93. Anti-inflammatory strategies in acute lung injury

P800

Recurrent recruitment manoeuvres are required to maintain lung functions during low tidal volume ventilation of healthy mice

Lucy Kathleen Reiss, Stefan Uhlig. *Institute of Pharmacology and Toxicology, Medical Faculty of RWTH Aachen University, Aachen, Germany*

Mechanical ventilation (MV) of mice is widely used in experimental studies. However, conditions for ventilation of mice producing stable lung mechanics, blood pressure and heart rate over several hours are not well defined. In the present study, for the first time, MV with and without recruitment manoeuvres (RM) was compared in healthy mice. The effects of RM on a variety of physiological parameters and pulmonary inflammation were studied over 6h.

C57BL/6 mice were ventilated for 6h at low-V_T=8mL/kg, f=180min^{-1} or high-V_T=16mL/kg, f=90min^{-1} and 3% inspiratory CO_2. FiO_2 was 0.5 and PEEP 2cmH_2O. RM were performed with 30cmH_2O for 1s every 5min, 60min or not at all. Lung mechanics were followed by the forced oscillation technique. Blood pressure (BP), ECG, heart frequency (HF), oxygen saturation and body temperature were monitored. Blood gases, histopathology, neutrophil recruitment, microvascular permeability and pro-inflammatory cytokines were examined.

MV with recurrent RM resulted in stable respiratory mechanics. Ventilation without RM worsened lung functions due to alveolar collapse, leading to impaired gas exchange. HF and BP were not affected. Microvascular permeability was highest in atelectatic lungs, whereas neutrophil recruitment and structural changes were strongest in lungs ventilated with high V_T. Although IL-6 and KC were markedly elevated in all ventilated mice, levels were clearly reduced by recurrent RM. In contrast, TNF-α and IP-10 remained at baseline, indicating that only moderate lung injury was induced.

We conclude that recurrent RM are required to prevent atelectasis and resulting lung injury during mechanical ventilation of healthy mice.

P801

Patterns of plasma membrane disruptions distribution in mechanically ventilated lungs

Matteo Pecchiari[1], Ario Monaco[1], Antonia Koutsoukou[2], Edgardo D'Angelo[1]. *[1]Dipartimento di Fisiologia Umana, Università degli Studi di Milano, Milan, Italy; [2]Department of Respiratory Medicine, University of Athens Medical School, Athens, Greece*

Rationale: Abnormally high stresses applied to a cell can result in the loss of cell membrane integrity and the formation of direct communications between intracellular and extracellular spaces, called plasma membrane disruptions (PMD). These lesions could be involved in the genesis of biotrauma as upregulators of proinflammatory mediators expression. Observations limited to the subpleural alveoli only indicate that PMD take place during mechanical ventilation with large tidal volumes (V_T) and a normal end-expiratory lung volume (EELV). It is unknown if PMD develop during low EELV ventilation with physiological V_T.

Objectives: To see if a) mechanical ventilation with physiological V_T at low EELV causes PMD; and b) the parenchymal distribution of PMD differs between ventilation with large V_T at normal EELV and ventilation with physiological V_T at low EELV.

Methods: PMD have been detected as red spots in gelatin included slices of rat lungs stained with ethidium homodimer-1 shortly after anesthesia, after prolonged ventilation at low EELV followed or not by the restoration of physiological EELV, and after prolonged ventilation with large V_T and normal EELV.

Main results: PMD increased during ventilation at low EELV, mainly at bronchiolar level. Resealing of most PMD occurred on restoration of a normal EELV. Ventilation with large V_T caused the appearance of PMD both bronchiolar and parenchymal, the latter to a much greater extent than with ventilation at low EELV. The increase of PMD correlated with the concomitant increase of interrupter resistance with both modes of mechanical ventilation.

Conclusions: Entity and distribution of PMD depends on the type of injurious mechanical ventilation.

P802

Dexamethasone reduces lung inflammation induced by alveolar stretch in mice

Jessica Hegeman[1,2,3], Marije Hennus[3], Pieter Cobelens[2,4], Annemieke Kavelaars[2], Nicolaas Jansen[3], Koen van der Sluijs[1], Marcus Schultz[1,5], Adrianus van Vught[3], Cobi Heijnen[2]. *[1]Lab. Experimental Intensive Care and Anesthesiology, Academic Medical Center Amsterdam, Amsterdam, Netherlands; [2]Lab. Neuroimmunology and Developmental Origins of Disease, University Medical Center Utrecht, Utrecht, Netherlands; [3]Department of Pediatric Intensive Care, University Medical Center Utrecht, Utrecht, Netherlands; [4]Department of Intensive Care Medicine, University Medical Center Utrecht, Utrecht, Netherlands; [5]Department of Intensive Care Medicine, Academic Medical Center Amsterdam, Amsterdam, Netherlands*

Background: Although mechanical ventilation is a lifesaving procedure, the associated alveolar stretch can provoke lung injury (ventilator-induced lung injury, VILI). At present, it is thought that ventilator-induced lung inflammation may precede lung injury. Activated granulocytes are known to induce oxidative stress and protease activity in alveoli, causing alveolar-capillary barrier disruption and lung dysfunction.

Aim: To study the anti-inflammatory action of dexamethasone, a widely used glucocorticoid, in mice exposed to either low or high alveolar stretch.

