P43

Procalcitonin, IL-10 and sCD25 as diagnostic and prognostic markers in critically ill patients

A Focà[1*], MC Liberto[1], L Rametti[1], A Giancotti[1], N Marascio[1], A Quirino[1], S Caroleo[2], A Renzulli[3], G Matera[1]
[1]University of Catanzaro Institute of Microbiology, Catanzaro, Italy; [2]University of Catanzaro Anesthesiology Unit, Catanzaro, Italy; [3]University of Catanzaro Cardiac Surgery Unit, Catanzaro, Italy
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Background: Diagnostic and prognostic markers are of outmost importance in the critical patient referred to the ICU. The aim of the present study was the evaluation of procalcitonin (PCT), IL-10 and soluble CD25 (sCD25) as potential markers in the diagnosis and prognosis of cohorts of critical patients evaluated following a prospective design in a tertiary-center university hospital.
Methods: We studied 52 consecutive SIRS patients with suspected sepsis that were firstly stratified based on culture results (culture-positive and culture-negative), then subsequently divided into survivors and nonsurvivors. Venous blood samples were obtained from each patient within 6 hours of hospital/ICU admission (T-0) and at the end of first week of hospital/ICU stay (T-1). Serum samples were collected and used for simultaneous determination of IL-10 and sCD25, using a cytokine biochip array on the Evidence Investigator analyser (Randox Laboratories Ltd, Crumlin, UK), while PCT was assayed by an enzyme-linked fluorescent assay (VIDAS BRAHMS PCT; bioMerieux, France). Statistically significant differences between groups were established by the Mann-Whitney U test. The receiver-operating characteristic (ROC) curve was used to evaluate the diagnostic accuracy (defined by the area under the ROC curve (AUROCC)) of the analyzed cytokines and to determine the sensitivity and specificity at selected cutoff values. The statistical analyses were performed using SPSS 14.0 software (SPSS, Chicago, IL, USA).
Results: PCT, IL-10 and sCD25 were significantly ($P < 0.05$) increased in infectious versus non-infectious SIRS patients at hospital admission (T-0) and after 1 week of hospital stay (T-1). Diagnostic accuracy of PCT, IL-10 and sCD25 was evaluated by AUROCC and exhibited a significance index of 0.0001, 0.0021 and 0.0095 respectively at T-0, while at T-1 the *P* values were 0.0011, 0.0016 and 0.0201 respectively. Regarding prognostic markers, PCT showed a prognostic significance only at T-1 ($P = 0.04$). However when IL-10 and sCD25 values from survivors were compared with those from nonsurvivors, significant differences were found for the former and the latter marker with $P = 0.0014$ and $P = 0.014$ respectively at T-0, as well as with $P = 0.0002$ and $P = 0.014$ respectively at T-1.
Conclusion: In our study, well-known PCT and IL-10 markers and novel sCD25 significantly contributed to diagnosis and prognosis of SIRS and septic patients.

P44

Ninjurin 1 contributes to TLR-induced inflammation in endothelial cells

C Jennewein[*], K Zacharowski
Hospital of the Goethe-University Frankfurt, Clinic of Anaesthesiology, Intensive Care and Pain Therapy, Frankfurt, Germany
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Background: Nerve injury induced protein 1 (Ninjurin 1 (Ninj1)) was first identified in Schwann cells and neurons contributing to cell adhesion and nerve regeneration. Recently, the role of Ninj1 has been linked to inflammatory processes in the central nervous system where functional repression reduced leukocyte infiltration and clinical disease activity during experimental autoimmune encephalomyelitis in mice [1]. But Ninj1 is also expressed outside the nervous system in various organs such as the liver and kidney as well as on leukocytes [2,3]. Therefore, we hypothesized that Ninj1 contributes to inflammation in general; that is, also outside the nervous system, with special interest in the pathogenesis of sepsis.
Methods: Ninj1 was repressed by transfecting HMEC-1 cells, a human dermal microvascular endothelial cell line with siRNA targeting Ninj1 (siNinj1) or a negative control (siC). Subsequently, cells were stimulated with 100 ng/ml LPS (TLR4 agonist), 3 µg/ml LTA (TLR2 agonist) or 100 n/ml poly(I:C) (TLR3 agonist) for 3 hours. The inflammatory response was analyzed by real-time PCR. In addition, transmigration of neutrophils across a HMEC-1 monolayer was measured using transwell plates (pore size 3 µm).
Results: Repression of Ninj1 by siRNA reduced Ninj1 mRNA expression in HMEC about 90% (Figure 1A). Reduced Ninj1 expression decreased neutrophil migration to 62.5% (Figure 1B) and TLR signaling. In detail, knockdown of Ninj1 significantly reduced TLR-2 and TLR-4 triggered expression of ICAM-1 and IL-6 (Figure 1C,D) while poly(I:C)-induced

