97. The many roads to lung injury

P819
PI3K signalling may explain differential response of lung cells to mechanical stretch
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Alveolar epithelial cells may be subjected to increased mechanical stretch (MS) during ventilation although low tidal volume is applied. High amplitude MS impairs PI3K activity and leads to apoptosis in alveolar type II (ATII) cells. The response of human pulmonary microvascular endothelial cells (HPMEC), fibroblasts (HPF), human AS49 cells and rat ATII cells to MS is compare in this study.

Stretching patterns (frequency/change in surface area) were chosen to mimic physiological breathing (P) and the effects of high frequency (hf), high amplitude (ha) and both (hfa). MS was compared with static cultures at 24h. Supernatant LDH, cell necrosis/apoptosis (Annexin-V binding/propidium iodide-staining) and cellular PI3K activity (measured as phosphorylated Pkt-kinase, pAkt) were analyzed. MS increased the release of LDH in all cell types. This effect increased with the hf, ha and hfa stretching patterns. Viable HPMEC and ATII cells decreased significantly in response to MS with a minimum in the hfa and ha group; predominately due to apoptosis. AS49 cells showed only a small decrease in viable cells with little change in necrotic and apoptotic cells. HPF, however, did not undergo apoptosis in response to MS. Cellular pAkt content was reduced in response to MS in HPMEC and ATII cells, unchanged in AS49 cells and increased in HPF. PI3K stimulation increases the percentage of apoptotic cells. In contradiction with endothelial and epithelial cells, pulmonary fibroblasts do not undergo apoptosis and show increased PI3K in response to MS. In lung injury fibroblast may remain as a scaffold for the pulmonary structure leading the way for repair.

P820
Remodelling of cytoskeleton towards a softer cell by latrunculin prevents stretch-induced apoptosis in alveolar type II cells
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Background: Cyclic stretching of alveolar type II (ATII) cells is associated with alterations of the actin cytoskeleton and has been shown to induce apoptosis.

Objectives: We hypothesize that the cytoskeleton of ATII cells and its viscoelastic properties are involved in the mediation of high amplitude mechanical stretch-induced apoptosis. Therefore we investigated the influence of the actin-modulating drug Latrunculin D on viscoelastic properties and stretch-induced apoptosis of ATII cells.

Methods: Alveolar type II (ATII) cells from Sprague Dawley rats were exposed to cyclic stretching using a pattern characterized by frequency of 40 [min-1] and an amplitude of 30 [%]. During stretching cells were treated with Latrunculin D. After stretching we determined apoptosis and cell injury using an Annexin V-FITC Apoptosis Detection Kit and the Cytotoxicity Detection kit. Further, elasticity measurements of Latrunculin treated cells were performed with the atomic force microscope (AFM) and the optical stretcher.

Results: Determination of elastic modulus using the AFM showed that Latrunculin decreased stiffness of ATII cells (83.5%). Using the optical stretcher ATII cells treated with Latrunculin showed a higher deformation than controls (25.3%). Treatment of overstretched cells with Latrunculin reduced significantly the number of apoptotic cells (control 30.6%, Latrunculin 19.1%) and decrease the level of LDH (control 0.12 U/ml; Latrunculin 0.13 U/ml).

Conclusions: We conclude that the remodelling of cytoskeleton towards a softer cell is a protective mechanism against stretch-induced apoptosis in ATII cells.

P821
Surfactant release into the alveolar space protects the lung from ventilation-induced injury
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Objective: The aim of this study was to investigate mechanisms involved in resistance to ventilation-induced lung injury.

Methods: Sprague-Dawley rats were randomly exposed to a non-injurious low-stretch ventilation (VT = 9 ml/kg, PEEP=5 cm H2O) (n=12) and an injurious high-stretch ventilation (VT = 25 ml/kg, PEEP=0 cm H2O) (n=29). Animals were continuously monitored for a maximum period of 2.5 h. The high-stretch ventilation group (HV) was subdivided in two groups: 1) animals showing a substantial PO2 reduction and peak airway pressure (Paw) increase, sacrificed at 60 min (HV60) (n=11); and 2) animals with insignificant PO2 and Paw changes at 60 min (n=18), sacrificed at 150 min (HV150).