Methods: C57Bl6 mice were mechanically ventilated for 5 hours with either an inspiratory pressure of 10 cmH_2O ("low" tidal volumes (V_T) ~7.5 ml/kg; LV_T) or 18 cmH_2O ("high" V_T ~15 ml/kg; HV_T). Dexamethasone was intravenously administered at initiation of ventilation. Non-ventilated mice served as controls. Inflammatory mediator expression and granulocyte influx were determined in lung homogenates. Differential cell counts were done on BALf cytospin preparations.

Results: Both LV_T and HV_T-ventilation increased inflammatory mediator ex-

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pression in lung tissue which was accompanied by granulocyte influx ($p<0.05$). BALf neutrophil numbers and inflammatory mediator expression (KC, IL-1β, IL-6, E-selectin) were enhanced in HV_T-ventilated mice compared to LV_T-ventilated mice ($p<0.05$). Dexamethasone inhibited lung inflammation caused by LV_T or HV_T-ventilation ($p<0.05$).
Conclusion: Dexamethasone prevents inflammatory mediator expression and granulocyte influx in lungs of mice exposed to low or high alveolar stretch. Dexamethasone treatment may be considered as a potential therapeutic strategy to inhibit the inflammatory response during mechanical ventilation.

P803
Intermedin stabilized endothelial barrier function and attenuated ventilator-induced lung injury in mice
Holger Christian Müller[1], Wolfgang Kummer[2], Uwe Pfeil[2], Katharina Hellwig[1], Daniel Will[1], Andreas Christian Hocke[1], Stefan Hippenstiel[1], Norbert Suttorp[1], Witzenrath Martin[1]. *[1]Department of Infectious Diseases and Pulmonary Medicine, Charité - Universitätsmedizin Berlin, Berlin, Germany; [2]Anatomy and Cell Biology - University of Giessen Lung Center, Justus Liebig Universität Giessen, Giessen, Germany*

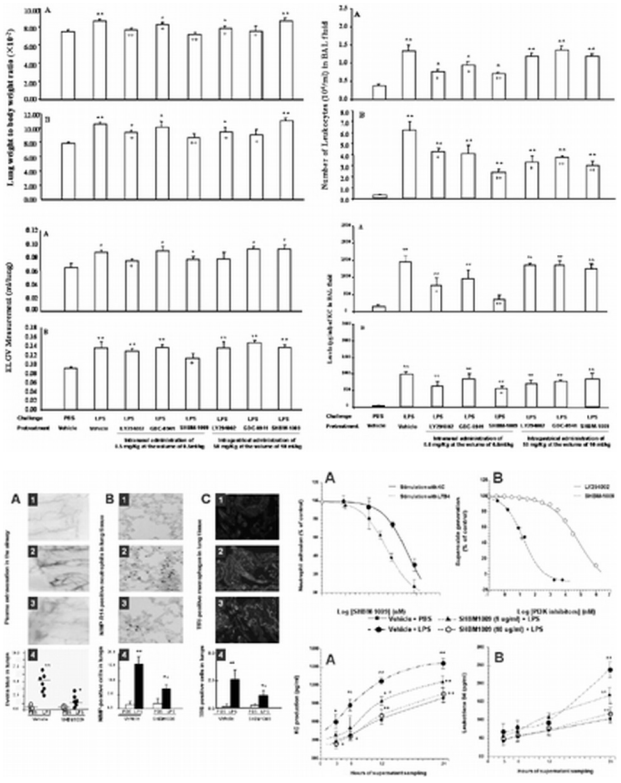
Rationale: Even protective ventilation may aggravate or induce lung failure, particularly in preinjured lungs. Thus, new adjuvant pharmacologic strategies are needed to attenuate ventilator induced lung injury (VILI). Intermedin/Adrenomedullin II (IMD) stabilized pulmonary endothelial barrier function in vitro. We hypothesized that IMD may attenuate VILI-associated lung permeability in vivo.
Methods: Human umbilical vein endothelial cell (HUVEC) monolayers were incubated with IMD and transendothelial electrical resistance was measured to quantify endothelial barrier function. Expression and localisation of endogenous pulmonary IMD, and its receptor complexes composed of CRLR und RAMP1-3 were analyzed by qPCR and immunoflourescence in unventilated mouse lungs and in lungs ventilated for 6h. In untreated and IMD treated mice, lung permeability and pulmonary leukocyte recruitment was assessed after mechanical ventilation.
Results: IMD stabilized endothelial barrier function in HUVECs. Mechanical ventilation reduced the expression of RAMP 3, but not of IMD, CRLR, and RAMP1 and 2. Mechanical ventilation induced lung permeability, which was ameliorated by IMD treatment. IMD did not reduce VILI associated pulmonary leukocyte recruitment.
Conclusion: We showed for the first time that IMD had endothelial barrier stabilizing properties in vivo. IMD may possibly provide a new approach to attenuate ventilator-induced lung injury.

