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Cytokine-mediated regulation of renal urea transporters during sepsis

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Background The pathogenesis of endotoxemic tubular dysfunction with failure in urine concentration is poorly understood. Urea plays an important role in the urinary concentrating mechanism and expression of the urea transporters UT-A1, UT-A2, UT-A3, UT-A4 and UT-B is essential for tubular urea reabsorption. The present study attempts to assess the regulation of renal urea transporters during severe inflammation in vivo.

Materials and methods By agreement of the animal protection committee C57BL/6J, mice were injected with lipopolysaccharides (LPS, 10 mg/kg) or proinflammatory cytokines. Hemodynamic, renal parameters and the expression of renal urea transporters were investigated. To clarify the role of cytokines and renal ischemia in the regulation of renal urea transporters, experiments were performed with cytokine knockout mice, mice treated with low-dose LPS (1, 5 mg/kg) as a sepsis model without induction of hypotension, glucocorticoid-treated mice, and mice with renal artery clipping serving as a model for renal ischemia.

Results and discussion LPS-injected mice (10 mg/kg) presented with reduced glomerular filtration rate, fractional urea excretion and inner medulla osmolality associated with a marked decrease in expression of UT-A1, UT-A2, UT-A3, UT-A4 and UT-B (Figure 1). Similar alterations were observed after application of TNFα, IL-1β, IFNγ or IL-6. LPS-induced downregulation of urea transporters was not affected in knockout mice with deficient TNFα, IL-receptor-1, IFNγ or IL-6. Glucocorticoid treatment inhibited LPS-induced increases of tissue TNFα, IL-1β, IFNγ or IL-6 concentration, diminished LPS-induced renal dysfunction and attenuated the downregulation of renal urea transporters. Injection of low-dose LPS (1, 5 mg/kg) also led to renal dysfunction paralleled by a downregulation of renal urea transporters without alterations in blood pressure. Renal ischemia induced by renal artery clipping did not influence the expression of urea transporters.

Conclusion Our findings demonstrate downregulation of renal urea transporters that probably accounts for tubular dysfunction during sepsis. Furthermore, they suggest that downregulation of...
(\(r = 0.345\) for CRP and \(r = 0.349\) for PCT), and less so in outpatients (\(r = 0.251\) for CRP and \(r = 0.257\) for PCT). The correlation between PMN CD64 and the soluble markers was higher than that between CRP and PCT (\(r = 0.331\) for hospitalized patients, \(r = 0.305\) for neonates, and \(r = 0.196\) for ambulatory patients). Soluble CD163 levels only weakly correlated with PMN CD64, CRP and PCT. The Saphire results were highly correlated with flow cytometry (\(r = 0.99\)). The measured level of imprecision of both assays was <12% CV for PMN CD64, monocyte CD64, and monocyte CD163 indices. The assay results were available in <1 hour.

Conclusions This study shows a moderate correlation of PMN CD64 with the ‘acute phase reactants’ CRP and PCT. Soluble CD163 is only weakly correlated with the other parameters and may independently define further subsets of patients based upon different anti-inflammatory responses to the clinical condition. The interrelationship of these parameters varies in different clinical situations. We demonstrate it is feasible to automate cellular assays for infection/sepsis in a routine hematology laboratory providing access to a larger patient population.

P21

Tolerance to lipopolysaccharide regulates apoptosis in B lymphocytes

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Background The most important event determining sepsis evolution is immune system cell apoptosis, the immune cell elimination compromises the host effective response, and prevention of apoptosis events improve survival in sepsis models. Our objective was to identify whether lipopolysaccharide (LPS) tolerance regulates apoptotic genes and caspase pathway.

Materials and methods Male Balb-C mice received LPS (1 mg/kg), a tolerant dose, and controls received 0.9% physiologic serum during 5 days, both receiving on day 7 a LPS lethal dose (20 mg/kg). Control, 2 and 4 hours after lethal dose, IL-10, IL-6, IL-1β, TNFα and MIF2 were measured by ELISA. Splenic B lymphocytes were separated through magnetic beads and genes were analyzed by microarray, comparing control and tolerant groups. The tolerant and control groups were followed during 5 days to analyze survival.