Results: The HV60 group was characterized by high amount of active surfactant, absence of edema, inflammatory marker, and surfactant data. The HV150 group was characterized by high amount of active surfactant, absence of edema, normal PO2. These animals exhibited a slight decrease of alveolar macrophages and increase of some BAL inflammatory markers (IL6, TNF-α, MIP-2, MCP-1), C-reactive protein, and acidic sphingomyelinase), pronounced decrease of alveolar macrophages, and an accelerated conversion of freshly secreted active surfactant to inactive surfactant. In contrast, the HV150 group was characterized by high amount of active surfactant, absence of edema, and normal PO2. These animals exhibited a slight decrease of alveolar macrophages and increase of some BAL inflammatory markers (IL6, TNF-α, MIP-2, MCP-1).

Conclusions: These results suggest that increased surfactant release into the alveolar space safeguards the lung from ventilation-induced injury and that surfactant alteration might directly contribute to lung dysfunction.
P822
Effects of PEEP-like cyclic stretch on the IL-6-protein production in normal human pulmonary artery endothelial cells in vitro
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Background and aim: Excessive cyclic stretch is one of the main causes of ventilator-induced lung injury. However, the molecular mechanisms of the injury by cyclic stretch have not yet been fully understood. The aim of the present study was to examine the effects of PEEP-like cyclic stretch on the IL-6-protein production in normal human pulmonary artery endothelial cells (HPAECs) in vitro.

Methods: Normal HPAECs were stretched by a Flexcell® FX-4000TM Tension System (Flexcell International). The stretching rate was 15 cycles/minute, and the cells were cyclically stretched from 0 to 5%, 0 to 10%, 0 to 20%, 3 to 20% and 5 to 20%. Stretch from 3 to 20% and from 5 to 20% simulated excessive stretch during mechanical ventilation with PEEP. The cells were stretched for various durations (0, 1, 3, 6 and 12 hours). During the experiments, culture medium was sampled at 0, 3, 6 and 12 hours after stretch started. The IL-6-concentration of the samples was determined by ELISA.

Results: Excessive stretch (0 to 20%) significantly increased the IL-6-production of the cells stretched for more than 3 hours compared to the unstretched cells (n=5, P<0.05), but moderate stretch (0 to 5% and 0 to 10%) did not. PEEP-like stretch (3 to 20% and 5 to 20%) produced no significant changes in the IL-6-production of the cells at any sampling points.

Conclusions: The IL-6-protein was produced by excessive cyclic stretch (0 to 20%) and IL-6-protein production of kallistatin against PGE2-induced lung injury. We found that the severity of lung injury was attenuated in kallistatin gene-transferred mice compared with untreated mice, indicating by decreasing cell counts, LDH and protein levels of bronchoalveolar lavage fluids (BALF). BALF levels of TNF-α, IL-1β, IL-6 were also lower in human kallistatin gene-transferred mice than untreated mice, suggesting a decline in inflammatory response after LPS treatment. In addition, the kallistatin gene-transferred mice showed less extent of epithelial cell apoptosis shown by TUNEL staining. Our data demonstrate for the first time that kallistatin protects against LPS-induced lung injury through attenuation of inflammation and epithelial cell apoptosis.

P823
LNC 2012 Abstract – Repair of the very immature lung following brief, injurious mechanical ventilation commences within 24 hours
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Background: Very preterm infants often require mechanical ventilation (MV). This intervention can cause lung injury that contributes to bronchopulmonary dysplasia. To investigate mechanisms of injury and repair in a novel model of ventilating the lungs of fetal sheep. Using this model MV-induced injury resolves within 15d, but it is not known when repair starts.

Methods: Pregnant sheep at 110d and 125d gestational age (GA, term = 147dGA) underwent aseptic surgery in which the fetal trachea was intubated and fetal lungs ventilated for 2h with an injurious MV protocol. Ewes and fetuses remained ventilated for 2h with an injurious MV protocol. Ewes and fetuses remained ventilated for 2h with an injurious MV protocol. Ewes and fetuses remained ventilated for 2h with an injurious MV protocol.

Results: Following MV, severe lung injury was present at 1d. At 1d potential repair genes (metallothionein and urokinase receptor) mRNA levels were increased in MV lungs at both stages of development. There was no difference in the mRNA levels of IL-1β, IL-6, IL-8 and TNFα (2 and 125dGA). Protein expression by Western blot was increased at 24h for IL-1β, IL-6, IL-8 and TNFα (2 and 125dGA).

Conclusions: Following MV injury, repair processes, commencing with normalization of early response and inflammatory gene expression and the activation of repair genes, occurs within 24h.

