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 A549 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 Akt-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. A549 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 A549 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 reduced 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 significant the number of apoptotic cells (control 30,0%, Latrunculin 19,1%) and decrease the level of LDH (control 0,21 U/ml; Latrunculin 0,13 U/ml).
Conclusions: We conclude that the remodelling of cytoskeleton toward 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 (V_T =9 ml/kg, PEEP=5 cm H_2O) (n=12) and an injurious high-stretch ventilation (V_T =25 ml/kg, PEEP=0 cm H_2O) (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 PO_2 reduction and peak airway pressure (Paw) increase, sacrificed at 60 min (HV60) (n=11); and 2) animals with insignificant PO_2 and Paw changes at 60 min (n=18), sacrificed at 150 min (HV150). Lung tissue, plasma, and bronchoalveolar fluid (BAL) were analyzed in the three groups. BAL provided native cytometric, inflammatory marker, and surfactant data.
Results: The HV60 group was characterized by leakage of plasma proteins into the alveoli, presence of hyaline membranes, high increase of inflammatory markers in BAL (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 PO_2. These animals exhibited a slight decrease of alveolar macrophages and increase of some BAL inflammatory markers (IL6, TNF-α, MIP-2, and 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 human pulmonary artery endothelial cells (HPAECs) *in vitro*.
Methods: Normal HPAECs were stretched by a Flexcell^R FX-4000T™ Tension System (Flexcell International). The stretching rate was 15 cycles/minute, and the cells were cyclicly 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 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 protein production of the cells at any sampling points.
Conclusions: The IL-6 protein was produced by excessive cyclic stretch (0 to 20%). However, the IL-6 production was significantly suppressed by PEEP-like cyclic stretch (3 to 20% and 5 to 20%). This result suggests that HPAECs would be protected by PEEP during mechanical ventilation even if the cells are excessively stretched.

P823

LSC 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 we have developed a novel method 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 healthy until necropsy, performed 1d later. Unventilated fetuses were controls, n=6.
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 early response genes (CTGF, CYR-61, and EGR-1) and inflammatory genes (IL-1β, IL-6, IL-8 and TNFα).
Conclusions: Following MV injury, repair processes, commencing with normalisation 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.
Objektives: 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. Remodelling 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, indicated by decreasing cell counts, LDH and protein levels of bronchoalveolar lavage fluids (BALF). BALF levels of TNF-α, IL-1β, MIP-2, and 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.

P826

Plant proteinase from Bauhinia bauhinioides Kallikrein inhibitor (BbKI) attenuates mechanics, inflammation and remodelling induced by elastase in mice

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Proteinases plays a key role on emphysema development. This study evaluated the capacity of the plant proteinase inhibitor BbKI in the inactivation of elastase and its response modulator.
Methods: C57Bl6 mice received elastase intratracheal or saline (Ve group). Afterwards, mice were treated with BbKI (2mg/kg) on days 1, 14, 21 after elastase instillation (I-E group) or saline instilation. On day 30 mice were anesthetized and mechanically ventilated and we analyzed respiratory system resistance (Rrs), elastance (Ers), tissue elastance (Htis), tissue damping (Gtis), airway resistance (Raw) and exhaled nitric oxide (ENO). Afterwards, bronchoalveolar lavage fluid (BALF) was performed and lungs were removed. By morphometry, we quantified the mean linear intercept (Lm), and the amount of collagen and elastic fibers in distal lung parenchyma.
Results: In elastase group there was a significant increase in the Ers, Rrs, Raw, Htis, Lm, ENO, total and, macrophages, neutrophils and lymphocytes in BALF, and elastic and collagen fibres compared to controls ($p<0.05$). The BbKI treatment of elastase group decreased the Lm (59.33±4.74 μm), Raw (0.33±0.05 cmH2O/ml/s), Ers (36.83±5.73 cmH2O/L), Rrs (0.843±0.19 cmH2O/mL/s), Htis (37.360±6.2 cmH2O/mL/s), total cells (69.25±20.98x10^4 cells/mL), neutrophils (19.38±9.11x10^4 cells/mL), lymphocytes (1.95±1.24x10^4 cells/mL) in the BALF, ENO (19,66±8,33 ppb) and elastic fibers content (30%±0.1%) compared to E-group ($p<0.05$).
Conclusions: This proteinase inhibitor (BBKI) reduced elastase-induced pulmonary inflammatory and extracellular matrix remodeling alterations.
Financial Support: FAPESP, CNPq, LIM-20 HCFMUSP.

