Critical Care Volume 13 Suppl 4, 2009

Sepsis 2009

Amsterdam, the Netherlands, 11–14 November 2009

Published online: 11 November 2009
These abstracts are available online at http://ccforum.com/supplements/13/S4

P1

Urokinase receptor is necessary for bacterial defense against Gram-negative sepsis (melioidosis) by facilitating phagoctytosis

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Critical Care 2009, **13(Suppl 4):**P1 (doi: 10.1186/cc8057)

Introduction Urokinase receptor (uPAR, CD87), a glycosylphosphatidylinositol-anchored protein, is considered to play an important role in inflammation and fibrinolysis. The Gram-negative bacterium *Burkholderia pseudomallei* is able to survive and replicate within leukocytes and causes melioidosis, an important cause of pneumonia-derived community-acquired sepsis in Southeast Asia. We here investigated the expression and function of uPAR both in patients with septic melioidosis and in a murine model of experimental melioidosis.
Methods Using a translational approach we conducted a patient study in patients with culture-confirmed sepsis caused by *B. pseudomallei*, *in vitro* experiments using wild-type (WT) and uPAR knockout (KO) cells, and mouse studies using WT and uPAR KO mice inoculated with *B. pseudomallei.*
Results uPAR mRNA and surface expression was increased in patients with septic melioidosis in/on both peripheral blood monocytes and granulocytes as well as in the pulmonary compartment during experimental pneumonia-derived melioidosis in mice. uPAR-deficient mice intranasally infected with *B. pseudomallei* showed an enhanced growth and dissemination of *B. pseudomallei* when compared with WT mice, corresponding with increased pulmonary and hepatic inflammation. uPAR KO mice demonstrated significantly reduced neutrophil migration towards the pulmonary compartment after inoculation with *B. pseudomallei*. Further *in vitro* experiments showed that uPAR-deficient macrophages and granulocytes display a markedly impaired phagocytosis of *B. pseudomallei*. Additional studies showed that uPAR deficiency did not influence hemostatic and fibrinolytic responses during severe melioidosis.
Conclusions These data suggest that uPAR is crucially involved in the host defense against sepsis caused by *B. pseudomallei* by facilitating the migration of neutrophils towards the primary site of infection and subsequently facilitating the phagocytosis of *B. pseudomallei.*

P2

A comparison of acute lung inflammation in *Klebsiella pneumoniae* B5055-induced pneumonia and sepsis in BALB/c mice

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Critical Care 2009, **13(Suppl 4):**P2 (doi: 10.1186/cc8058)

Introduction Lungs play an important role in the body's defense against a variety of pathogens, but this network of immune system mediated defense can be deregulated during acute pulmonary infections.
Objective The present study compares the acute lung inflammation (ALI) occurring during *Klebsiella pneumoniae* B5055-induced pneumonia and sepsis in BALB/c mice.
Methods Pneumonia was induced by intranasal instillation of bacteria (10^4 CFU) while sepsis was developed by placing the fibrin–thrombin clot containing a known amount of bacteria (10^2 CFU) into the peritoneal cavity of animals. Various cytokine (TNFα and IL-1α) levels were estimated using ELISA and the degree of lung inflammation (that is, inflammatory cell infiltration) was evaluated by histopathological analysis. The other markers of inflammation (that is, nitric oxide (NO), malondialdehyde (MDA) and myeloperoxidase (MPO)) were estimated by standard biochemical methods.
Results Mice with sepsis showed 100% mortality within 5 post infection days whereas all the animals with pneumonia survived. In animals suffering from *K. pneumoniae* B5055-induced pneumonia all the inflammatory parameters (TNFα, IL-1α, MPO, MDA and NO) were found to be maximum until the third post infection day, after that a decline was observed, whereas in septic animals all the above-mentioned markers of inflammation kept on increasing until death of the animals. Histopathological study showed that inflammatory damage to the lungs in pneumonia was not very severe as lesser neutrophil infiltration and pulmonary damage (that is, alveolitis, bronchiolitis, endothelitis and perivascular congestion) was seen as compared with lungs taken from septic animals. This can be further strengthened by the presence of alternatively activated alveolar macrophages (AAMacs) or foam cells in lungs of mice with pneumonia after the third post infection day and their number kept on increasing until the seventh post infection day, which might have contributed to the induction of resolution of inflammation and clearance of the infection. But no such AAMacs or foam cells were seen in lungs of septic mice on histopathological examination, lungs were seen to be infiltrated with only neutrophils on all experimental days.
Conclusions Hence, during pneumonia controlled activation of AAMacs or foam cells led to the resolution of inflammation and infection as well.