P824
Influence of protein kinase C on stretch-induced apoptosis in rat alveolar type II cells
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Background: Cyclic stretching of alveolar type II (ATII) cells has been shown to induce apoptosis and is associated with alterations of F-actin cytoskeleton. Protein kinase C is able to promote alterations in the actin cytoskeleton. Objectives: We hypothesize that PKC is involved in the regulation of apoptosis in stretched cells. Therefore we investigated the PKC expression in stretched cells and their influence on actin cytoskeleton and stretch-induced apoptosis.

Methods: Alveolar type II (ATII) cells were exposed to cyclic stretch using a stretching pattern characterized by frequency of 40 [min^-1] and an amplitude of 30 [%]. After stretching we determined PKC-expression by Western blot. Next, we treated ATII cells with phorbol ester (PMA, a PKC stimulator), measured (a) F-actin by phalloidin staining, (b) elasticity with atomic force microscope (AFM), and (c) determined the effect of PMA on stretch-induced apoptosis using an Annexin V-FITC Apoptosis Detection Kit. Further, we tested the effect of the PKC inhibitor Staurosporin on stretch-induced apoptosis.

Results: Our results showed an increase of PKC in stretched ATII cells after 1h. The stimulation of PKC with phorbol ester (PMA) induced a decrease of F-actin (21.5%) and elastic modulus (24.9%). We observed that treatment of ATII cells with PMA during stretching reduced the number of apoptotic cells (control 32.8%, PMA 16.1%). Inhibition of PKC using staurosporin increased the number of apoptotic cells (control 27.7%, Staurosporin 36.1%).

Conclusions: We conclude that activation of PKC is able to prevent apoptosis in stretched cells. Remodeling of cytoskeleton to a softer cell by PKC seems to be a possible mechanism for it.

P825
Kallistatin protects against LPS-induced mouse lung injury
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Acute lung injury (ALI) is caused by overwhelming lung inflammation, resulting in diffuse alveolar damage, edema, and subsequently respiratory dysfunction. The mortality remains high, and the treatments are exclusively supportive because of lacking selective and efficacious pharmaceutical agents targeting on the pathogenesis of ALI. Kallistatin is a serine proteinase inhibitor that exhibits pleiotropic functions in vasodilation, anti-angiogenesis, anti-inflammation, and anti-apoptosis, which may contribute to its therapeutic effects in a variety of human diseases. Kallistatin is also found in the lungs, implicating its involvement in the regulation of lung functions. However, the role of kallistatin in the pathophysiology of ALI is still unclear. Herein, we hypothesized that kallistatin plays a role in protection against lung injury. Using kallistatin gene-transferred mice by delivering plasmid DNA encoding human kallistatin into the lungs, we examined the protective effect of kallistatin against LPS-induced lung injury. We found that the severity of lung injury was attenuated in kallistatin gene-transferred mice compared with untreated mice, indicating by decreasing cell counts, LDH and protein levels of bronchoalveolar lavage fluids (BALF). BALF levels of TNF-α, IL-1β, IL-6 were also lower in human kallistatin gene-transferred mice than untreated mice, suggesting a decline in inflammatory response after LPS treatment. In addition, the kallistatin gene-transferred mice showed less extent of epithelial cell apoptosis shown by TUNEL staining. Our data demonstrate for the first time that kallistatin protects against LPS-induced lung injury through attenuation of inflammation and epithelial cell apoptosis.
A single dose of a specific serine protease inhibitor attenuated the protease-antiprotease imbalance in an experimental model of emphysema

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We showed that a single dose of specific serine protease inhibitor in mice attenuated parenchymal destruction with an improvement in lung function in emphysema.

Objectives: To study the possible effects of this inhibitor in pathological mechanisms that contribute to emphysema.

Methods: Mice were subcutaneously injected with either saline or elastase. Animals were divided into two groups: (1) a group receiving a single dose of elastase or saline and (2) a group receiving a single dose of elastase followed by a single dose of the serine protease inhibitor for 5 days. After 21 days, we evaluated the number of macrophages in BALF and macrophage elastase activity in the lung. We also measured the inflammatory cytokines in BALF and serum.

Results: In the group receiving elastase followed by elastase, there was a significant increase in the number of macrophages and elastase activity in the lung compared to the group receiving saline followed by saline. The inflammatory cytokines were also increased in the group receiving elastase followed by elastase.

Conclusions: The use of a specific serine protease inhibitor attenuates the protease-antiprotease imbalance in emphysema.

