## 469. Lung cell injury and repair

**P4580**



**P4581**

**Intrinsic phenotypic differences of the asthmatic epithelium and its inflammatory responses to RSV and particulate matter**

Tillie-Louise Hackett, Gurpreet Singhera, Furquan Shaheen, Del Dorsheid, Darryl Knight. *James Hogg Research Centre, University of British Columbia, Vancouver, BC, Canada*

Within the airway, the epithelium forms the first structural cell defense against common environmental insults such as respiratory syncytial virus (RSV) and particulate matter. These stimuli have been shown to be associated with exacerbations of the disease, which contribute to the financial burden of asthma.
**Objective:** Characterize the intrinsic phenotype of asthmatic and non-asthmatic airway epithelium and determine their inflammatory responses to RSV and particulate matter (EHC-93) exposure.
**Methods:** Air-liquid interface cultures (ALICs) were generated from asthmatic and non-asthmatic airway epithelial cells. Airway tissue and ALICs were analyzed by immuno for Cytokeratin-5, E-cadherin, Ki67, Muc5AC, and apoptosis. ALI cultures were exposed to RSV ($4\times10^6$ PFU/ml) or EHC-93 (100μg/ml) for 24, 48, and 96 h and supernatants analyzed for pro-inflammatory cytokines using ELISA.
**Results:** The airway epithelium of asthmatics *in vivo* and in ALIC demonstrated a less differentiated epithelium characterized by elevated numbers of cells expressing basal cell markers CK-5 and Ki67 and less adherens junction protein E-cadherin ($p<0.01$), though trans-epithelial resistance was not different compared to non-asthmatic ALICs. In response to RSV infection and PM10 exposure, asthmatic ALICs released greater levels of IL-6, IL-8 and GM-CSF compared to non-asthmatic ALICs ($P<0.05$). This enhanced cytokine expression was not due to enhanced p38 or NF-kB expression.
**Conclusion:** This parallel *ex vivo* and *in vitro* study demonstrates that the asthmatic epithelium displays an intrinsic alteration in phenotype and responds with an enhanced inflammatory profile to RSV and particulate matter.

**P4582**

**Down-regulation of SLPI by cysteinyl leukotrienes in human bronchial epithelial cells can be reverted by glucocorticoids**

Siiri Altraja[1], Kaido Värbu[2], Alan Altraja[2]. [1]*Institute of General and Molecular Pathology, University of Tartu, Tartu, Estonia;* [2]*Department of Pulmonary Medicine, University of Tartu, Tartu, Estonia*

Secretory leukoproteinase inhibitor (SLPI) protects the lung against proteinases implicated in the pathogenesis of diseases like COPD, but has also antimicrobial and anti-inflammatory properties. A fall in SLPI contents in the airways has been detected in COPD and asthma, however, the factors involved in the regulation of SLPI gene expression in the airway epithelial cells have insufficiently studied.
To evaluate whether pro-inflammatory cysteinyl leukotrienes (CysLT) exert regulatory effects on SLPI expression in normal human bronchial epithelial cells, the cultured cells were exposed to 0-50 nM $LTE_4$ for 3-6 h. Expression of SLPI mRNA was analyzed by RT-qPCR. The potential of glucocorticoids (GC) to revert the CysLT-induced effect on SLPI expression was addressed by incubating the cells for additional 3 h with 10 nM fluticasone propionate (FP) and 20 nM budesonide (BUD). Montelukast at $10^{-6}$ mol/L was used to block the possible $CysLT_1$-mediated action of $LTE_4$.
Exposure of the cells to 50 nM $LTE_4$ resulted in a significant drop in SLPI expression after 6 h ($p<0.001$). Both GC alone tended to increase the expression of SLPI. More importantly, in the cells exposed to $LTE_4$, both FP and BUD fully reverted the CysLT-induced drop in SLPI ($p=0.001$ and $p=0.003$, respectively). The presence of montelukast did not additionally influence the potency of GC.
In conclusion, by down-regulation of SLPI in bronchial epithelial cells, CysLT may augment the pathogenetic events leading to COPD providing a pathway for more rapid decline in lung function in COPD patients with a concomitant CysCT-mediated disorder like asthma. Fortunately, this can be overpowered by treatment with GC.