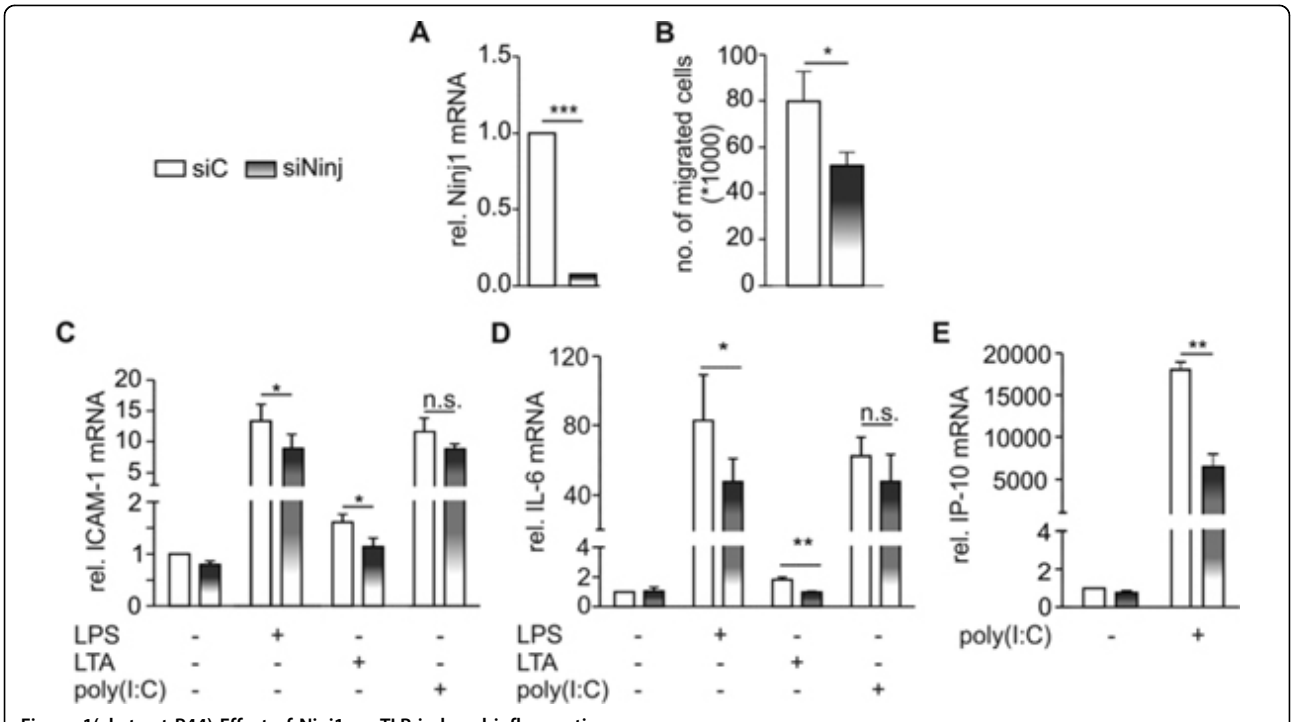

Figure 1(abstract P44) Effect of Ninj1 on TLR-induced inflammation.

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.

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P45

Effect of *Calotropis procera* latex extracts on the hypothalamic TNFα and PGE_2 levels in the rat model of yeast-induced pyrexia

B Guruprasad*, P Chaudhary, VL Kumar
All India Institute of Medical Sciences, New Delhi, India
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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 TNFα and IL-1β. *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-arthritic 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 TNFα 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 TNFα 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 TNFα (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 TNFα 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.

P46

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

E Christaki[1*], E Diza[1], EJ Giamarellos-Bourboulis[2], N Papadopoulou[3], A Pistiki[2], D Droggiti[2], A Machova[3], M Georgitsi[2], D Lambrelli[4], G Karkavelas[1], A Iliadis[1], N Malisiovas[1], P Nikolaidis[1], SM Opal[5]
[1]Aristotle University of Thessaloniki Medical School, Thessaloniki, Greece; [2]University of Athens Medical School, Athens, Greece; [3]University of Cologne, Institute for Medical Microbiology Immunology and Hygiene, Cologne, Germany; [4]University of Macedonia, Thessaloniki, Greece; [5]Alpert School of Medicine of Brown University, Providence, RI, USA
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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 IFNγ were significantly increased and splenocytes from NKT depleted animals, incubated *ex vivo* in the presence or absence of IL-2, produced more IFNγ 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 IFNγ production, which were associated with specific changes in splenocyte miRNA expression.

P47

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

THN Tran[1*], W Kanczkowski[2], V Hoesker[1], SR Bornstein[2], K Zacharowski[1]
[1]Hospital of the Goethe-University Frankfurt, Clinic of Anaesthesiology Intensive Care Medicine and Pain Therapy, Frankfurt am Main, Germany; [2]Technical University Dresden, Germany
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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