Late-breaking abstract: Soluble immune complex exaggerates LPS-Induced acute lung injury (ALI) by transfusion – A novel mechanism of transfusion-related acute lung injury (TRALI)

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Rationale: TRALI is a major cause of morbidity and mortality related to transfusion therapy. Soluble immune complex (ICx) could aggravate LPS-induced lung injury. We speculate that ICx formation during transfusion may play a role in the pathogenesis of TRALI.

Method: We designed a double-hit mice model to simulate TRALI development. The first hit is LPS i.t. injection. The second hit is transfusion of conditioned blood 24 hours after LPS injection. Mice were sacrificed 6 hours later. The conditioned blood was generated by transfusing 0.1cc/day C57 mice blood to BALB/C mice consecutively for two weeks. Animal were divided into four groups XX, XB, LP and LB (X: no treatment, L: LPS i.t.; B: transfusion of conditioned blood, P: transfusion with PBS)

Result: 1. The LB group has higher protein, TNF-α and MIP-2 level in BALF.

<table>
<thead>
<tr>
<th></th>
<th>XX (n=5)</th>
<th>XB (n=5)</th>
<th>LP (n=9)</th>
<th>LB (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cell count (x 10^5)</td>
<td>0.73±0.21</td>
<td>0.73±0.17</td>
<td>3.74±1.20</td>
<td>4.70±1.25</td>
</tr>
<tr>
<td>Neut. Count (x 10^5)</td>
<td>0.01±0.01</td>
<td>0.01±0.01</td>
<td>3.14±1.12</td>
<td>4.03±1.12</td>
</tr>
<tr>
<td>Neut. (%)</td>
<td>1.40±0.75</td>
<td>0.50±0.22</td>
<td>9.8±13.2</td>
<td>70.6±10.6</td>
</tr>
<tr>
<td>Protein (μg/ml)</td>
<td>0.12±0.04</td>
<td>0.06±0.02</td>
<td>0.41±0.07</td>
<td>0.69±0.02*</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>42.0±16.8</td>
<td>78.2±17.7</td>
<td>473.0±53.2</td>
<td>871.2±186.7*</td>
</tr>
<tr>
<td>MIP-2 (pg/ml)</td>
<td>21.0±5.2</td>
<td>31.1±12.7</td>
<td>348.5±84.3</td>
<td>871.2±186.7*</td>
</tr>
</tbody>
</table>

*p<0.05 as compared with LP.

2. Soluble ICx is present in LB only.

<table>
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<th>LP (n=9)</th>
<th>LP (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICx in plasma</td>
<td>0 / 5</td>
<td>0 / 5</td>
<td>1 / 9</td>
<td>8 / 9</td>
</tr>
</tbody>
</table>

Conclusion: We demonstrated that transfusion with 2cc conditioned blood aggravated LPS-induced ALI and generated soluble ICx in plasma. This probably indicates ICx may aggravate pre-existing ALI which implies a novel mechanism of the pathogenesis of TRALI.

Haplotype of the endothelial protein C receptor gene is associated with reduced risk of acute lung injury in critically ill patients


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Background: Activation of the coagulation system is present in the pulmonary circulation of patients with acute lung injury and acute respiratory distress syndrome (ALI/ARDS). Endothelial Protein C Receptor (EPCR) is involved in the activation of the protein C cytoprotective and anticoagulant pathway. Functionally relevant EPCR gene mutations have been described but their impact on ALI/ARDS development remains unclear.

Aims and objectives: To investigate the role of EPCR mutations as predisposing factors to ALI development in severely ill critically ill patients.

Methods: 92 severely ill (APACHE score ≥25) ICU patients were screened for variations in the EPCR gene. Multivariate logistic regression analysis was performed in order to determine the prognostic significance of EPCR haplotypes compared to other potential risk factors, including disease severity, age and the presence of sepsis, to the development of ALI/ARDS.
Results: Depending on the haplotype-defining point-mutations, patients were divided into three haplotypes: H1 (n=59), H2 (n=17) and recombant H1/H3 (n=16). ALI occurred in 53% of H2 carriers, 24% of H1 carriers and 50% of H1/H3 carriers with odds ratios 1.0, 0.284 (Confidence Interval= 0.090-0.904) and 0.963, respectively (p=0.033). Severe sepsis/septic shock was positively correlated with ALI/ARDS development, while APACHE II, SOFA, age and soluble EPCR levels were independent predictors of ALI/ARDS.

Conclusion: EPCR genotype seems to be a determinant of ALI/ARDS development in critically ill patients. H1 haplotype carriers have a reduced risk of ALI/ARDS, as compared to H2 haplotype and H1/H3 carriers.