P7

Faster differentiation of *Staphylococcus aureus* versus coagulase-negative *Staphylococci* from blood culture material: a comparison of different bacterial DNA isolation methods

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Critical Care 2009, **13(Suppl 4):**P7 (doi: 10.1186/cc8063)

Introduction Frequent usage of medical devices, such as intravenous lines, often results in sepsis, which is characterized by high morbidity and mortality. Rapid and reliable detection and differentiation between *Staphylococcus aureus* and coagulase-negative *Staphylococci* (CNS) is therefore clinically relevant to be able to provide adequate early treatment. Blood culture is still the gold standard method in identifying these pathogens but is time consuming. Molecular diagnostics might be a promising alternative to reduce this time-to-result delay.
Objective This study aims to compare different DNA extraction methods from two commonly used blood culture materials, BACTEC (BD) and Bact/ALERT (Biomerieux), to accelerate differentiation between *S. aureus* and CNS.
Methods Two fast real-time PCR duplex test assays, targeting the Tuf gene, to differentiate *S. aureus* from CNS, were developed in order to select the most sensitive one. This Tuf RT-PCR was used to compare three different DNA isolation methods on two different blood culture systems. Negative blood culture material was spiked with *S. aureus*; bacterial DNA was isolated with: automated extractor EasyMAG (Biomerieux), automated extractor MagNA Pure (Roche), and a manual kit MolYsis Plus (MolZyme).
Results The best Tuf RT-PCR method appeared to have a sensitivity of 100 CFU/ml. Approximately 50 positive blood cultures containing Gram-positive cocci in clusters were tested in the Tuf RT-PCR and all were identified correctly. Bacterial DNA isolation, from spiked blood culture material, with the EasyMAG showed the highest analytical performance with a detection limit of 10^3 CFU/ml in Bact/ALERT material, whereas using BACTEC resulted in a detection limit of 10^4 CFU/ml. Hand-on time, for 26 samples, was lowest for the EasyMAG (10 minutes) and highest for the manual kit of MolZyme (2 hours). Total handling time was highest for the MolYsis Plus kit (3.5 hours) and lowest for the automated extractor EasyMAG (50 minutes).
Conclusions A sensitive RT-PCR was developed for detection and differentiation of *S. aureus* versus CNS. Bacterial DNA isolation from Bact/ALERT blood culture material seems to show better reproducibility compared with isolation from BACTEC blood culture material. In this preliminary study the EasyMAG performed better when compared with MolYsis Plus and the MagNA Pure system. In future work this method will be further evaluated with reduced culture times.

P8

Effect of canine hyperimmune plasma on TNFα and inflammatory cell levels in a lipopolysaccharide-mediated rat air pouch model of inflammation

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Critical Care 2009, **13(Suppl 4):**P8 (doi: 10.1186/cc8064)

Introduction Unregulated elevated levels of serum TNFα have been associated with proinflammatory cytokine cascades that are characteristic in diseases such as septic shock. Endotoxic shock, which has a poorer prognosis than found with other forms of septic shock, is mediated by lipopolysaccharide (LPS), a molecule that is released from the outer membrane of Gram-negative bacteria. LPS is a potent stimulator of TNFα secretion by serum monocytes and tissue macrophages. Whilst the use of monotherapeutic TNFα antagonists has been trialed, none have been registered for use in patients with sepsis.
Objective The purpose of this study was to test the effect of canine hyperimmune frozen plasma (HFP), which is known to contain elevated levels of soluble TNFα receptor 1 (sTNFR1), on TNFα and inflammatory cell levels in a LPS-mediated rat air pouch model of inflammation.
Methods A dorsal air pouch in 175 to 200 g Sprague–Dawley rats was formed by 20 ml subcutaneous infusions of sterile air. Prophylactic subcutaneous injections of canine HFP, canine fresh frozen plasma (FFP) or carprofen were administered daily for 3 days into the lateral flank of the right foreleg at doses recommended by the manufacturers ($n = 10$ for each treatment group). Pouch fluid was harvested by syringe at 1, 6, 12, 24 and 48 hours post LPS administration and subjected to histological and cytokine/cytokine receptor analysis. TNFα and sTNFR1 levels were determined by ELISA and an immunofluorescent dot blot assay.
Results Pouch fluid analysis: maximal effects were detected at 6 hours post LPS administration. TNFα levels were significantly depressed in animals dosed with HFP, but not in animals treated with FFP or carprofen ($P < 0.05$). sTNFR1 levels were significantly elevated in HFP, but not in FFP or carprofen dosed animals ($P < 0.05$). Neutrophil numbers were significantly depressed in HFP dosed but not in FFP or carprofen treated animals ($P < 0.05$).
Conclusions There appears to be a correlation between elevated levels of sTNFR1 and depression of TNFα and neutrophil levels in the pouch fluid of HFP dosed rats ($r = -0.73$, $P < 0.0001$). The data suggest that canine HFP, which has been demonstrated to contain elevated levels of sTNFR1 compared with FFP, has a direct effect on depressing TNFα levels and neutrophil sequestration in the rat air pouch model of inflammation. These data suggest that HFP may be worthy of further investigation to determine whether such preparations have a therapeutic potential for treatment of acute inflammatory diseases in which TNFα is implicated.

P9

Clinical impact of a PCR-based assay for pathogen detection in critically ill patients with evidence of infection

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Critical Care 2009, **13(Suppl 4):**P9 (doi: 10.1186/cc8065)

Introduction Blood cultures are often negative even in patients with clinical signs of severe sepsis. Furthermore, the long time to result of culture-based methods does not allow the results to guide empiric antimicrobial therapy. PCR-based pathogen detection promises a higher rate of positivity and a faster time to result.
Objective To report the performance of PCR-based pathogen detection compared with blood culture in ICU patients with evidence of infection, and the impact of this test on the antimicrobial therapy.
Methods Patients treated on an interdisciplinary ICU were included into this observational study if a blood culture (BC) was