P827

A single dose of a specific serinoprotease inhibitor attenuated the protease-antiprotease imbalance in an experimental model of emphysema

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We showed that a single dose of specific serinoprotease 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 submitted to either a nasal instillation of porcine pancreatic elastase (PPE) or saline (S) and 1h after, animals received a second nasal instillation of either a protease inhibitor (r-BmTIA, 35.54pmol) or vehicle (VE). After 21 days,we evaluated the number of macrophages (MAC) and cells expressing metalloproteinase 12 (MMP12) to assess the protease-antiprotease imbalance. We quantified caspase-3 to evaluate apoptosis and expression of 8-isoprostane as a marker of oxidative stress.
Results: We observed an increase in MAC in groups that received PPE and only PPE-VE group showed higher values of MMP-12. There were no differences between groups when we analyzed caspase-3 and 8-isoprostane.

	SAL-VE	SAL-BMTIA	ELA-VE	ELA-BMTIA
MAC-2 (cells/μm^2)	1.593±0.406	1.854±0.287	2.607±0.307*	2.202±0.217
MMP-12 (cells/μm^2)	1.474±0.254	1.609±0.180	2.579±0.235**	1.711±0.166
Caspase-3 (cells/μm^2)	1.302±0.218	1.479±0.154	1.461±0.202	1.147±0.143
8-isoprostane (%)	2.658±0.477	4.054±0.477	2.892±0.624	2.470±0.441

*Compared to control groups; **compared to other groups.

Conclusions: Although this inhibitor treatment has not diminished the macrophages in animals that received PPE, it reduced MMP-12 positive cells, suggesting that the improvement in parenchyma destruction and in lung function occurred due to attenuation in protease-antiprotease imbalance.
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P828

Plant proteinase inhibitor from *Enterolobium contortisiliquum* (*EcTI*) attenuates elastase-induced pulmonary inflammatory, remodeling and mechanical alterations in mice

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Aims: To evaluate if a plant *Kunitz* proteinase inhibitor *EcTI* contributes to inactivation of elastase-induced mechanical, inflammatory and remodelling alterations.
Methods: C57Bl6 mice received elastase (E group). Control group received saline (Ve group). Mice were treated with *EcTI* (2mg/kg) on days 1, 14 and 21 after elastase instillation (I-E group). On day 30, mice were anesthetized, mechanically ventilated and we analyzed respiratory system resistance (Rrs) and elastance (Ers), tissue elastance (Htis), tissue damping (Gtis), airway resistance (Raw) and exhaled nitric oxide (E_{NO}). Bronchoalveolar lavage fluid (BALF) was performed, lungs were removed and by morphometry, we quantified the mean linear intercept (Lm), collagen and elastic fibers in lung parenchyma.
Results: In E-group there was a significant increase in the Ers, Rrs, Raw, Htis, Lm, E_{NO}, total cells and macrophages,neutrophils and lymphocytes in BALF, elastic and collagen fibres compared to controls ($p<0.05$). In I-E group there was a decreased in Lm (57.63±5.2μm), Raw (0.29±0.05cmH_2O/ml/s), Ers (34.71±3.16cmH_2O/L), Htis (36.30±4.42cmH_2O/mL/s), macrophages (94.86±2.36%), neutrophils (3.29±2.31%), and lymphocytes (0.91±0.28%) in the BALF, E_{NO} (31.67±2.23ppb) and collagen fibers (0.69%±0.04%) compared to E-group ($p<0.05$).
Conclusions: This proteinase inhibitor (*EcTI*) reduced 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.
Financial support: FAPESP, CNPq, LIM-20 HCFMUSP.

P829

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

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Bauhinia bauhinioides Cruzipain Inhibitor (BbCI) is a proteinase inhibitor that neutralizes neutrophil elastase and cathepsin 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 (ELABI group) or saline instillation (SALBI group). At day 28, mice were ventilated and respiratory resistance (Rrs), elastance (Ers), tissue elastance (Htis), tissue damping (Gtis), airway resistance (Raw), and exhaled nitric oxide (ENO) were analyzed, and BALF was obtained. We also quantified, mean linear intercept (Lm), elastic and collagen fibers.
Results: In ELA group, there was a significant increase in the Ers, Rrs, Raw, Htis, Lm, ENO, total cells, macrophages, neutrophils and lymphocytes in BALF, elastic and collagen fibres compared to controls ($p<0.05$). In ELABI group, we observed a decrease in Ers (37.08±1.6 cmH2O.mL.-1), Rrs (0.76±0.1 cmH2O/mL.-1.s), Raw (0.27±0.1 cmH2O/mL/s), Htis (39.47±1.7 cmH2O/mL/s (1-α)), Lm (58.2±2.7 μm), elastic content (0.34±0.02%), total cells (1.17±0.1 104 cells/mL) and neutrophils (0.00±0.0 104 cells/mL) in BALF compared to ELA group ($p<0.05$).
Conclusions: The treatment with BbCI reduced inflammatory, mechanics and extracellular 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