P804
Plasma levels of LL37 in patients with and at risk of acute lung injury
Rachel Dancer[1], Gavin Perkins[2], Daniel Park[1], David Thickett[1]. *[1]Department of Respiratory Science, University of Birmingham, Birmingham, United Kingdom; [2]Clinical Trials Unit, University of Warwick, Coventry, United Kingdom*

Cathelicidins are a group of antimicrobial peptides with a wide range of functions including immunomodulation, chemoattraction, angiogenesis and antimicrobial effects. LL-37 is the active form of the only known human example of a cathelicidin, HCAP-18. It is induced by the local action of 1,25-OH vitamin D. LL37 is expressed by many different cells including lung epithelial cells, neutrophils and other immune cells. We have shown that patients with severe vitamin D deficiency have an increased risk of Acute Lung Injury post-oesophagectomy and hypothesized that this may be related to the effects of vitamin D on LL37.
Methods: Plasma levels of LL37 were determined by ELISA in a cohort of patients with Acute Lung Injury and in a cohort of patients at risk of Acute Lung Injury due to undergo oesophagectomy.
Results: Average pre-operative plasma LL37 levels in the oesophagectomy cohort were 117.2ng/ml and post operative levels are significantly lower ($p=0.027$, paired t test). In our ALI cohort, median levels of LL37 on day 0 lower than normal levels ($p=0.017$). LL37 levels correlate with vitamin D status, however, preoperative LL37 levels did not predict Acute Lung Injury.
Conclusion: LL37 levels are lower than normal in patients with Acute Lung Injury and fall perioperatively in patients undergoing oesophagectomy. The mechanism underlying this is unclear and requires further evaluation, but may be related to vitamin D deficiency in these patients.

P805
Preventive and therapeutic effects of phosphoinositide 3-kinase inhibitors on acute lung injury
Xiaocong Fang[1], Chengshui Chen[2], Yaoli Wang[3], Xuwen Chen[4], Chunxue Bai[1], Xiangdong Wang[1,5]. *[1]Department of Pulmonary Medicine, Zhongshan Hospital, Fudan University, Shanghai, China; [2]Department of Respiratory Medicine, Wenzhou Medical College and The First Hospital, Wenzhou, Zhejiang, China; [3]Intensive Care Unit, Daping Hospital,The Third Military Medical University, Chongqing, China; [4]Shanghai Biomedical Ltd, Shanghai Biomedical Ltd, Shanghai, China; [5]Biomedical Research Center, Zhongshan Hospital, Fudan University, Shanghai, China*

Background: Phosphoinositide 3-kinases (PI3Ks) are involved in a number of biological responses. Recent preclinical studies demonstrated that PI3K-dominent signal pathway could play an important and critical role in the development of acute lung injury, while the number of studies are limited and the mechanism remains unclear.
Methods: CD-1 mice were intranasally or intragastrically administered with different PI3K inhibitors once a day for three days before intratracheal instillation of LPS for 4 and 24 hours. Effects of SHBM1009 on LPS-induced capillary permeability, leukocyte distribution and epithelial cells were measured. Besides, the therapeutic effects of SHBM1009 on pancreatic elastase induced lung injury was evaluated in wistar rats.
Results: Local delivery of PI3K inhibitors had more effective roles in the prevention from LPS-induced ALI than systemic delivery. PI3K inhibitors prevented both LPS- or elastase-induced lung injury in mice and rats probably through the directly inhibitory effects on airway epithelial cells, activating neutrophils and macrophages.



Conclusion: PI3K may be a therapeutic target for lung injury.

P806
Adenosine but not the A2A adenosine receptor agonist, CGS 21680 attenuates the endothelial cell barrier disruption induced by lipopolysaccharide
Joyce Gonzales, Alexander Verin. *Pulmonary, Georgia Health Sciences University, Augusta, GA, United States*

Rationale: Acute lung injury and acute respiratory distress syndrome (ALI/ARDS) are severe disorders. Pulmonary injury is at the endothelial cell (EC) barrier with increased permeability. Our *in vitro* studies demonstrate that adenosine protects from the bacterial toxin lipopolysaccharide (LPS) induced barrier dysfunction.
Hypothesis: Based on the known activation of the A2A adenosine receptor (A2AR) with adenosine it was hypothesized that the A2A agonist, CGS 21680 (CGS), would attenuate the LPS disrupted EC barrier *in vivo*.
Methods: Control and A2AR knock out (KO) mice were given normal saline (NS) or LPS intratracheally. NS or CGS was instilled intravenously (IV) at the same time or 3hrs later. At 22 hours Evans Blue Dye albumin (EBD) was instilled IV, at 24hrs the samples were collected. Bronchoalveolar lavage (BAL) was done and the lungs harvested. Cell counts, protein and EBD extravasation were analyzed.
Results: LPS caused an increased number of cells in the LPS/adenosine treated mice and the KO mice treated with CGS at 3hrs consistently demonstrated a decreased cell count. LPS challenge increased the protein in BAL and EBD from lung, the protein and EBD were significantly attenuated in the LPS/adenosine treated mice but not in the CGS treated mice.
Conclusions: Adenosine significantly attenuates the LPS induced EC barrier dysfunction in mice. The A2AR agonist, CGS, attenuates the cell count but not the protein or EBD extravasation. Other adenosine receptors or mechanisms may be involved in the significant improvement of protein and EBD barrier disruption that occurs with adenosine treatment.

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P807

Use of lipids in a murine model of ALI and lymphocyte apoptosis – Harmful or beneficial?