1686
Diagnostic value of Von Willebrand factor (VWF) in patients suffering from respiratory distress
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Introduction: Acute respiratory distress syndrome (ARDS) is characterized by an extensive alveolar capillary leak. Von Willebrand Factor antigen (VWF) is a macromolecular antigen that is considered as a marker of endothelial activation.

Aim: To investigate the diagnostic value of VWF antigen in patients with ALI/ARDS caused by poisoning or non-poisoning etiology.

Patients and methods: VWF antigen was measured in 52 patients with ALI/ARDS, 13 poisoned patients without ALI/ARDS and 20 control subjects.

Results: There was a highly significant difference between VWF level in patients and control groups (P< 0.001). VWF level had a significant negative correlation with the ratio between PaO2 and FiO2 in patients with respiratory distress. There was a non significant difference in VWF level between poisoned and non-poisoned cases. There was a significant relationship between the level of VWF and the severity of poisoning in patients with respiratory distress. Among the poisoned patients, the highest level of VWF was in patients with anticholinesterase poisoning. The difference between VWF level in poisoned patients with ALI/ARDS and those without was highly significant. The level of VWF didn’t affect patients’ need for mechanical ventilation or their mortality. The cut-off value of VWF at 100% sensitivity and 20% specificity was 0.99 units, while the cut-off value of VWF at 100% specificity and 60% sensitivity was 1.87 units.

Conclusion: VWF has a diagnostic value for ALI/ARDS but it does not differentiate between poisoning or non-poisoning etiology, nor does it predict outcome of the illness. VWF is significantly increased in patients with anticholinesterase poisoning.

1687
Stimulation of NOD1 induces RIP2, TAK1 and p38 MAPK dependent pro-inflammatory signalling in human lung microvascular endothelial cells

Gram-negative bacteria are an important cause of septic shock. NOD1 receptors recognize peptidoglycan in the bacterial cell wall and initiate pro-inflammatory responses. We have previously shown in rodents that stimulation of NOD1 induces vascular dysfunction in vitro and profound shock in vivo. In this study we investigated the role of NOD1 in human lung microvascular endothelial cells (HMVEC) which represent a site of key importance in the pathophysiology of sepsis and acute lung injury.

HMVEC from healthy donors were cultured in 96-well plates. Cells were treated for 24 hours with vehicle ± LPS (TLR4) or iE-DAP (NOD1). In additional experiments cells were pre-treated for 1 hour with specific signalling inhibitors prior to addition of agonists (n=4-5). Cell activation was assessed by multiplex ELISA and with specific ELISAs for CXCL8 and 6-keto Prostaglandin F1α (PGF1α). iE-DAP induced significant release of CXCL8, 6-Keto Prostaglandin F1α (PGF1α), IL-1β, IL-2, and TNFα. 5Z-7-oxo-zeaxanin or BIRB0796 similarly inhibited responses to iE-DAP (Figure 1) or LPS (P<0.05;two-way ANOVA). By contrast, PP2 was more potent an inhibitor of iE-DAP (Figure 1) than LPS (P<0.05; two-way ANOVA).

In conclusion NOD1 is active in human microvascular endothelium and converges with TLR4 signalling at the level of TAK1 and p38 MAPK. NOD1 thus represents a potential target in the treatment of gram-negative sepsis.

1688
Lactate, pH and angiogenic markers in exhaled breath condensate correlate with outcome and disease severity in patients with acute lung injury
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Acute lung injury (ALI) is characterized by increased capillary permeability, interstitial and alveolar oedema, influx of circulating inflammatory cells, and formation of hyaline membranes. Vascular endothelial growth factor (VEGF) has been correlated to a favourable prognosis in ARDS in a number of investigations. VEGF plays a role in regulating vascular permeability to water and protein. The aim of this investigation was to characterize the role of VEGF, Angiogenin, basic fibroblast growth factor (bFGF), lactate and pH in exhaled breath condensate (EBC) in mechanical ventilated patients with acute lung injury. For this purpose, exhaled breath condensate was collected from 30 patients with ALI at 24 to 72 hours from start of mechanical ventilation and correlated with ventilatory parameters, clinical scores, and outcome. Cytokines were measured by a cytometric bead array (CBA).

We observed a significant lower value of VEGF in EBC in the group with lethal outcome compared to survivors; Mann-Whitney test: p<0.0001 but no significant difference for Angiogenin, bFGF, IL-8, or TFN-alpha. In addition EBC-lactate and EBC-pH correlated with lung injury severity indices. There was a further correlation of bFGF and IL-8 in EBC with lung injury severity indices.

We conclude that measurement of lactate, pH, bFGF, and VEGF in EBC may provide information on prognosis in ALI.