Results See Table 1. The mRNA of caspasas 2, 7, 8 and 11, Bid, Apaf-1 and FAS genes were reduced in the tolerant mice. The IL-6 levels reduced in the tolerant mice (724 ± 15 pg/ml) versus control mice (1,488 ± 96 pg/ml) in 2 hours. IL-1β was reduced at 0 hours and at 4 hours in the tolerant group (657 ± 25 pg/ml) versus control (1,117 ± 20 pg/ml). MIP2 also showed a reduction at 4 hours in tolerant (1,803 ± 159 pg/ml) versus control mice (2,173 ± 252 pg/ml). The tolerant animals had 100% survival, controls had zero survival. In all mentioned data, \(P < 0.05\).

Conclusions Tolerance was able to reduce cytokine plasma levels, immune cell apoptosis and mortality to LPS lethal doses.

Physiological parameters, location of infection and organ failure are significant predictors of misdiagnosing severe sepsis

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Background Severe sepsis and septic shock are common disease processes in the critically ill and are associated with substantial morbidity and mortality. The importance of the early identification and diagnosis of severe sepsis has been highlighted by the Surviving Sepsis Guidelines with the aim to provide early and aggressive management in order to improve outcome. In contemporary practice, all clinicians have the responsibility of identifying severe sepsis. Therefore the objectives of this study were to determine whether emergency department and intensive care clinicians could identify and diagnose severe sepsis in those patients in their care within the first 24 hours of admission, and to identify predictors of failing to diagnose sepsis.

Methods The patient cohort were prospectively screened and enrolled on admission to intensive care within the first 24 hours. Severe sepsis was defined as new-onset acute organ dysfunction, using consensus criteria. Clinical data and physiological parameters were collected prospectively. Diagnosis was based on microbiologically confirmed clinical findings. Clinicians caring for each patient were prospectively surveyed.

Results All 402 subjects had infection. Infection sites included 52% pneumonia, 17% urinary, 15% abdominal, 6% wound and skin, and 10% isolated organs and bone. Single-organ failure was evident in 21%, 42% had two-organ failure, 29% had three-organ failure and 8% had four-organ failure. Nurses identified sepsis in 141 of the 402 patients (\(P < 0.001\)) whereas physicians did so in 265 of the 402 patients (\(P < 0.05\)). Misdiagnosis of severe sepsis by the attending nurse or physician was more likely to be associated with pneumonia (odds ratio (OR) = 4.2 (95% confidence interval (CI) = 3.6–4.2), \(P < 0.01\)), urinary sepsis (OR = 2.9 (95% CI = 2.6–3.4), \(P < 0.5\)), less than three-organ failure (OR = 3.1 (95% CI = 2.4–3.7), \(P < 0.01\)), Gram-negative infection (OR = 2.3 (95% CI = 1.6–3.5), \(P < 0.5\)) and presenting without fever (OR = 3.5 (95% CI = 3.1–3.9), \(P < 0.05\)). Thirty-two percent of clinicians did not know the criteria for severe sepsis and 57% missed the patient diagnosis in their care at that time.

Conclusion In this study, misdiagnosis of severe sepsis is still an acknowledged problem in meeting the goals of early resuscitation.

Table 1 (abstract P21)

<table>
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<tr>
<th>Genebank analysis number</th>
<th>Name</th>
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<tr>
<td>X92346</td>
<td>TNF receptor-associated factor 4 (TRAF4)</td>
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<tr>
<td>U06948</td>
<td>Fas antigen ligand (FASL)</td>
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<tr>
<td>U37522</td>
<td>TNF-related apoptosis-inducing ligand (TRAIL)</td>
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<tr>
<td>M83649</td>
<td>Fas l receptor</td>
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</tr>
<tr>
<td>U88990</td>
<td>Inhibitor of apoptosis protein 3</td>
<td>Down</td>
</tr>
<tr>
<td>D28492</td>
<td>Caspase 2 precursor</td>
<td>Up</td>
</tr>
<tr>
<td>U39613</td>
<td>Caspase 7, apoptosis-related cysteine protease</td>
<td>Up</td>
</tr>
<tr>
<td>U59463</td>
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<tr>
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<td>Caspase 2, apoptosis-related cysteine protease</td>
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</tr>
<tr>
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<td>Apoptotic protease activating factor 1</td>
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<tr>
<td>U39643</td>
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<td>X67914</td>
<td>Programmed cell death 1 protein precursor</td>
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<tr>
<td>NM022864</td>
<td>Bid, apoptotic protease</td>
<td>4.6</td>
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<tr>
<td>XM232860</td>
<td>Caspase 8, apoptosis-related cysteine protease</td>
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*Ratio = controls/tolerants: down, down to 0.05; up, up to 5.0.
Protocols and monitoring tools may assist the early identification of severe sepsis so appropriate care can be prioritised and resuscitation implemented early in their admission.