Juliane Ott, Britta Bausch, Matthias Rose, Andrea Mohr, Matthias Hecker, Werner Seeger, Konstantin Mayer. *Internal Medicine, University of Giessen Lung Center, Giessen, Germany*

Acute lung injury (ALI) and sepsis remain major challenges in critical care. While a massive inflammation determines early sepsis, apoptosis of lymphocytes is a hallmark of late sepsis. Lipid emulsions (LE) based on soybean oil (SO) are used in critically ill to maintain caloric intake. Fish oil (FO) based LE are considered as alternative with immunmodulatory impact. We investigated the effects of LE in a murine model of ALI.

Mice were infused with SO, FO or NaCl. 24h after intratracheal instillation of 10μg lipopolysaccharid (LPS), a bronchoalveolar lavage (BAL) was performed to determine numbers of leukocytes, protein and cytokines. Lymphocytes were isolated from spleen and apoptosis was determined by FACS.

LPS induced a massive invasion of leukocytes into the airspace compared to unstimulated controls. Infusion of SO amplified whereas FO attenuated the rise. Both, protein and TNF exhibited a LPS-induced rise which was further increased by SO. Infusion of FO reduced protein as well as TNF after LPS.

Before LPS, infusion of SO induced a significant rise in apoptosis of lymphocytes. After LPS, a reduced number of lymphocytes accompanied with a rise in apoptosis were detected in all groups with FO infused mice showing significantly less apoptosis compared to SO.

In a murine model of ALI the choice of lipid emulsions is able to influence inflammatory parameters. Induction of ALI is paralleled by reduced lymphocytes with increased apoptosis in the spleen. SO leads to massive apoptosis in lymphocytes even before ALI. Infusion of FO attenuated the rise in ALI-induced apoptosis. Modulating the lipid emulsions used for nutrition may be relevant for critically ill and may have impact on outcome.

P808

Eugenol dose-dependent improvement of pulmonary lesions in lipopolysaccharide acute lung injury

Clarissa Magalhães[1], Natália Casquilho[1], Mariana Machado[1], Douglas Riva[1], Rodrigo Fortunato[1], José-Henrique Leal Cardoso[2], Leonardo Travassos[1], Walter Zin[1]. *[1]Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil; [2]Biomedical Sciences Institute, State University of Ceará, Fortaleza, Ceará, Brazil*

Background: Eugenol, a methoxyphenol component of clover oil, inhibits NF-κB activation induced by TNF-α in LPS-stimulated macrophages.

Aim: To evaluate the effects of different doses of eugenol on lung mechanics, histology and cytokines in LPS-injured lungs.

Methods: 59 female BALB/c mice were randomly divided in 9 groups (n=5-8/group). Mice received intratracheally sterile saline solution (0.05 ml) or LPS (10 μg in 0.05 ml of saline); 6 h later they received sterile saline (0.2 ml) and Tween 1% (C, and L groups) or different doses of eugenol: 16, 65, 114, 160, 650 or 1140 mg/kg in saline (0.2 ml) and Tween 1% (LE1, LE2, LE3, LE4, LE5, LE6 and LE7 groups, respectively) by gavage. Mice were evaluated 24 h after receiving LPS. In another 18 mice [C=6, L=6, E=3 (saline followed by eugenol) and LE=3]; in these animals TNF-α and IL-1β were detected by ELISA in lung homogenates at 6 (C=3 and L=3) and 24 h (C=3, L=3, E=3 and LE=3) after LPS administration. One-way ANOVA followed by Tukey test was used (α=5%).

Results: Static elastance, viscoelastic component of elastance and viscoelastic resistive pressure were higher in L group (33.05, 5.01 cmH_2O/ml, and 1.00 cmH_2O, respectively) than in C (22.13, 3.62 cmH_2O/ml, and 0.71 cmH_2O), accompanied by alveolar collapse and collagen fiber deposition; eugenol reduced the parameters in LE4 group (except alveolar collapse) and abolished them from LE5 (23.34, 3.62 cmH_2O/ml, and 0.72 cmH_2O) onwards. LE group showed smaller TNF-α and IL-1β levels than L mice. LE behaved similarly to C and E groups.

Conclusion: Eugenol exhibits an *in vivo* anti-inflammatory dose-dependent action in LPS-induced lung injury. Supported by: CNPq, FAPERJ, MCT.

P809

Increased uric acid levels in bronchoalveolar lavage fluid of mice infected with H1N1 influenza

Maria Kuipers, Maria Hegeman, Hamid Aslami, Geartsje Jongsma, Catharina Wieland, Marcus Schultz, Koenraad van der Sluijs. *Laboratory of Experimental Intensive Care and Anesthesiology, Academic Medical Center, Amsterdam, Netherlands*

Rationale: Lower respiratory tract infections with influenza are associated with severe inflammatory responses, which may result in ALI/ARDS. Tissue injury results in the release of damage-associated molecular patterns (DAMPs), such as uric acid and ATP, leading to NLRP3/inflammasome activation and IL-1β release.

Hypothesis: We hypothesized that influenza-induced lung injury is associated with the release of DAMPs into the airway lumen.

Methods: C57Bl/6 mice were inoculated with 10TCID50 influenza A/PR/8/34 (H1N1) and sacrificed 4, 8 and 14 days later to collect BALF to determine uric acid, extracellular ATP and markers of inflammation and lung injury. Non-infected mice were sacrificed on day 0 for control measurements.