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P828
Plant proteinase inhibitor from Entrobolomobus contortispiliquum (EcTTI) attenuates elastase-induced pulmonary inflammatory, remodeling and mechanical alterations in mice

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Aims: To evaluate a plant proteinase inhibitor EcTTI contributes to inactivation of elastase-induced mechanical, inflammatory and remodeling alterations.

Methods: C57Bl/6 mice received elastase (E group). Control group received saline (Ve group). Mouse received EcTTI (2mg/kg) on days 13 and 15 after elastase instillation in the E group. On day 30, mice were anesthetized, mechanically ventilated and we analyzed respiratory system resistance (Rrs) and elastance (Ers), tissue elastance (Gtis) and collagen fibers compared to controls (p < 0.05). The model of elastase-induced emphysema in mice used in this study was approved by the Ethical Committee for Animal Experimentation of Universidade Federal de São Paulo (IAG-02011). In addition, all procedures were performed in accordance with the guidelines established by the Brazilian Society for Experimental Therapeutics (SBE).

Results: In E group there was a significant increase in the Ers, Rrs, Htis, Lm, ENO, total cells, macrophages, neutrophils and lymphocytes in BALF, elastic and collagen fibers compared to controls (p < 0.05). In E group there was a significant increase in the Ers, Rrs, Htis, Lm, ENO, total cells, macrophages, neutrophils and lymphocytes in BALF, elastic and collagen fibers compared to controls (p < 0.05).

Conclusions: The use of a plant proteinase inhibitor EcTTI reduces the elastase-induced pulmonary inflammatory, remodeling and mechanical alterations induced by elastase.

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P829
Treatment with proteinase inhibitor, BbCI, modulates inflammatory response, mechanic alterations, and remodeling on elastase-induced emphysema in mice

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Bauhinia bauhinoides Zcrapnian Inuphi (BbCI) is a proteinase inhibitor that neutralizes neutrophil elastase and cationin G. The present study evaluated the capacity of BbCI in the treatment of elastase-induced emphysema.

Methods: Mice received elastase intratracheally (ELA group) or saline (SAL group). Afterwards, mice were treated with BbCI (2 mg/kg) at days 1, 15 and 21 after elastase (ELA group) or saline instillation (SAL group). At day 28, mice were ventilated and respiratory resistance (Rs), elastance (Es), tissue elastance (Htis), tissue damping (Gtis), airway resistance (Raw), and exhaled nitric oxide (ENO) were analyzed, and BbCI was observed. We also quantified, mean linear intercept (Lm), elastic and collagen fibers.

Results: In ELA group, there was significant increase in the Ers, Rs, Raw, Htis, Lm, ENO, total cells, macrophages, neutrophils and lymphocytes in BbCI after a decrease in Ers (P = 0.16 cmH2O/mL/s), Rs (P = 0.16 cmH2O/mL/s), Ers (P < 0.05 cmH2O/mL/s), Htis (P < 0.05 cmH2O/mL/s), and collagen fibers (P < 0.05 cmH2O/mL/s) compared to controls (p < 0.05). In ELA group, we observed a decrease in Ers (37.08 ± 1.6 cmH2O/mL/s), Rs (39.41 ± 1.7 cmH2O/mL/s), Htis (39.41 ± 1.7 cmH2O/mL/s), Lm (0.27 ± 0.2 cmH2O/mL/s), elastic content (0.34 ± 0.03% cells/mL/s),total cells (1.17 ± 0.104 cmH2O/mL/s) and neutrophils (0.00 ± 0.104 cmH2O/mL/s) in BALF compared to ELA group (p < 0.05).

Conclusions: The treatment with BbCI reduced inflammatory mechanisms, mechanical and structural matrix remodeling alterations induced by elastase. Although more studies need to be performed to elucidate the mechanisms involved in this process, but we may considerate BbCI as a therapeutic tool for COPD management.

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P830
Angiopoietsins: Possible biomarkers in severe pneumonia

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In severe pneumonia, endothelial permeability may develop due to an inadequate host-pathogen interaction and might lead to acute lung injury (ALI). The angiopoietins Ang-1 and Ang-2 are centrally involved in inflammation and permeability. The objective of this study was to evaluate the capacity of a new Ang-2 assay to detect increased permeability in patients with severe pneumonia.

Methods: We enrolled 244 patients with severe pneumonia (68.6% PLE type, 31.4% non-pleural) in a prospective observational study (EVIDENCE). Patients were included if pneumonia was confirmed and there was an acute increase in permeability on admission. The primary endpoint was the change in permeability in patients with pneumonia during hospitalization. The secondary endpoints were the change in permeability from baseline to follow-up and the change in permeability from admission to follow-up.