Angiopoietins: 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. Ang-1 reduces endothelial inflammation whereas Ang-2 enhances the impact of inflammatory stimuli on the endothelium. In murine pneumococcal pneumonia, we previously observed that therapeutic application of Ang-1 attenuated lung injury in severe pneumonia, and pretreatment with specific siRNA against Ang-2 reduced pneumolysin (PLY) induced permeability in isolated perfused mouse lungs (ERS2011).
In this study, Ang-1 and Ang-2 were quantified in serum of patients with lethal (n=58) and non-lethal (n=42) pneumonia (CRB65 0-3;CAPNETZ) as well as patients with pneumonia-induced ALI (VALIDstudy, n=44). Stimulating human umbilical vein endothelial cells (HUVEC) with PLY, we measured Ang-2 levels after 3 hours and investigated the impact of Ang-2 on endothelial barrier function in vitro.
At the time of pneumonia diagnosis, patients with subsequently lethal pneumonia had higher Ang-2 serum levels than patients with non-lethal pneumonia. Ang-2 levels correlated with procalcitonin concentrations. In pneumonia-induced ALI reduced serum Ang-1 and increased serum Ang-2 converged to normal amounts within 7 days. PLY stimulation increased Ang-2 release from HUVEC, and Ang-2 caused a rapid permeability increase in HUVEC monolayers.
Our combined results suggest that angiopoietins may play an important role in the development of lung failure in pneumonia. Current further investigations in larger patient cohorts may validate Ang-2 as biomarker for improved risk stratification in pneumonia.

P831

Reduction of endogenous acetylcholine contributes to pulmonary inflammation in a model of emphysema in mice

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Emphysema is characterized by pulmonary inflammation and destruction. Acetylcholine (ACh) regulates inflammation due to the activity of the cholinergic

anti-inflammatory system in several diseases and ACh release depends on neurotransmitter storage in synaptic vesicles mediated by the vesicular acetylcholine transporter (VAChT).
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 VAChT (VAChT KDHOM 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 tissue elastance (Htis) and an increase in Lm, total cells and macrophages in BALF, and collagen and elastic fibers in lung parenchyma compared to saline groups ($p<0.05$). The HOM-treated elastase animals presented high values of total cells, macrophages, lymphocytes and neutrophils in BALF compared to WT-treated elastase group ($P<0.05$). There were no differences between WT-treated elastase and HOM-treated elastase groups in Htis, Lm, collagen and elastic fibers content.
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 the 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 (Cl_2) is a highly irritant and reactive gas produced in large quantities throughout the world. The accidental release of large amounts of Cl_2 in 30 large cities world-wide, caused significant mortality and morbidity to humans and animals. Our previous findings show that exposure of rodent to Cl2 causes both pulmonary and systemic injury (Zarogiannis et al. Am J Respir Cell Mol Biol. 2011;45(2):386-92; Honavar et al.Am J Respir Cell Mol Biol. 201145(2):419-25.) Herein we tested the hypothesis that exposure to Cl_2 activates intraalveolar and systemic coagulation cascades which in turn may contribute to the development of lung and other end-organ injury. Male C57Bl/6 mice (6-8 weeks) were exposed to either Cl_2 (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 Cl_2 had much higher levels of Thrombin/anti-Thrombin (TAT) complexes (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 ± 0.1 ng/ml vs. 0.1 ± 0.05; mean + SE; n=6; $p<0.01$) at 1 h post exposure. In addition clotting time in the blood (measured by thromboelastometry) was significantly prolonged in Cl2 exposed mice as compared with air controls (275 ± 25 sec. vs. 150 2 ± 10; 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 demonstrates a strong activation of the coagulation within the airspaces as well as the development of a systemic disseminated intravascular coagulation after Cl_2 exposure in mice.