Results: Influenza virus infection resulted in bodyweight loss between day 6 and day 11 ($p<0.05$) and returned to normal values on day 14 after infection. Uric acid levels in BALF were significantly increased on day 8 after viral infection (52.7-115.9 μM *vs* 12.2-25.8 μM in control mice, 95% CI, $p<0.01$), while ATP was undetectable. Uric acid in BALF was associated with increased levels of inflammatory markers (IL-6, KC and IFN-γ) as well as markers of lung injury (sRAGE and total protein in BALF). However, increased IL-1β levels, indicative for inflammasome activation, were only observed on day 4 after influenza infection ($p<0.01$).

Conclusion: Uric acid in BALF is increased during influenza infection and associates with biomarkers of inflammation and lung injury, but not with markers of inflammasome activation. Whether uric acid fails to activate NLRP3/inflammasome or that IL-1β is scavenged by IL-1 receptor antagonist during influenza infection remains to be determined.

P810

Effects of vitamin D on alveolar epithelial wound repair and cell survival

Rachel Dancer[1], Shengxing Zheng[1], Vijay D'Souza[1], Fang Gao[2], David Thickett[1]. *[1]Department of Respiratory Science, University of Birmingham, Birmingham, United Kingdom; [2]Clinical Trials Unit, Warwick Medical School, Coventry, United Kingdom*

It is increasingly recognised that vitamin D is important in both adaptive and innate immunity with significant immunomodulatory effects. Patients undergoing oesophagectomy are at significant risk of post-operative Acute Lung Injury. They are severely vitamin d deficient and those with the lowest levels of vitamin D have a greater systemic inflammation with increased epithelial damage and extra-vascular lung water. We hypothesised that vitamin d has a trophic role to protect against alveolar epithelial damage.

Methods: A549 cells were mechanically wounded using a pipette tip and then wound repair after 24 hours culture with or without 25 D3 was assessed by videomicroscopy. Cell proliferation was measured by BRDU incorporation. Cells were incubated with FasL alone or with 25 D3 for 24 hours. Cell viability was measured using a Celltiter Aqueous solution (Promega).

Results: A549 and primary alveolar type II cells increased wound repair in response to a physiological dose of vitamin 25D3. BRDU incorporation was similarly increased supporting proliferative effect of vitamin D. sFasL induced cell death was reduced by addition of 50nmol/L 25-OH vitamin D ($p=0.001$) when added before or up to 1.5 hours after addition of sFasL suggesting that vitamin d reduced apoptotic cell death. sFasl inhibited proliferation of ATII in actions that were blocked by vitamin d.

Conclusions: Our data suggests that Vitamin D plays a role in the maintenance of an intact alveolar epithelial barrier through effects on wound repair and is protective against apoptotic cell death.

P811

Modeling resolution of direct acute lung injury

Brijesh Patel, Michael Wilson, Masao Takata. *Section of Anaesthetics, Pain Medicine and Intensive Care, Imperial College London, London, United Kingdom*

Translational animal models allowing the study of both inflammatory and resolution phases of acute lung injury (ALI) are lacking. Widely used models including intratracheal endotoxin or bleomycin are difficult to translate. Here we developed and optimized a reproducible model of resolving aspiration pneumonitis that mimics human ALI.

C57Bl6 mice were instilled intratracheally with 75μl of 0.1M hydrochloric acid by direct laryngoscopy under anesthesia. At specified timepoints up to 10 days after instillation, respiratory mechanics, arterial blood gases, soluble mediators in bronchoalveolar lavage fluid (BALF), and alveolar fluid clearance (AFC) using an in situ preparation were measured. Acid instillation produced significant increases in BALF protein levels and respiratory elastance. BALF levels of TNF and soluble RAGE, a marker of epithelial damage, both increased, while AFC deteriorated. All of these injury parameters peaked at days 1-3 post-acid, but resolved by days 5-10. These results indicate that the model replicates major hallmarks of ALI, i.e. changes in respiratory mechanics, gas exchange, lung permeability, inflammation

Abstract P811 – Table 1

	Control (untreated)	3 hrs	Day 1	Day 2	Day 3	Day 5	Day 10
PaO2:FiO2	555±62	119±28**	162±71**	206±130**	262±156**	413±189	521±90
BALF Protein Conc. (mg/ml)	0.15±0.02	–	4.86±0.47**	3.36±0.56**	1.72±0.89**	0.73±0.20	0.31±0.09
BALF TNF (pg/ml)	Undetectable	–	223±121*	338±202**	45±23	Undetectable	Undetectable
AFC (%/30 mins)	10.95±0.97	8.11±2.4	7.19±2.3*	6.36±1.5**	9.67±2.3	13.0±1.6	12.9±2.7

One-Way ANOVA with Bonferroni test. Mean±SD. **P<0.01, *P<0.05 vs control. N = 3–6/each time point.

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and AFC, but shows full resolution. This model will allow future investigation of novel resolving pathways.
Supported by the Wellcome Trust.