Results: In the pneumonia group, the change in permeability from admission to follow-up was significant (p < 0.001). The change in permeability from admission to follow-up was also significant (p < 0.001). The change in permeability from admission to follow-up was significant (p < 0.001).

Conclusions: The use of a plant proteinase inhibitor EcTTI attenuates the elastase-induced pulmonary inflammatory, remodeling and mechanical alterations induced by elastase. Although more studies need to be performed, this inhibitor may contribute as potential therapeutic tool for COPD management.

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P831
Reduction of endogenous acetylcholine contributes to pulmonary inflammation and destruction.

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Emphysema is characterized by pulmonary inflammation and destruction. Acetylcholine (ACH) regulates inflammation due to the activity of the cholinergic nervous system. The use of a plant proteinase inhibitor EcTTI attenuates the elastase-induced pulmonary inflammatory, remodeling and mechanical alterations induced by elastase. Although more studies need to be performed, this inhibitor may contribute as potential therapeutic tool for COPD management.
anti-inflammatory system in several diseases and ACh release depends on neuromuscular transmitter storage in synaptic vesicles mediated by the vesicular acetylcholine transporter (VACT).

**Aim:** To investigate the role of the cholinergic system on emphysema in mice with reduced levels of ACh release.

**Methods:** Mice with decreased expression of VACT (VACT KD/HOM 70%) (HOM) and littermate wild-type mice (WT) received intranasal elastase or saline on day 0. On day 28, pulmonary mechanics, bronchoalveolar lavage fluid (BALF), mean linear intercept (Lm), elastic and collagen fibers in alveolar septa were evaluated.

**Results:** WT-treated elastase animals presented a reduction in total cells, macrophages, lymphocytes and neutrophils in BALF compared to WT-treated group (p<0.05). There were no differences between WT-treated elastase and HOM-treated elastase groups (p>0.05). HOM, collagen and elastic fibers content were increased.

**Conclusions:** Reduction of endogenous ACh worsens pulmonary inflammation in mice with emphysema without changing pulmonary function and remodeling, suggesting that inflammation per se is not major determinant of pulmonary function in this model. Our results suggest however a major role of the cholinergic anti-inflammatory system in the control of inflammatory response induced by elastase.

P832

**Activation of the coagulation system following exposure of mice to chlorine**

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Chlorine (Cl₂) is a highly irritant and reactive gas produced in large quantities throughout the world. The accidental release of large amounts of Cl₂ in 30 large cities worldwide, caused significant mortality and morbidity to humans and animals. Our previous findings show that exposure of rodents to Cl₂ causes both pulmonary and systemic injury (Zarogiannis et al. Am J Respir Cell Mol Biol. 2011;45(2):386-92; Honavkar et al Am J Respir Cell Mol Biol 2011;44(2):219-25.) Herein we tested the hypothesis that exposure to Cl₂ activates intraalveolar and systemic coagulation cascades, which in turn may contribute to the development of lung injury. Male C57BL/6J mice (6-8 weeks) were exposed to either Cl₂ (600 ppm for 45 minutes in environmental chambers) or air (0 ppm). Mice were returned to room air and sacrificed immediately or at 1 h post-exposure and their lungs were lavaged. Mice exposed to Cl₂ had much higher levels of thrombin-activated F1+2 (measured by ELISA) as compared to those exposed to air both in the BAL (10±±2 ng/ml vs. 0.5±±0.1; mean + SE; n=6; p<0.01) and plasma (25±±1 ng/ml vs. 0.1±±0.05; mean + SE; n=6; p<0.01). In contrast, there was no significant change in the clotting time blood taken from mice immediately after Cl₂. Taken together, these data demonstrate a strong activation of the coagulation system throughout the airways as well as development of a systemic disseminated intravascular coagulation after Cl₂ exposure in mice.

P833

**Novel swine model of transfusion-related acute lung injury (TRALI)**

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**Background:** TRALI is a life-threatening complication of blood transfusion. Antibodies against human leukocyte antigens in donors’ serum are major causes of TRALI. Several animal models of TRALI have been developed, and the mechanisms underlying TRALI development have been extensively investigated using rodent models. Although sheep models of nonimmune TRALI have been developed, large-animal models of immune TRALI have not yet been developed. Because the incidence of TRALI is low and prospective human studies are difficult to conduct, large-animal models mimicking TRALI might be useful for developing treatment options.