**P4583**

**Identification of mesenchymal stromal cells in lung tissue from lung-transplanted patients**

Sara Rolandsson[1], Annika Andersson-Sjöland[1], Jan Claas Brune[2], Leif Eriksson[3], Stefan Scheding[2,4], Leif Bjermer[3], Gunilla Westergren-Thorsson[1]. [1]*Experimental Medical Science, Lung Biology, Lund, Sweden;* [2]*Lund Stem Cell Center, Laboratory Medicine, Lund, Sweden;* [3]*Clinical Medical Sciences, Lung Medicine and Allergology, Lund, Sweden;* [4]*Hematology, Laboratory Medicine, Lund, Sweden*

**Background:** Multipotent mesenchymal stromal cells (MSC) have been isolated from a variety of human tissues. Previous studies have shown that MSC are present in the bronchoalveolar fluid from lung-transplanted patients where they were suggested to be an indication of a future bronchiolitis obliterans syndrome (BOS) onset. We hypothesize that MSC are lung-resident cells that are present in lung tissue from lung-transplanted patients.
**Objective:** Our aims were to examine whether MSC are present in lung tissue from lung-transplanted patients and to evaluate whether these cells showed typical MSC characteristics such as adherent clonal growth and multi-lineage differentiation capacity.
**Methods:** MSC were isolated from central- and distal biopsies obtained from lung-transplanted patients (3 mo. – 13 yrs. post transplantation). After dissociation, the resulting single cell suspension was subjected to colony-forming unit-fibroblast (CFU-F) assays to determine the frequency of mesenchymal progenitor cells. Further, the cells were assayed for their differentiation capacity towards adipocytes, chondrocytes and osteoblasts. The surface marker profile of lung-derived MSC was examined by flow cytometry.
**Results:** MSC isolated from lung tissue adhered to tissue culture treated plastic, formed colonies when cultured in CFU-f assays and possessed multi-lineage potential. Immunophenotyping showed that lung-derived MSC were positive for the surface markers such as CD73, CD90 and CD105 and negative for CD45 and CD34.
**Conclusion:** Our study suggests that there are MSC present in the lung tissue of lung-transplanted patients that possess multi-lineage potential and give rise to typical colonies *in vitro*.

**P4584**

**Lung on Chip: In vitro HGF effects on injured alveolar A549 epithelial cells in microfluidic system**

Pauline Sallin[1,3], Laurent Barbe[1,3], Amiq Gazdhar[2], Thomas Geiser[2], Oliver Guenat[1,3]. *[1]CSEM, CSEM Centre Suisse d'Electronique et de Microtechnique, Landquart, Switzerland; [2]Division of Pulmonary Medicine, University Hospital Bern, Bern, Switzerland; [3]ARTORG, University of Bern, Bern, Switzerland*

Microfluidic systems have become competitive tools in the invitro modelling of diseases and promising alternatives to animal studies. They allow obtaining more invivo like conditions for cellular assays. Research in idiopathic pulmonary fibrosis could benefit from this novel methodological approach to understand the pathophysiology of the disease & develop efficient therapies. The use of hepatocyte growth factor (HGF) for alveolar reepithelisation is a promising approach. In this study, we show a new microfluidic system to analyse the effects of HGF on injured alveolar epithelial cells. Microfluidic systems in polydimethylsiloxane were fabricated by soft lithography. The alveolar A549 epithelial cells (10,000 cells) were seeded and studied in these microfluidic systems with media perfusion (1µl/30min). Injury tests were made on the cells by the perfusion with media containing H2O2 or bleomycin. The degree of injury was then assessed by a metabolic and an apoptotic assays. Wound assays were also performed with a central laminar flow of trypsin. Monitoring of wound closure with HGF vs control media was assessed. The alveolar A549 epithelial cells grew and proliferated in the microfluidic system. In the wound closure assay, the degree of wound closure after 5 hours was (53.3±1.3%) with HGF compared to (9.8±2.4%) without HGF ($P < 0.001$). We present a novel microfluidic model that allows culture, injury and wounding of A549 epithelial cells and represents the first step towards the development of an invitro reconstitution of the alveolar-capillary interface. We were also able to confirm that HGF increased alveolar epithelial repair in this system.

