEM (n ≥ 6). Statistical significance was determined by one-way ANOVA and Bonferroni's post test (vs. PBS within one group) or t test (WT vs. TLR2^{-/-}). *P < 0.05, **P < 0.01, +P < 0.005, ++P < 0.001, #P < 0.0005, ##P < 0.0001.

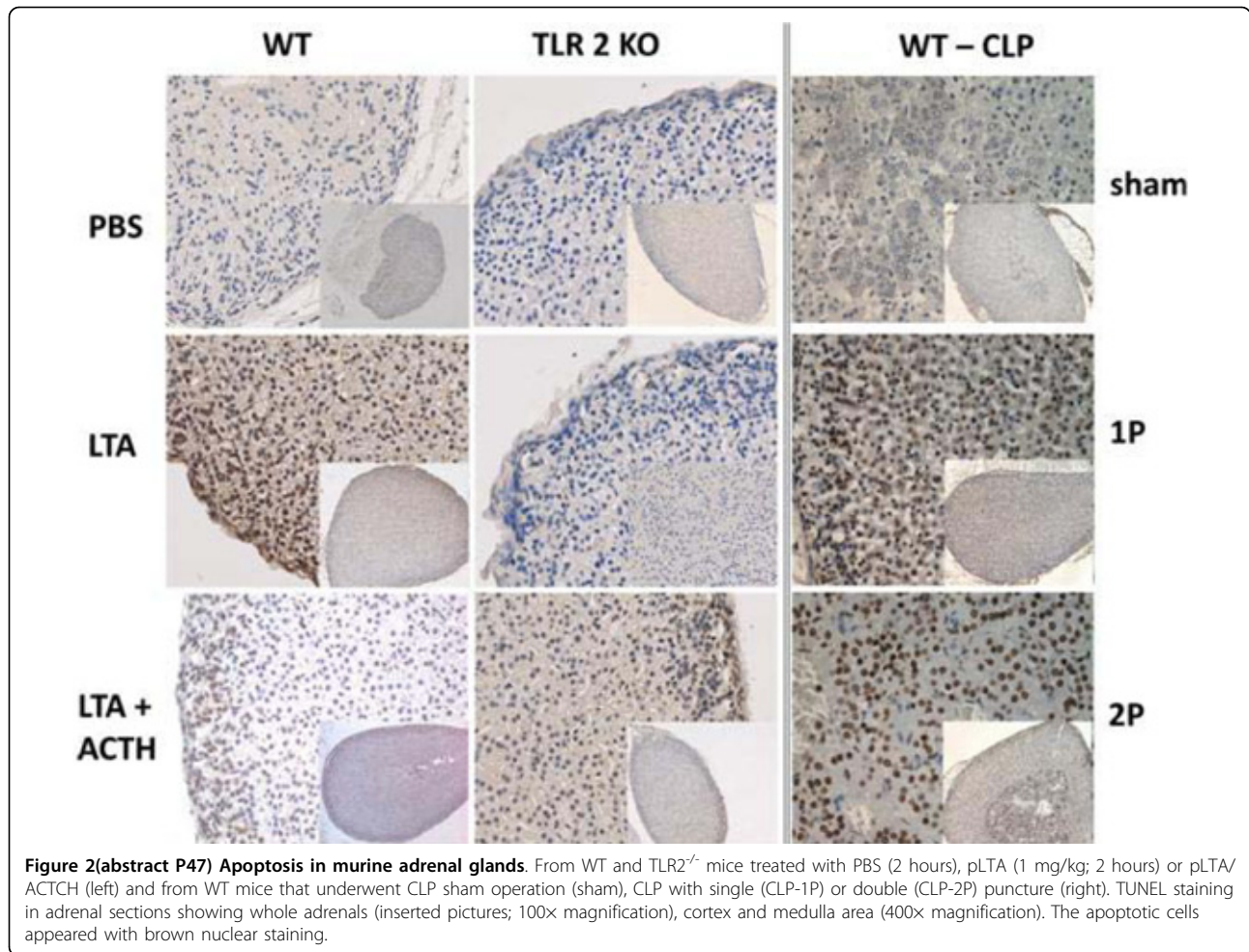


Figure 2(abstract P47) Apoptosis in murine adrenal glands. From WT and TLR2^{-/-} mice treated with PBS (2 hours), pLTA (1 mg/kg; 2 hours) or pLTA/ACTCH (left) and from WT mice that underwent CLP sham operation (sham), CLP with single (CLP-1P) or double (CLP-2P) puncture (right). TUNEL staining in adrenal sections showing whole adrenals (inserted pictures; 100x magnification), cortex and medulla area (400x magnification). The apoptotic cells appeared with brown nuclear staining.

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Effects of a TREM-like transcript-1 derived peptide during septic shock in pigs

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Critical Care 2012, **16**(Suppl 3):P48

Background: Triggering receptor expressed on myeloid cells-1 (TREM-1) is expressed on innate immune cells and plays a crucial role during the onset of sepsis by amplifying the host immune response. TREM-like transcript-1 (TLT-1) belongs to the TREM family and is selectively expressed on activated platelets. We recently showed that TLT-1 and a TLT-1-derived peptide (LR12) exhibit anti-inflammatory properties by dampening TREM-1 signalling and thus behave as naturally occurring TREM-1 inhibitors [1]. We also, however, demonstrated that the same peptide modulates *in vivo* the inflammatory cascade triggered by infection, thus inhibiting hyper-responsiveness, organ damage and death during sepsis in mice. As mouse models of septic shock are far from recapitulating the human physiology, we investigated the effects of LR12 during peritonitis in adult mini-pigs. Here we show that sepsis-induced cardiovascular dysfunction and organ failure was prevented by LR12 administration. The objective was to determine the effects of a TLT-1

derived peptide (LR12) administration during septic shock in pigs (13 adult male mini-pigs).

Methods: Two hours after induction of a fecal peritonitis, anesthetized and mechanically ventilated mini-pigs were randomized to receive LR12 ($n = 6$) or its vehicle alone (normal saline, $n = 5$). Two animals were operated and instrumented without the induction of peritonitis and served as controls (sham). Resuscitation was achieved using hydroxyethyl starch (up to 20 ml/kg) and norepinephrine infusion (up to 10 µg/kg/minute).

Results: Hemodynamic parameters were continuously recorded. Gas exchange, acid-base status, organ function, and cytokines were measured at regular intervals until 24 hours after the onset of peritonitis when animals were sacrificed under anesthesia. Peritonitis induced profound hypotension, myocardial dysfunction, lactic acidosis, coagulation abnormalities, and multiple organ failure. These disorders were largely attenuated by LR12. In particular, cardiovascular failure was prevented as attested by better mean arterial pressure, cardiac index, cardiac power index, and SvO₂, despite lower norepinephrine requirements (Figure 1). Finally, 24-hour mortality rates were respectively 60% and 0% for control and LR12 groups.

Conclusion: LR12, a TLT-1 derived peptide, exhibits salutary properties during septic shock in adult mini-pigs.

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