**Aims:** To study circulating levels of IL-18 during one week of hypoxia exposure in mice, and to investigate whether induction of IL-18 corresponds with inflammatory changes in lung parenchyma.

**Methods:** IL-18 levels in blood was determined in C57Bl/6j mice (n=4 at each time point) exposed to hypoxic conditions at 6 hours (h) and 1.7 days. Lungs were harvested at each time point for histological analyses. Concentration of MIP-2 in blood was determined at 6h, 12h, 1.3 days.

**Results:** The levels of circulating IL-18 were significantly increased at all time points peaking at day 1 (Figure 1A) compared to normoxic controls. Histology revealed perivascular infiltration of neutrophil granulocytes increasing from day 1 to day 5.
to day 3. At day 7 neutrophils were still present, but to a lesser extent than at day 3. The concentration of MIP-2 was significantly increased at day 1 (Figure 1B).

**Conclusions:** The increase in IL-18 induced by alveolar hypoxia may promote the subsequent influx of neutrophils in lung parenchyma, possibly mediated through the neutrophil chemotactic agent MIP-2.

### P836

**Mesenchymal stem cells and recombinant erythropoietin treatment in an experimental sepsis model**

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As recently found the surface of mesenchymal stem cells (MSCs) have receptors for erythropoietin (EPO), we hypothesized that the introduction of EPO together with MSCs may enhance their effect and improve efficiency of sepsis treatment.

**Aim:** To evaluate effects of combined treatment with EPO and MSC in an experimental LPS sepsis model.

**Methods:** 50 Wistar rats were randomized into 5 groups: Group 1 - the healthy controls, Groups 2-5 were intraperitoneally introduced bacterial LPS 20 mg/kg. Two hours later LPS injection animals received the following intravenous treatments:

- Group 2: 0.5×10⁶ cells/ml. Serum IL-1β level in groups 2 and 4 was significantly higher than in healthy and treated with MSCs and EPO animals. Histologically in the group 5 we observed significantly less leukocyte lung interalveolar septal infiltration and kidney tubular necrosis. The most significant differences in the LPS + EPO group were found in the lymphoid tissue - considerable hyperplasia of spleen white pulp up to 64,9% and thymus cortex up to 69,7% in contrast to LPS controls, Groups 2-5 were intraperitoneally introduced bacterial LPS 20 mg/kg. Two hours later LPS injection animals received the following intravenous treatments:

**Results:** The increase in IL-18 induced by alveolar hypoxia may promote the subsequent influx of neutrophils in lung parenchyma, possibly mediated through the neutrophil chemotactic agent MIP-2.

**Conclusions:** Combined treatment with EPO and MSCs can reduce acute lung injury and kidney damage, cause hyperplasia of lymphoid tissue and enhance the immune response more than separate treatment in an experimental model of sepsis in rats.

### P837

**Derivation and characterization of young and aged stem cell populations in an interleukin 1 receptor antagonist mouse model system**

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Recent evidence suggests that the IL1 receptor antagonist (IL1RN) assumes an important role in regulating stem cell senescence, and a deficiency of IL1RN may contribute to impaired lung tissue repair associated with COPD pathogenesis. Here we isolated stem cells from the teeth of transgenic mice and compared them to bone marrow (BM) derived stem cells from control B6CO mice; B6 IL1RN overexpressing transgenic mice (T16); and B6 IL1RN knockout mice (IL1RN KO) and tested the hypothesis that the IL1 pathway would also regulate stem cell functions in this unique dental stem cell pool. BM cells were obtained by flushing the medullar space of both femurs and establishing adherent cultures. Dental cells were obtained by digesting excised teeth and mandibular pocket overnight, followed by plating single cell suspensions for culture. Cells were then analyzed by immunohistochemistry for stem cell associated as well as for endothelial progenitor and pluripotency-associated markers. A viable stem cell population was obtained and established from the BM and the dental tissues of all mice strains. BM stem cell populations displayed CD105 and KDR surface markers and contained populations expressing the VEGFR2 endothelial progenitor marker, indicating a possible population of circulating endothelial progenitors. Dental cells from both young (8 wks) and old (10 mo) animals contained populations expressing pluripotency-associated markers OCT4, Sox2, and NANOG, as well as the CD105 and Connexin43 surface markers. These results demonstrate that dental tissue derived precursor cells may be obtained from transgenic mice and tested in experimental emphysema models.

### P838

**WITHDRAWN**