**P4585**

**Lung on Chip: Co-culture of alveolar epithelial cells & bone marrow derived stromal cells in microfluidic system**

Anna Schoeni[1,3], Laurent Barbe[1], Amiq Gazdhar[2], Thomas Geiser[2], Olivier Guenat[1,3]. *[1]CSEM, Centre Suisse d'Electronique et de Microtechnique, Landquart, Switzerland; [2]Division of Pulmonary Medicine, University Hospital Bern, Bern, Switzerland; [3]ARTORG, University of Bern, Bern, Switzerland*

**Background:** Microfluidics system are novel tools to study cell-cell interactions in vitro. This project focuses on the development of a new microfluidic device to co-culture alveolar epithelial cells and mesenchymal stem cells to study cellular interactions involved in healing the injured alveolar epithelium.
**Methods:** Microfluidic systems in polydimethylsiloxane were fabricated by soft lithography. The alveolar A549 epithelial cells were seeded and injury tests were made on the cells by perfusion with media containing H2O2 or bleomycin during 6 or 18hrs. Rat Bone marrow derived stromal cells (BMSC) were then introduced into the system and cell-cell interaction was studied over 24 hrs.
**Results:** A successful co-culture of A549 alveolar epithelial cells and BMS was achieved in the microfluidic system. The seeded alveolar epithelial cells and BMSC adhered to the bottom surface of the microfluidic device and proliferated under constant perfusion. Epithelial injury to mimic mechanisms seen in idiopathic pulmonary fibrosis was induced in the microchannels by perfusing with H2O2 or bleomycin. Migration of BMSC towards the injured epithelium was observed as well as cell-cell interaction between the two cell types was also seen.
**Conclusion:** We demonstrate a novel microfluidic device aimed at showing interactions between different cell types on the basis of a changing microenvironment. Also we were able to confirm interaction between injured alvolar epithelium and BMSC, and showed that BMSC try to heal the injured epitelium.

**P4586**

**TGF-β 1 suppresses IRF-1 expression in pulmonary fibroblasts and modulates its induction by IFN α and IFN γ**

Patricia Leoni[1], Xu Shi-Wen[2], Carmel Stock[1], David Abraham[2], Andrew Nicholson[3], Toby Maher[1], Athol Wells[1], Elizabeth Renzoni[1], Gisela Lindahl[1]. *[1]Interstitial Lung Disease Unit, Royal Brompton Hospital, London, United Kingdom; [2]Department of Rheumatology, Royal Free Hospital, London, United Kingdom; [3]Department of Histopathology, Royal Bropmton Hospital, London, United Kingdom*

**Background:** In our microarray study of lung fibroblasts from control subjects (n=10) and patients with scleroderma-associated interstitial lung disease (SSc-ILD) (n=8), the Interferon Stimulated Genes (ISGs) are an overrepresented group of genes with reduced expression in SSc-ILD fibroblasts. Since interferon gamma is known to interfere with TGF-β signalling and reduce the expression of individual pro-fibrotic genes, we asked whether TGF-β signalling could in turn downregulate ISGs in lung fibroblasts, and whether TGF-β could affect the induction of ISGs by interferons. In this study, we have focused on the expression of IRF-1, a key transcription factor regulating ISGs.
**Methods:** Human primary pulmonary fibroblasts were grown to near confluence, serum starved in 0.1% BSA for 24 hrs, and treated with or without TGF-β (2ng/ml), IFNα (2500u/ml), and IFNγ (10ng/ml), for a further 24 hrs. Specific antibodies were used to assess protein expression in cell layers by Western blot analysis.
**Results:** IRF-1 mRNA expression was significantly reduced in SSc-ILD fibroblasts (-3.6-fold, p=0.0001). TGF-β treatment suppressed baseline IRF-1 protein expression to undetectable levels in fibroblast preparations from control and SSc-ILD lungs. In both SSc-ILD and control fibroblasts, TGF-β had no effect on IFNγ-induced IRF-1 protein expression, but completely inhibited the induction of IRF-1 by IFNα. By contrast, IFNγ reduced TGF-β induced CTGF expression, while IFNα had no effect.
**Conclusion:** TGF-β signalling suppresses baseline and IFNα-induced IRF-1 expression in lung fibroblasts, suggesting a possible mechanism for the reduced expression of ISGs expression observed in SSc-ILD fibroblasts.

**P4587**

**Alveolar lymphocyte phenotype and respiratory distress syndrome**

Karine Risso[1], Céline Sanfiorenzo[2,3], Gaurav Kumar[4], Francine de Salvador-Guillouet[1], Alain Bernard[4], Gilles Bernardin[5], Pierre-Marie Roger[1]. *[1]Infectiology, Hospital Archet, Nice, France; [2]Pneumology, Hospital Pasteur, Nice, France; [3]EA4319, University of Nice Sophia, Nice, France; [4]Inserm Unit 576, Hospital Archet, Nice, France; [5]Intensive Unit Care, Hospital Archet, Nice, France*