P812
Fas activation impairs the alveolar epithelial function in mice by mechanisms involving apoptosis
Raquel Herrero[1], Lincoln S. Smith[1], Osamu Kajikawa[1], Stephen Mongovin[1], Venus A. Wong[1], Gustavo Matute-Bello[2], Thomas R. Martin[1]. *[1]VA Puget Sound Medical Center. Division of Pulmonary and Critical Care Medicine, University of Washington, Seattle, WA, United States; [2]Center for Lung Biology. Division of Pulmonary and Critical Care Medicine, University of Washington, Seattle, WA, United States*

Background: Alveolar epithelial damage is a critical event that leads to protein-rich edema in acute lung injury (ALI). Even though Fas activation induces apoptosis of alveolar epithelial cells, its role in the formation of lung edema is unclear.
Aim: We investigated whether inhibition of caspase-dependent apoptosis protects against Fas-mediated epithelial injury in mouse lungs.
Methods: We administered the pan-caspase inhibitor Z-VAD.fmk (10 mg/kg) or vehicle subcutaneously to mice treated with one intratracheal dose of recombinant human sFasL (rh-sFasL, 25 ng/g) or PBS, then studied the mice 16 h later. We measured alveolar fluid clearance (AFC) by intratracheal instillation of FITC-human albumin, and protein permeability by measuring IgM in bronchoalveolar lavage fluid. Caspase-3 activity and cytokines (IL-1β, IL-6, KC, TNF-α) were measured in lung homogenates.
Results: Compared with PBS-treated mice, the intratracheal instillation of rh-sFasL decreased AFC (PBS= 20.0±1.3% vs rh-sFasL= 1.9±2.4%, $p<0.05$), and increased protein permeability (PBS= 34.7±18.3 vs rh-sFasL= 350±40.4 ng/mL, $p<0.05$), caspase-3 activity and cytokine production. In contrast, mice treated with rh-sFasL and Z-VAD.fmk had normal AFC (17.0±2.3%, $p<0.05$) and a smaller increase in protein permeability (152.5±18.4 ng/mL, $p<0.05$), associated with a reduction of caspase-3 activity and an increase in cytokine production. Z-VAD.fmk was not harmful in PBS-treated mice.
Conclusion: Activation of the Fas pathway impairs the alveolar epithelial function in mouse lungs by mechanisms involving caspase-dependent apoptosis, suggesting that targeting apoptotic pathways could reduce the formation of lung edema in ALI.

P813
Resolvin D1 attenuates lung inflammation in LPS induced-ALI partly through PPARγ/NF-κB pathway
Zenglin Liao[1], Jiajia Dong[1], Tao Wang[2], Fuqiang Wen[1]. *[1]Department of Respiratory Medicine, West China Hospital of Sichuan University, Chengdu, Sichuan, China; [2]Division of Pulmonary Diseases, State Key Laboratory of Biotherapy of China, Chengdu, Sichuan, China*

Objective: Acute Lung Injury (ALI) is characterized by tissue damage and respiratory failure with uncontrolled inflammation. Since docosahexaenoic acid (DHA) and DHA-derived lipid mediators have recently been shown to possess anti-inflammatory and pro-resolving properties, we sought to investigate the effect of a novel DHA-derived mediator termed resolvin D1 (RvD1) on LPS-induced ALI.
Methods: BALB/c mice were randomly divided into seven groups: saline group; RvD1 (600ng) group; LPS group; LPS+RvD1 (300ng) group; LPS+RvD1 (600ng) group; GW9662 (a PPARγ antagonist) group; LPS+RvD1 (600ng) + GW9662 group. LPS (50ug/mouse in 100ul saline) or saline was instilled intratracheally. RvD1 was injected intravenously 24h and 30min before LPS instillation. GW9662 was injected intravenously 30min before RvD1 injection. Mice were killed at 6, 12, and 24h. BALF samples were collected for cell counts and cytokine analysis. Lung tissues were collected for histological analysis and western blot analysis.
Results: RvD1 significantly decreased total leukocyte counts by 44±7%, and also reduced TNF-α, IL-6 expression levels in BALF in comparison to the LPS group ($p<0.05$, n=9). H&E staining of histological sections showed that RvD1 markedly attenuated LPS-induced lung inflammation. Western blot analysis revealed that RvD1 activated PPARγ and suppressed I-κBα degradation and p65 nuclear translocation. PPARγ inhibition with GW9662 could partially reverse the effect of RvD1 in suppressing I-κBα degradation and p65 nuclear translocation.
Conclusions: These results suggest that RvD1 may attenuate lung inflammation of LPS-induced ALI through suppressing NF-κB activation, which is partly dependent on PPARγ activation.

P814
A CXCR2 antagonist inhibits polyI:C-induced airway neutrophil, but not lymphocyte infiltrate
Paul Harris, Ruoqi Peng, Lisa Burns, Jonathan E. Phillips, Jay S. Fine, Christopher S. Stevenson. *Inflammation Discovery, Hoffmann-La Roche, Nutley, NJ, United States*