1689
Which is the best source of mesenchymal cells to treat acute lung injury?
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Mesenchymal stem cells (MSC) may derive from bone marrow, adipose tissue or lung. Previous studies have shown that bone marrow derived mesenchymal stem cells (BM-MSC) exert beneficial effects in acute lung injury (ALI), but the effects of adipose tissue and lung derived mesenchymal cells (AD-MSC and L-MSC, respectively) have not been evaluated so far. The aim of this study was to investigate the effects of BM-MSC, AD-MSC, L-MSC on lung mechanics and morphometry, as well as inflammation and remodeling in an experimental model of ALI Forty-eight female Wistar rats (200-250g) received Escherichia coli lipopolysaccharide (LPS) intratracheally (100 μg; ALI) or saline (C). At 48 hours, ALI and C groups were further randomized into subgroups receiving saline (0.05 mL), BM-MSC, AD-MSC, and L-MSC (1x10^6) intravenously. Bone marrow cells were extracted from four male Wistar rats. The induction of differentiation showed that cells from bone marrow, adipose tissue and lung were able to generate osteocytes, adipocytes and chondrocytes besides presenting CD34-, CD90+ and CD29+ profile. At day 7, mesenchymal cells promoted a reduction in lung static elastance, resistive and viscoelastic pressures, alveolar collapse, collagen fiber content and number of neutrophils in lung tissue, independent of the source. However, the beneficial effects of BM-MSC and AD-MSC on lung parenchyma remodeling were greater than those observed with L-MSC. In conclusion, in the present LPS-induced ALI model, BM-MSC and AD-MSC therapies were more effective than L-MSC at modulating inflammatory and fibrogenic processes.

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Mesenchymal stem cells prevent early inflammation in a rat model of ventilator induced lung injury
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Background: Recent studies have suggested that bone marrow-derived mesenchy-
mal stem cells (MSC) might have potential therapeutic effects in acute lung injury (ALI), specifically in bleomycin-induced and bacterial LPS-induced lung injury models. However, whether MSC could induce an early anti-inflammatory response in lung injury induced by overventilation remains to be elucidated.

**Aim:** To assess the potential role of MSC in preventing or modulating early inflammation in healthy rats subjected to ventilator induced lung injury (VILI).

**Methods:** Adult male Sprague-Dawley rats (250-300 g) were anaesthetised, tracheotomised, intubated and paralysed by intravenous instillation of pancuronium bromide. Nine rats were ventilated with a high tidal volume of 25 ml/kg (VILI) for 3 h. Five millions of MSC were intravenously injected to 4 of these rats 30 min before starting ventilation. Spontaneously breathing anaesthetised rats (N=4) served as controls. After 3 h of VILI/control the animals were sacrificed and broncoalveolar lavage inflammatory cells were assessed.

**Results:** In VILI, MSC significantly (p<0.01) decreased total neutrophil counts from 7620±3710 to 1580±470 (cells/μL), which was close to control values (787±344; p=0.6).

**Conclusion:** This preliminary result suggests that infusion of bone marrow-derived MSC prevents early inflammation in VILI.

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1691 Impact of bone marrow mononuclear cell administration route on lung and distal organs in pulmonary and extrapulmonary acute lung injury

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The pathophysiology of acute lung injury (ALI) differs according to the type of insult. We hypothesized that the administration route of bone marrow-derived mononuclear cell (BMDMC) therapy might have different effects on lung and distal organs in models of pulmonary (p) or extrapulmonary (exp) ALI. Forty-eight C57BL/6 mice: 36 females and 12 males (20-25 g) were used. In control animals, sterile saline solution was intratracheally or intraperitoneally injected. whereas ALI animals received *Escherichia coli* lipopolysaccharide intratracheally (40 μg, ALIp) or intraperitoneally (400 μg, ALIexp). Six hours after lipopolysaccharide administration, ALIp and ALIexp animals were further randomized into subgroups receiving saline or BMDMC (2×10⁶) intravenously (BMDMC iv) or intratracheally (BMDMC it). At day 7: 1) BMDMC iv and it decreased static elastance, alveolar collapse, collagen fiber content, and bronchoalveolar lavage fluid cellularity; 2) BMDMC it increased the number of green fluorescent protein (GFP)+ cells in lung in ALIp, while BMDMC iv decreased GFP+ cells in kidney and liver in ALIexp; 3) BMDMC it induced greater reduction of lung apoptotic cells in ALIp, while BMDMC iv decreased lung, kidney and liver apoptosis in ALIexp; 4) BMDMC iv resulted in greater reduction in interleukin (IL)-6, KC (murine IL-8 homolog), and IL-10 compared to BMDMC it; 5) the beneficial effects of BMDMC were independent of engraftment. In conclusion, BMDMC therapy was effective in modulating the inflammatory and fibrogenic process in both models, but greater beneficial effects were achieved with BMDMC iv.

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