**Introduction:** The role played by adaptive immunity in the physiopathology of acute lung injury (ALI) and acute respiratory distress syndromes (ARDS) is largely unknown. We characterized lymphocytes phenotype in broncho-alveolar lavage (BAL) during respiratory distress syndromes.
**Patients and method:** Analyses were carried out on BAL samples of three patients groups: ALI/ARDS patients (ARDS group), healthy patients (control group) and inflammatory pulmonary diseases patients (ID group). Lymphocyte and monocyte/macrophage phenotype determination used cytofluorometric technics. CD45RA+, CD45RA- and CD25FoxP3+ cells were respectively identified as naïve, memory and regulatory T cells. HLADR, KI67 and annexin V were used as markers for cell activation, proliferation and apoptosis respectively. CD4, CD8, CD80, CD86, CD28, CD40/CD40L and CTLA-4 were used as costimulatory cell markers.
**Results:** 9 BAL were obtained from 8 ARDS patients, 8 BAL from 8 controls and 8 from ID patients. A significant increase of HLADR, KI67 and CTLA-4 on T CD4+ lymphocytes was observed in ARDS group compared to control. Concerning CTLA-4, the difference still persisted when comparing ID to ARDS group. KI67 expression was significantly increased on T CD8 T. HLA-DR and CD86 were significantly decreased on alveolar macrophages (AM) compared to others groups.
**Discussion:** During ALI/ARDS activation and proliferation of T lymphocyte are noticeable, associated with alteration of the CD28/CTLA-4 costimulatory pathway and its ligand on AM.
**Conclusion:** This is the first human study on adaptive immunity in ARDS/ALI identifying relevant modifications on T cell phenotype which may account for the dramatic alveolar inflammation observed in these syndromes.

**P4588**

**IL-18 and IL-12 induce inflammatory cytokines, chemokine ligand 9 (CXCL9) and T cell infiltration in pulmonary tissue**

Fadila Telarevic Cero[1,2], Karl Otto Larsen[1], Vigdis Hillestad[2], Geir Christensen[2], Ole Henning Skjønsberg[1]. *[1]Department of Pulmonary Medicine, Oslo University Hospital Ullevål, University of Oslo, Oslo, Norway; [2]Institute for Experimental Medical Research and Center for Heart Failure Research, Oslo University Hospital Ullevål, University of Oslo, Oslo, Norway*

**Background:** Increased levels of interleukin (IL)-18 and IL-12 have been found during experimental alveolar hypoxia and in patients with COPD.
**Aims:** To study the presence of IL-18 and IL-12 receptors (IL-18R/IL-12R) in the lungs and the effect of IL-18 and IL-12, alone or in combination, on the lungs with regard to inflammatory response and induction of emphysema related metalloproteinases (MMP2, MMP9, MMP12).
**Methods:** Expression of IL-18R and IL-12R was studied in lungs, heart, liver and spleen in C57Bl/6 mice. 24 hours after a single i.p. dose of recombinant murine IL-18 alone, recombinant IL-12, IL-18+IL-12 or PBS (controls), lungs were harvested for immunohistochemical (IHC) and RT-PCR analyses.
**Results:** The expression of IL-18R mRNA was 16-630-fold higher in lungs than in other organs. The expression of IL-12R was 4-fold higher in the spleen than in lungs. Mice treated with IL-18 or IL-12 alone showed significantly higher mRNA levels of interferon-γ, tumor necrosis factor-α and MMP12, than in controls ($p<0,05$). Mice treated with IL-18+IL-12 showed an even more pronounced induction of these mediators, as well as a significant increase in IL-6 and IL-1β($p<0,05$). A marked increase in CXCL9 mRNA was induced by IL-18 (12-fold), IL-12 (19-fold), IL-18+IL-12 (153-fold). IHC showed perivascular T-cell infiltration following co-stimulation.
**Conclusions:** High levels of IL-18 receptor suggest that the lungs might be a target organ for IL-18. IL-18+IL-12 exert a synergistic effect on the lungs by inducing inflammatory cytokines and MMP12 which may promote inflammation and emphysema. Induction of CXCL9 may be of importance for the observed T cell infiltration in lung tissue.