Introduction: PolyI:C is a synthetic dsRNA that triggers an innate immune response through the activation of TLR3 and RNA helicases. Our aim was to dissect the mechanism driving polyI:C-induced airway neutrophilia.
Methods: Male, BALB/c mice were intranasally administered polyI:C (30 mg/animal) or saline under isofluorane. Bronchoalveolar lavage fluid (BALF) was collected 2-168h later (n=8). BALF cells were counted and inflammatory mediators were measured in BALF supernatants by ELISA. The efficacy of a CXCR2 antagonist, SCH563750 (3-30 mg/kg) or its vehicle (2% Klucel, 0.1% Tween 80 in water), was assessed by administering p.o. 1h prior to polyI:C challenge. Airway hyper-responsiveness (AHR) and BALF inflammation were assessed 24h later.
Results: Administration of polyI:C increased several mediators in the BALF. Greatest amongst these was KC, peaking at 2h (2366±397 in polyI:C vs 8±1 control animals; $P<0.01$) and remaining elevated up to 72h. Neutrophil infiltration followed the increased KC levels, increasing from 6h before resolving back to baseline at 96h. Lymphocytes, predominantly CD49b+ NK cells, increased from 24h and were still elevated 168h post-challenge. SCH563750 attenuated the neutrophils in the BALF by 92% at a dose of 30 mg/kg ($P<0.01$). SCH563750 had no effect on BALF lymphocytes or AHR, although there was a trend towards a reversal of the latter.
Conclusion: Poly I:C administration to the airways increased chemokine levels, including KC. The influx of neutrophils was abrogated by a CXCR2 inhibitor. The lack of neutrophil infiltrate did not affect lymphocyte migration to the airways, suggesting an independent mechanism drives this response.

P815
Temporal characterization of murine genomic response to poly I:C stimulation reveals tri-phasic inflammatory response signatures
Sriram Sridhar, Paul Harris, John Allard, Ruoqi Peng, Palani Ravindran, Hans Bitter, Michael E. Burczynski, Jay S. Fine, Christopher S. Stevenson. *Inflammation Discovery, Hoffmann-La Roche, Nutley, NJ, United States*

Introduction: Poly I:C mimics a viral infection through the activation TLR3 and RNA helicases. Our aim was to characterize molecular changes occurring in the lung after polyI:C exposure in mice.
Methods: BALB/c mice were administered saline or poly I:C (30 μg/animal). At 7 timepoints after dosing (2-168h), poly I:C- and saline-treated mice were sacrificed, bronchoalveolar lavage was performed and lungs were snap-frozen. RNA from lung homogenates was isolated using the NuGen labeling method and assessed on Affymetrix standard murine whole genome arrays (MOE430 2.0) (n=6 per group). Differentially expressed genes were determined using an ANOVA, with pairwise comparisons between poly I:C and saline treatment at each timepoint. Functional ontologies were determined using GO annotations. Gene set enrichment analysis (GSEA) was determined for cytokine and immune cell signatures compiled from the literature.
Results: The peak response occurred 6-48h post-treatment. Hierarchical clustering across the study revealed 3 distinct temporal clusters (early, mid, and late phase). Inflammatory processes were enriched in the early phase (2-6h), TLR/IL-1 signaling genes in the mid phase (6-48h), and cell cycle pathways in the late phase (>72h). GSEA revealed activated NK and dendritic cell signatures up to 96h post-challenge, while several immune and myeloid related gene modules were also up-regulated between 6-96h post-challenge.
Conclusions: Inflammatory signatures including TLR and IL1-related genes were highly up-regulated in response to poly I:C challenge, indicating a strong inflammatory response potentially driven by NK and dendritic cells in the lungs of mice.

P816
Molecular mechanisms are involved in ethanol mediated lung endothelial barrier dysfunction
Umapathy Siddaramappa, Alexander Verin. *Vascular Biology Center, Georgia Health Sciences University, Augusta, GA, United States*

Recent studies have uncovered a significant but previously unknown correlation between alcohol (ethanol) abuse and the risk of acute respiratory distress syndrome (ARDS) (1). Despite studies aimed at improving outcomes in patients with ARDS, the mortality remains high at > 40%. For those who abuse alcohol, the mortality is even higher, at 65% (2) and, therefore, alcohol abuse causes tens of thousands of excess deaths annually. One of the important functions of lung endothelium is to provide a barrier against the penetration of bacterial toxins in the circulation and endothelial junctional proteins act as a structural barrier against the paracellular permeation (3). We hypothesized that excessive alcohol could negatively regulate the integrity of the lung endothelial barrier and increases the risk during bacterial infections. Our novel preliminary data using human lung microvascular endothelial cells (HLMVEC) demonstrate that ethanol dose-dependently disrupts the EC barrier properties as evidenced by Electric Cell-Substrate Impedance Sensing (ECIS) based transendothelial electrical resistance measurement. Pretreatment of the HLMVEC with LPS and subsequent challenge with ethanol disrupts the tight junctional proteins and increased phosphorylation of Myosin light chain compared to LPS or ethanol alone treatment suggesting an increased damage to the endothelium when one takes alcohol during bacterial infection. We showed that ethanol inhibit the endothelial wound healing process.
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Effect of proteinase inhibitor from *crataeva tapia* (cratabl) in distal lung mechanical, inflammatory and remodeling alterations induced by elastase in mice

Leandro V. Oliva[1], Rafael Almeida-Reis[1], Osmar Theodoro Jr.[1], Bruno Tadeu Martins-Oliveira[1], Clarice R. Olivo[1], Tales V. Lopes[1], Alessandra C. Toledo[1], Rodrigo S. Ferreira[2], Marlon V. Brito[2], Milton A. Martins[1], Maria L.V. Oliva[2], Iolanda F.L.C. Tiberio[1]. *[1]Clinical Medicine, School of Medicne University of Sao Paulo, Sao Paulo, Brazil; [2]Biochemistry, Federal University of Sao Paulo, Sao Paulo, Brazil*