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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 mechanism underlying TRALI development has 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.
Aim: To determine whether a swine model of TRALI can be developed using a monoclonal antibody (mAb) to swine leukocyte antigens (SLAs).
Materials and methods: Male Clawn strain miniature pigs (9-10 months) were used. A mAb against SLA class I (4G8: 0.3 or 1.0 mg/kg body weight (BW)) and a control antibody (1.0 mg/kg BW) were prepared and injected into the peripheral vein after priming with or without 1 μg/kg BW of lipopolysaccharide (LPS) (n=3; each arm). Lung injury was assessed using the PaO_2/FiO_2 (P/F) ratio and by chest X-ray monitoring. Histopathological analysis was also performed.
Results: Lung injury was induced only when the 4G8 mAb with a concentration of 1.0 mg/kg BW was injected after LPS priming. The P/F ratio 90 min after the administration of the antibody had significantly decreased ($p<0.05$). Chest X-ray showed bilateral infiltration. Lung injury was confirmed by histopathological analysis.
Conclusions: Lung injury in pigs was successfully induced by the SLA mAb. Priming with LPS is prerequisite for inducing lung injury and the dose of the antibody is critical to inducing lung injury.

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The role of epithelial-mesenchymal transition in the possibility of chronic process in the lungs during influenza pneumonia, caused by the virus AH1N1
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Despite numerous influenza pandemics and morphological studies of influenza pneumonia, the question of the possible mechanisms of chronisity of the process has not been studied. It is known that in the process of chronic inflammation and sclerosis of various organs plays an important role of epithelial-mesenchymal transition. The aim of our study - to test this hypothesis in the lungs. Studied 30 sectional cases of influenza pneumonia using immunohistochemistry studies: evaluated the expression of cytokeratin and AE1/AE3 pantcytokeratina 18, vimentin, alpha-smooth muscle actin (monoclonal antibody, imaging system En Vision, Daco). The material was divided in 2 groups based on dominant lokalization. First group included 20 cases with significant damage of alveolar epithelium (necrosis, apoptosis, desquamation) and intaalveolar exudate (fibrinous, hemorrhagic, suppurative and often mixed). The second group included 10 cases with predominant changes of interalveolar septae (thickening due to edema, infiltration by inflammatory cells, stromal proliferation with transition fibrosis into myofibroblasts, accumulation of intercelular matrix. In only half of the cases in first group showed features of proliferation of alveolar epithelium in the form of small groups and sometimes numerous type 2 pneumocytes, which are more resistent to damaging factors, capable to division and differentiation toward type 1 pneumocytes and producing surfactant. All cases of the second group were found significant hyperplasia of type 2 pneumocytes. The results of our study allow to suggest that this mechanism may underlie the chronic influenza pneumonia.

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Hypoxia induces IL-18 and neutrophil influx in lung parenchyma possibly mediated by MIP-2
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Background: Increased levels of interleukin (IL)-18 have been found during experimental alveolar hypoxia and IL-18 have previously been shown to induce neutrophil migration, production of macrophage inflammatory protein 2 (MIP-2) and enhanced neutrophil functions (Verri, W.A. et al. Eur J Immunol 2007; 37:3373-80, Kinoshita, M. et al. Infect Immun. 2011; 79:2670-80).
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

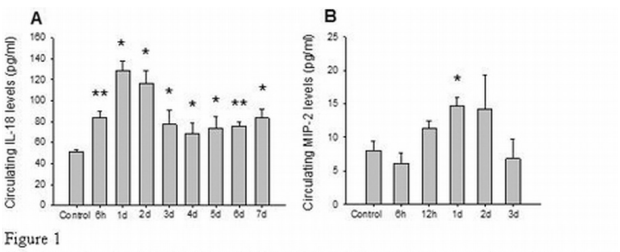

Figure 1
Circulating levels of IL-18 (A) and MIP-2 (B) at different time points during hypoxia compared to normoxia (Control). Values are presented as mean±SE. *$P<0.05$, **$P<0.001$ vs control group.

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 chemoattractant MIP-2.

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WITHDRAWN

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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 3: 4 x10^5 allogeneic MSCs, Group 4: 8.5 μg of recombinant EPO-beta, Group 5: MSCs and EPO in the same doses. Surviving animals were euthanased on the 4th day.
Results: The highest white blood cells count was determined in group 5 (8.15 x 10^6 cells/ml) compared with controls (2,15 $\times 10^6$ cells/ml) and LPS controls (6,52 $\times$ 10^6 cells/ml). Serum IL-1β level in groups 2 and 4 was significantly higher than in healthy and treated with MSCs and MSCc + 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 the atrophy of the corresponding zones in the other groups.
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.

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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 ILRN 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.