**P4589**

**Characterization of human mesenchymal stem cells phenotype and secretome in a in vitro model of acute lung injury inflammation**

Arnaud Goolaerts[1], Valérie Vanneaux[2,3], Jérôme Larghero[2,3], Michael Matthay[1,2], Christine Clérici[1,2,4]. *[1]Unité 773, INSERM, Paris, France; [2]Physiologie, Université Paris Diderot, Paris, France; [3]Assistance Publique des Hôpitaux de Paris, APHP F-75010, Paris, France; [4]Assistance Publique des Hôpitaux de Paris, APHP F-75018, Paris, France*

Intra-tracheal instillation of MSCs have therapeutic efficacy in models of acute lung injury (ALI). Protective effects of MSC are reported despite low engraftment rates supporting that paracrine mediators may be involved. In ALI, MSCs delivered by intra-tracheal route are exposed to alveolar hypoxia and cytokines, both known to independently modify MSC phenotype, which may affect their survival or their reparative properties. We therefore investigated the effects of a typical alveolar ALI microenvironment consisting in hypoxia (HYP) and cytomix (CYT; IL-1β, TNFα and INFγ) on MSC phenotype, apoptosis and secretion profile. Testing the effects of such conditions is important to better understand the fate and behavior of MSC instilled into injured lungs and to detect potential modification of their secretion profile. In order to decide whether MSC preconditioning might be an interesting strategy, MSC were exposed 24 hours to CYT 50ng/ml and/or HYP (0% O2). CYT and/or HYP neither modify the expression of the typical MSCs markers (CD90, CD105, CD45) nor the degree of apoptosis/necrosis when compared to control MSCs. We measured KGF, PGE2 and IL-1 receptor antagonist (IL-1rA) in the supernatant because they are known to be important for resolution of alveolar edema. Compared to control conditions, CYT plus HYP increased the release of PGE2 (190±160 vs 2999±261 ng/ml), IL1-rA (0 vs 134±26 ng/ml) but decreased by two fold the release of KGF (345±8 vs 115±12,8 ng/ml). In conclusion: Hypoxic and inflammatory environment mimicking ALI does not affect the survival and phenotype of MSC but differentially modulates KGF, PGE-2 and IL-1rA secretion.

**P4590**

WITHDRAWN

**P4591**

**The nitration mediated inhibition of Rac1 is involved in the endothelial barrier dysfunction induced by lipopolysaccharide**

Christine Gross, Ruslan Rafikov, Saurabh Aggarwal, Yali Hou, Stephen Black. *Vascular Biology Center, Georgia Health Sciences University, Augusta, GA, United States*

Lipopolysaccaharide (LPS) induced acute lung injury (ALI) is a respiratory condition characterized by alveolar epithelial and endothelial cell damage resulting in increased lung leak. Recent studies have shown that protein nitration plays a key role in the endothelial barrier dysfunction in LPS exposed mice. The molecular mechanisms involved in this process are not well understood. Immunoprecipitation analyses, using human lung microvascular endothelial cells, demonstrated that LPS mediates an increase in Rac1 nitration and a decrease in activity which were attenuated by the cell-permeant peroxynitrite scavenger, manganese(III) tetrakis(1-methyl-4-pyridyl)porphyrin (MnTMPyP). Utilizing mass spectroscopy, we found that LPS induces a single tyrosine nitration in human Rac1 located at amino acid 32 (Y32). The crystal structure analysis of Rac1 indicated that Y32 lies adjacent to the nucleotide-binding site within Switch I, a region known to modulate GTP/GDP binding. Molecular dynamic simulations predicted the nitration of Y32 of Rac1 would preferentially stabilize the closed conformation of Switch I. To test this prediction, we utilized stopped-flow analysis to measure changes in GDP and GTP binding and found that the nitration of Y32 did not alter GTP binding, but enhanced GDP binding to Rac1. LPS also induced Rac1 nitration and decreased Rac1 activity in the mouse lung. These changes were attenuated in mice pretreated with MnTMPyP. Lung permeability studies using Evans blue dye showed that peroxynitrite scavenging decreased LPS induced lung leak. We conclude that agents that prevent Rac1 nitration may have clinical importance in the management of ALI.

**P4592**

**The role of leptin in pulmonary neutrophilia during murine acute lung injury**

N.D. Ubags[1,2], J.H. Vernooy[2], E. Burg[1], C. Lenox[1], L.L. Kordonowy[1], J. Petty[1], K. DiPalma[1], E.F. Wouters[2], B.T. Suratt[1]. *[1]Department of Medicine, University of Vermont College of Medicine, Burlington, VT, United States; [2]Department of Respiratory Medicine, Maastricht University, Maastricht, Netherlands*