Aims: The leading role of elastase in the emphysema physiopathology has been recognized. The present study aimed to evaluate if a plant proteinase inhibitor *CratabL* contributes to inactivation of elastase-induced mechanical, inflammatory and extracellular matrix remodelling alterations.
Methods: C57Bl6 mice received elastase intranasally (50ml/animal E group). Control group received saline (Ve group). Afterwards, mice were treated with *CratabL* (2mg/kg) at days 1, 15, 21, 28, 35 after elastase instillation (I-E group). At day 40, mice were anesthetized and mechanically ventilated and we analyzed respiratory system resistance and elastance, tissue elastance, tissue damping and airway resistance. Afterwards, BAL was performed and lungs were removed. By morphometry, we quantified the mean linear intercept (Lm) and the collagen and elastic fibers in distal lung parenchyma.
Results: We did not observe any differences in pulmonary mechanics comparing all groups. In E group, there was an increase in BAL-total cells, BAL-lymphocytes, BAL-neutrophils, collagen and elastic fibers and Lm compared to Ve group ($p<0.05$). The *CratabL* treatment in elastase treated animals decreased Lm ($105.9\pm10.0\mu m$) compared to E group ($p<0.05$). BAL-neutrophils ($7.2\pm1.4\times10^4$cells/mL), and collagen content ($0.59\pm0.02\%$) were decreased in I-E group compared to E animals ($p<0.05$).
Conclusions: This plant proteinase inhibitor (*CratabL*) reduced elastase-induced pulmonary inflammatory and remodeling alterations which may be considered as a new and potential therapeutic strategy for COPD treatment.
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P818

Intravenous immunoglobulin in community acquired pneumonia

Usman Farkhutdinov, Albert Mirchaidarov, Shamil Farkhutdinov. *Department of Internal Diseases, City Hospital 21, Ufa, Russian Federation Department of Internal Diseases, City Hospital 21, Ufa, Russian Federation Department of Internal Diseases, City Hospital 21, Ufa, Russian Federation*

Immunity disorders play an important role in the inflammatory formation in patients with lung diseases.
The aim was to study the efficacy of i.v. immunoglobulin immunovenin (IMV) in patients with community acquired pneumonia (CAP).
Methods: The study included 35 patients with CAP. The patients blood was tested to estimate the level of CD3+, CD4+, CD8+, CD16+, CD20+ lymphocytes, the content of A,G,M immunoglobulins (Ig), circulating immunocomplexes (CIC), γ-interferon and TNFa. 17 patients with CAP were treated with standard therapy. The other 18 patients received combined treatment with IMV.
Results: Compared to healthy subjects in patients with CAP the level of CD3+,CD4+,CD8+, CD16+ lymphocytes was lower by 1,2-1,4 times, the content of IgA, IgG and γ-interferon was lower by 1,3 times. Conversely, the level of CD20+ lympocytes, IgM, CIC was higher by 1,3-1,8 times, and the level of TNFa was higher by 1,9 times. Symptoms of inflammation and impairment of the immune status have been found in patients who received standard therapy. The use of IMV in patients with CAP eliminated immunity disorders, improved the results of the treatment.
Conclusion: In patients with CAP immunovenin improved the immunity status and increased the treatment efficacy.

P819

PTX3 as a component of innate immunity in the role of captopril in acute lung injury induced by bacterial endotoxin

Xiaolin He, Shi Wang, Shuyue Xia, Xu Liu, Ning Song. *Department of Respiratory Medicine, Shenyang Feng-Tian Hospital Affiliated to Shenyang Medical College, Shenyang, Liaoning, China*

Objective: Innate immunity is an important mechanism for the development of acute lung injury (ALI). Long pentraxin PTX3 is an inflammatory mediator and a component of innate immunity. Recent evidence implies that angiotensin-converting enzyme (ACE) plays an important role in the pathogenesis of ALI. We speculated that inhibition of ACE play the protective effect on ALI through the presence of PTX3, therefore, protect the lung from severe injury.
Methods: Lung injury was induced by intratracheal instillation of lipopolysaccharide (LPS) in rats, followed by i.p. administration of captopril, an ACE inhibitor, or saline control, and the PTX3 expression, fibrin deposition, tissue factor expression and lung injury were determined. Local and systemic inflammatory responses were assessed by measuring cytokines in the lung and plasma.
Results: Treatment with captopril dramatically attenuated LPS-induced lung injury, alveolar fibrin deposition and inflammatory cell infiltration 6 h after LPS challenge compared to that in the saline control rats. Local and systemic PTX3 expression were significantly decreased by the captopril therapy, accompanied by decreased interleukin (IL)-6, IL-10 and monocyte chemoattractant protein-1 levels in the plasma.
Conclusion: These results support that inhibition of ACE with its clinically used inhibitor offers protective effects on ALI; PTX3, acting as both anti-inflammation component and the component of innate immunity, may reflect severity of lung injury and serve as the potential therapeutic "target" during ALI. Captopril treatment through the presence of PTX3 could be a potential mechanism that mediates lung injury.

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