**P23**

**In vitro and in vivo determination of anti-TNFα activity in canine plasma from donors subject to preconditioning with endotoxin**

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**Background**

Septic shock is characterized by cardiovascular and vasomotor failure that is induced by an uncontrolled cascade of inflammatory mediators such as TNFα, IL-1β and IL-6. In dogs, systemic bacterial infections, haemorrhage, trauma, gastric dilatation/volvulus and pancreatitis are the major causes of septic shock. While endotoxin is a recognized effector molecule that can initiate an inflammatory cascade, it has been reported that preconditioning with endotoxin can downregulate inflammatory cytokine responses to subsequent endotoxin challenge. This study reports the effect of endotoxin preconditioning on anti-TNFα activity present in plasma from canine donors.

**Materials and methods**

Plasma from preconditioned (Caniplas®) and normal dogs (FFP) was provided blind to the study by a commercial supplier (Plasvacc Pty Ltd). In vitro anti-TNFα activity in canine donor plasma was determined by a L929 murine cell TNFα inhibition bioassay using recombinant murine TNFα. In vivo effects were tested by a rat subcutaneous skin pouch model. Rats were pretreated for 3 days with either Caniplas®, FFP, saline (2 ml/day, s.c.) or carprofen (5 mg/kg, s.c.) and inflammation was induced by injecting monosodium urate crystals into the pouch (5 mg/ml in 5 ml saline). Fluid was taken from pouches at specified intervals for cell count, TNFα and IL-6 analysis. Data analysis: normalized data were fitted to a four-parameter logistic curve. The fitted midpoints were compared statistically for datasets using an F-test and calculated fitted hill slopes.

**Results**

In the rat skin pouch model, both Caniplas® and FFP reduced TNFα levels and Caniplas® was a more potent antagonist. The heightened anti-TNFα activity of Caniplas® compared with FFP was confirmed in the in vitro cell bioassay (Figure 1). Neither Caniplas® nor FFP reduced inflammatory cell infiltration or the levels of IL-6.

**Conclusion**

While we need to confirm the mechanism, we report that preconditioning with endotoxin does illicit specific anti-TNFα activity and that this observation has been confirmed in both in vitro testing and in vivo animal models. It is plausible that preconditioning animals with endotoxin induces an increase in the concentration of soluble TNFα receptors I and II in donor plasma, and that this is the probable source of TNFα antagonism. This report suggests that preconditioned plasma may be a beneficial treatment where inflammation causes increased expression of TNFα.

**Figure 1 (abstract P23)**

![In vitro TNFα dose-response to canine sera.](http://ccforum.com/supplements/11/S4)


**P24**

**The human antimicrobial peptide LL-37 induces endothelium-dependent vascular smooth muscle relaxation mediated via the lipoxin A4 receptor**

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**Background**

Septic shock includes blood vessel dilatation and activation of innate immunity. The activation causes release of antimicrobial peptides such as LL-37. It has been shown that LL-37 can attract leukocytes via the lipoxin A4 receptor (ALX). ALX is also present in vascular endothelial cells. To explore possible ways of pharmacological intervention in septic shock, we investigated whether LL-37 can affect vascular tone.

**Materials and methods**

Human omental arteries and veins were obtained during abdominal surgery. The circular smooth muscle activity, in the wall of the vessel segments, was studied in organ baths. Gene expression was studied using reverse transcriptase PCR.

**Results**

LL-37, at micromolar concentrations, induced a concentration-dependent and endothelium-dependent relaxation in vein segments but not in artery segments precontracted by endothelin-1. The relaxation was profoundly reduced by potassium chloride (30 mM) to inhibit endothelin-derived hyperpolarizing factor (EDHF), while it was less affected by the NOS inhibitor L-NAME and not at all by indomethacin. The ALX agonist, WKYMVm, did also induce a relaxation, and both the relaxations induced by LL-37 and WKYMVm were inhibited by the ALX antagonist WR14613. ALX was expressed in the endothelium.

**Conclusion**

We demonstrate for the first time that the human antimicrobial peptide LL-37 induces endothelium-dependent relaxation of human arteries and veins.