**Rationale:** One of the hallmarks of Acute Lung Injury (ALI) is the extensive recruitment of neutrophils to the lung. Recent evidence shows that leptin expression is increased in the injured human lung, and is known to have diverse effects on leucocytes. We hypothesized that leptin contributes directly to neutrophil recruitment in the lung during ALI.
**Methods:** C57/Bl6 wild type (WT) mice were examined in a lipopolysaccharide (LPS) model of lung injury with or without oropharyngeal aspiration of recombinant leptin. We measured bronchoalveolar lavage (BAL) neutrophilia, leptin concentrations and inflammatory cytokine levels at 24 hours after injury. In addition, the effects of LPS induced lung injury were examined in leptin resistant (*db/db*) obese and Diet Induced Obese (DIO) mice and their lean controls. Lastly, we examined chemotaxis to leptin in neutrophils isolated from lean and obese uninjured mice.
**Results:** BAL leptin levels were significantly increased in injured WT mice (1324 pg/ml ± 320) relative to their saline-treated controls (96 pg/ml ± 74) 24 hours after LPS exposure. Recombinant leptin augmented airspace neutrophilia after LPS exposure in WT mice, yet inflammatory cytokine levels in BAL remained unchanged. In addition, we observed attenuation in BAL neutrophilia in both *db/db* and DIO obese mice compared to their lean controls. Lastly, neutrophils were found to migrate to leptin *in vitro*, and obesity blunted this response.
**Conclusion:** Our findings indicate that leptin plays a role in neutrophil recruitment to the lung during ALI. This appears to occur in part via leptin-induced neutrophil chemotaxis, which is diminished in obesity.

**P4593**

**Preferential activation of alveolar macrophages versus epithelial cells during initiation of ventilator-induced lung injury in mice**

Alicia Waite, Kieran O'Dea, Michael Wilson, Masao Takata. *Section of Anaesthetics, Pain Medicine and Intensive Care, Imperial College, London, London, United Kingdom*

**Background:** It has been suggested that stretch-induced deformation of alveolar epithelial cells (AEC) plays a role in the initiation of ventilator-induced lung injury (VILI). *In vitro* stretch of AEC upregulates intracellular mitogen-activated protein kinases (MAPK) leading to mediator release, but this may not reflect the situation *in vivo*. We have developed a novel method to measure MAPK intermediates within discrete lung cell populations following *in vivo* VILI.
**Methods:** Anaesthetised C57BL6 mice were ventilated with high (Vt=40ml/kg) or low stretch (Vt=10ml/kg). At various time points lungs were removed and single cell suspensions were produced by mechanical disruption, with cells immediately fixed and permeabilised. Levels of intracellular phosphorylated (p-) p38 and MK2 in alveolar macrophages (AM, CD11c+ cells), type I (T1α+) and II (Pro-SPC+) AEC were determined by flow cytometry.
**Results:** p-p38 and p-MK2 expression (mean fluorescence intensity, MFI) increased in AM within 5 minutes of high stretch. In contrast, both type I and II AEC showed little p38 and MK2 phosphorylation, even up to 1 hour.

MAPK phosphorylation

| | AM | Type I AEC | Type II AEC |
|---|---|---|---|
| p-p38 (MFI) | | | |
| 5 mins low | 4.1±1.9 | 0.7±0.4 | 0.4±0.2 |
| 5 mins high | 12.8±1.0† | 1.4±0.4 | 0.6±0.2 |
| p-MK2 (MFI) | | | |
| 5 mins low | 23.1±4.9 | 15.6±3.9 | 18.6±5.1 |
| 5 mins high | 35.4±5.3* | 20.8±5.7 | 21.4±5.5 |

Mean ± s.d. *p<0.05, †p<0.001 by t-test. N=3-4.

**Conclusion:** Contrary to previous suggestions that stretch-induced activation of AEC is the key initiator of VILI, our results indicate an immediate, direct activation of AM with high stretch, placing AM as potentially more important than AEC in triggering the early inflammatory response during VILI.

**P4594**

**Immunomodulatory function of chemerin and its receptor ChemR23 in the physiopathology of viral pneumonia and acute lung injury**

Benjamin Bondue[1], Olivier Vosters[2], Patricia De Nadai[2], Stephanie Glineur[4], Olivier De Henau[2], Souphalone Luangsay[2], Frederic Van Gool[3], Daniel Desmecht[4], Paul De Vuyst[1], Marc Parmentier[2]. *[1]Department of Pneumology, Erasme Hospital, Université Libre de Bruxelles, Brussels, Belgium; [2]Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire (IRIBHM), Université Libre de Bruxelles, Brussels, Belgium; [3]Institut de Biologie et de Médecine Moléculaires (IBMM), Université Libre de Bruxelles, Gosselies, Belgium; [4]Department of Pathology, Université de Liège, Liège, Belgium*

Chemerin, the natural ligand of the ChemR23 receptor, acts as a chemoattractant agent for macrophages, immature dendritic cells, and NK cells. In this study, we investigated the role of the chemerin/ChemR23 system in the physiopathology of inflammatory lung diseases using wild type (WT) and knock-out mice for the receptor (ChemR23$^{-/-}$). Whereas no differences are observed in models of lung fibrosis and asthma, ChemR23$^{-/-}$ mice present higher mortality and morbidity in a model of viral pneumonia induced by the pneumonia virus of mice (PVM). ChemR23$^{-/-}$ mice display delayed viral clearance, and impaired acquired immunity contrasting with an excessive innate response and recruitment of neutrophils. Lower recruitment of type I interferon-producing plasmacytoid dendritic cells (pDCs) may explain the delayed acquired response and viral clearance in ChemR23$^{-/-}$ mice. However, the stronger inflammation observed in these mice is not due to defective pDC recruitment but rather to the loss of an anti-inflammatory role of chemerin on non-leukocytic cells. Indeed, experiments involving adoptive transfer of pDCs and bone marrow transplantation exclude the role of ChemR23-expressing pDCs and more generally leukocytes in the protection against excessive inflammation. Moreover, a strong anti-inflammatory role of chemerin is observed in a model of acute lung injury induced by instillation of lipopolyssacharide (LPS), which is strictly dependent of ChemR23. Altogether, our data suggest a role of the chemerin/ChemR23 system in the modulation of innate immunity and this is likely mediated by non-leukocytic cells, such as lung endothelial or epithelial cells.

**P4595**

**Temporal behavior of lung injury induced by instillation of microcystin-LR in mice**

Viviane Cagido[1], Natália Casquilho[1], Douglas Barbosa[1], Raquel Soares[1], Sandra Azevedo[1], Samuel Valença[2], Walter Zin[1]. *[1]Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; [2]Institute of Biomedical Sciences, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil*

Cyanotoxins present in the water for human use may yield serious health problems. We aimed to evaluate the effects of microcystin-LR (MC-LR) on lung mechanics, lung and liver histology and inflammation, and oxidative stress. Male Swiss mice (n=149) were randomly divided in 2 groups: control (CT) received sterile saline intratraqueally (*i.t.*, 50 μL), and MC a sublethal dose of MC-LR (40 μg/kg, *i.t.*). CT (n=13), and MC mice were evaluated at 2 (n=16), 8 (n=13), 24 (n=24), 48 (n=15) e 96 h (n=17) after receiving MC-LR. In the remaining animals (CT=10 and MC=41) oxidative stress and damage were analyzed. Alveolar collapse increased in MC groups at 2, 8, 24 and 48 h compared to CT, accompanied by deterioration of lung mechanics on the same experimental time points, except for 24 h, when mechanics returned to baseline. The amount of polymorphonuclear cells and mieloperoxidase activity in lung augmented in all MC groups until 96 h, indicating a neutrophilic inflammatory cell infiltrate. Hepatic histology showed necrosis and hepatocyte disarrangement beginning at 8 h. Free MC-LR was detected in lung and liver homogenates, with a time-dependent toxin accumulation in liver. Antioxidative enzymes activities and thiobarbituric acid reactive substances were altered in MC-LR exposed animals. *i.t.* administration of MC-LR led to a biphasic compromise of pulmonary mechanics: an early deterioration (2 h and 8 h), normalization at 24 h, and a later worsening (48 h). Also, alveolar collapse, lung and liver inflammation, imbalance of antioxidants enzymes, and oxidative damage were identified. This work reinforces the airways as an important route of intoxication by MC-LR.

Supported by: PRONEX/FAPERJ, FAPERJ, CNPq, MCT

**P4596**

**Toxicological assessment of indoor airborne fine particulate matter induced oxidative stress in alveolar epithelial cells**

Yasuo Takano[1], Tomomi Komura[2], Kennichi Yagyu[3], Takeshi Minami[4]. *[1]Health and Nutrition, Kio University, Kitakatsuragi-gun, Nara, Japan; [2]Interdisciplinary Studies for Advanced Aged Society, Osaka City University Graduate School of Human Life Science, Osaka, Japan; [3]The Science Research Center, Kochi University, Nankoku, Kochi, Japan; [4]Life Science, Kinki University, Higashi-Osaka, Osaka, Japan*

**Background:** Exposure to airborne particulate matter (PM) has long been associated with adverse health problems. These fine PM particles can penetrate the distal regions of the lung causing severe respiratory problems.
**Objectives:** As relatively little information of indoor fine PM is available to evaluate the actual risks, we will focus our examination on lung injuries caused by fine indoor PM, by investigating the mechanism of the cell injury using primary culture of alveolar type II cells.
**Method:** Indoor PM was collected in Nara prefecture, Japan using an Andersen sampler by the impact cascade method. Particle samples of PM were collected on five Teflon filters in accordance with the diameter of particles (diameter: >11μm, 2.1-11 μm, 1.1-2.1 μm, 0.4-1.1 μm, <0.4 μm). Alveolar type II cells from rats were exposed to PM at the concentration of 500 μg/ml for 24 hrs respectively. At the end of the experiment, lactate dehydrogenase (LDH) activity released into the culture medium was determined and the percentage of LDH leakage was then calculated to reflect the cytotoxicity of PM.
**Results:** In relation to the control cells, the PM-induced LDH leakage was more significant among the smaller fine particles of indoor PM than the larger ones. However this did not occur with the above mentioned 1.1 μm diameter size of PM. Superoxide dismutase (SOD) significantly reduced LDH leakage-induced indoor PM. In the analysis of the elements in indoor PM, the most abundant element was iron (Fe).
**Conclusions:** Indoor PM causes oxidative cellular damage, which may be closely associated with a transition metal, Fe in rat alveolar epithelial cells.

**P4597**

**Effect of nanoparticle and $NO_2$ exposure on thoracic gas volume in Brown Norway rat**

Skander Layachi[1], Françoise Rogerieux[2], Christelle Gamez[2], Kelly Blazy[2], Franck Robidel[2], Anthony Lecomte[2], Ghislaine Lacroix[2], Sam Bayat[1,3]. *[1]EA4285 - INERIS UMI01, Université de Picardie - UFR de Médecine, Amiens, France; [2]Direction des Risques Chroniques, INERIS, Verneuil-en-Halatte, France; [3]Paediatric Lung Function Laboroatory, CHU Amiens, Amiens, France*

**Rationale:** Air pollutant exposure, has been associated with increased asthma morbidity in children. The aim of the present study was to determine whether exposure to $NO_2$ and carbon nanoparticles (CNP) affect the rate of Thoracic gas volume (TGV) change with growth in 7-week old Brown-Norway (BN) rats.
**Methods:** Animals were anesthetized and tracheally intubated. TGV was measured using whole-body plethysmography at age 49 days, and following a 4-wk exposure to air, $NO_2$ (10 ppm) 5h/d, 5d/wk, CNP (FW2, Ø13 nm; 3 instillations at 7-day intervals), or CNP+$NO_2$. Serum cytokines were quantified using multi-array immunoassay.
**Results:** TGV significantly increased after 4 weeks in all groups. When normalized to body weight, TGV increased significantly only in the air-exposed group.

| | Control | Air | NO2 | Air-CNP | NO2-CNP |
|---|---|---|---|---|---|
| n | 32 | 6 | 6 | 6 | 6 |
| Age (days) | 49 | 77 | 77 | 77 | 77 |
| Wt (g) | 158.7±12.4 | 239.7±13.9* | 226.3±21.8* | 237.3±8.5* | 229.1±14.3* |
| TGV (ml) | 2.6±0.4 | 4.9±0.6* | 4.1±0.8*# | 3.6±0.5*# | 3.8±0.7*# |
| ΔTGV (%) | 0 | 94.5±26.3* | 56.8±31.5*# | 41.2±21.8*# | 50.5±29.4*# |
| TGV/Wt (ml/kg) | 16.2±2.8 | 20.7±3.4* | 18.1±3.5 | 15.2±2.2# | 16.8±3.1 |
| TNF-α in serum(pg/ml) | NA | 74.6±88.1 | 525±374.4# | 27.1±13.9§ | 157.1±207.1§ |
| IFN-γ in serum(pg/ml) | NA | 17,3±10,1 | 20,5±14,9 | 21,1±12.0 | 125,6±145,9#§ |

Data are mean±SD; *p<0.05 vs. control; #p<0.05 vs. Air; §p<0.05 vs. NO2, by one-way ANOVA. NA: not available.

**Conclusions:** These data show that a 4-wk exposure to either $NO_2$ or CNP significantly decreases TGV and the rate of TGV increase with age in BN rat, suggesting a reduced lung growth rate. CNP, $NO_2$ and combined exposure produced distinct lung inflammatory cytokine profiles.