369. Experimental pulmonary hypertension

P3330

Effect of interferon α preparations on IP10 and ET-1 release from human pulmonary artery smooth muscle cells

Rekha Badiger[1], Tobias Manigold[2], Hime Gashaw[1], Heather J. Hinton[2], Thomas Singer[2], Peter M. George[1], Trevor T. Hansel[3], Jane A. Mitchell[1].
[1]Cardiothoracic Pharmacology, National Heart and Lung Institute, Imperial College London, London, United Kingdom; [2]Non Clinical Safety, Roche, Basel, Switzerland; [3]Imperial Clinical Respiratory Research Unit, St. Mary's Hospital, Paddington, London, United Kingdom

Pegylated (PEG) interferons (IFN), used to treat hepatitis C, are associated with lung toxicity and pulmonary hypertension. Pegylation increases stability of the IFN moiety and *in vivo* half-life, but reduces *in vitro* anti-viral activity. These effects are related to the size/shape/position of the PEG attachment. There are two marketed PEGIFNα preparations for hepatitis C; PEGIFNα2a and PEGIFNα2b, which are conjugated to 40 KDa and 12 KDa moieties respectively.
Endothelin-1 (ET-1) and IP10 are associated with lung inflammation and are induced by IFNs. We investigated the effect of IFNα preparations on ET-1 and IP10 release from human pulmonary artery smooth muscle (HPASM) cells.
HPASM cells were treated with IFNs (0.33ng/mL to 30ng/mL). For ET-1, TNFα (10ng/mL) was added. IP10 and ET-1 immunoreactivity was measured by ELISA at 24h.
IFNα preparations induced IP10 with PEGIFNα2a being the weakest inducer (Fig. 1A; n=6; *p<0.05 by two way ANOVA). In TNFα treated cells, IFNα2a, IFNα2b and PEGIFNα2b induced ET-1 above baseline release (Fig. 1B; n=8; *p<0.05 by one way ANOVA vs control; #p<0.05 by two way ANOVA).

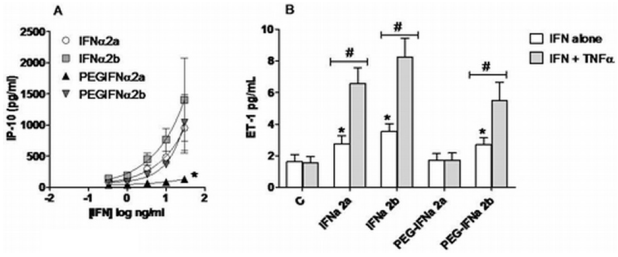

Figure 1

We conclude that IFNα preparations activate HPASM cells and this may contribute to the lung inflammation seen in some patients. PEGIFNα2a has the larger PEG moiety and induced least ET-1/IP10. Our results suggest ET-1/IP10 are important when considering mechanisms of pulmonary toxicity of IFNs.

P3331

Could platelet-activating factor acetylhydrolase (PAF-AH) predict adverse event in pulmonary hypertension?

Rozenn Quarck[1], Hervé Durand[2], Ewa Ninio[2], Marion Delcroix[1]. *[1]Respiratory Diseases, Katholieke Universiteit Leuven, Leuven, Belgium; [2]UMRS937, INSERM, Paris, France*

Chronic thromboembolic pulmonary hypertension (CTEPH) and pulmonary arterial hypertension (PAH) are threatened conditions mostly diagnosed at late stages. Inflammation could play a role in the pathogenesis. The need of new biomarkers, non-invasively measurable may help to improve the diagnosis and follow up. PAF-AH, a plasmatic enzyme, is a predictive risk factor for cardiovascular events. In a prospective study, we have investigated a potential role of PAF-AH in

predicting the outcome in PAH and CTEPH. Circulating PAF-AH activity has been measured in consecutive patients diagnosed with PAH (n=152) and CTEPH (n=115), at the time of right heart catheterization and compared to a control group of healthy subjects (n=115).
Circulating PAF-AH activity was lower in CTEPH and PAH patients compared to controls (37, 95% CI: 33-41; 41, 95% CI: 37-45; 54, 95% CI: 50-60 nmol.mL-1.min; p<0.0001). In PAH, PAF-AH activity is correlated to total cholesterol (r=0.29, p=0.0002) and to LDL-cholesterol (r=0.26, p=0.001). In CTEPH, PAF-AH activity is correlated to pulmonary vascular resistance (PVR; r=0.21, p=0.02) and to LDL-cholesterol (r=0.22, p=0.01). In PAH, clinical worsening is associated with an elevated PAF-AH activity (36, 95% CI: 32-41 vs. 45, 95% CI: 40-51; p=0.04). PAH patients with a mean pulmonary arterial pressure>50 mmHg, and with CRP>4mg.L-1 have increased PAF-AH activity (35, 95% CI: 30-41 vs. 47, 95% CI: 43-52; p=0.02 - 37, 95% CI: 32-42 vs. 45, 95% CI: 40-51; p=0.02, respectively). Non-operable CTEPH patients with PAF-AH activity>50 nmol.mL-1.min have a better survival (p=0.02).
Our results suggest that PAF-AH could be a prognostic factor in pulmonary hypertension.

P3332

Hypoxia-mediated alterations in adenosine receptor expression in rat lung

Jonas Salys, Adelheid Kratzer, Martin Zamora, Laima Taraseviciene-Stewart.
Medicine, University of Colorado Denver, Aurora, United States

Background: Chronic hypoxic exposure induces pulmonary arterial remodeling, resulting in pulmonary hypertension (PAH) and right ventricular hypertrophy. The role of adenosine (Ado) receptors in the pathogenesis of PAH has not been addressed.
Aims and objectives: To investigate the role of Ado and Ado receptor signaling in hypoxia-induced pulmonary vascular remodeling.
Methods: Sprague Dawley rats were exposed to hypobaric hypoxia (5,000 m altitude) for 1 and 3 weeks. The adenosine receptor expression profile in the normoxic and hypoxic rat lungs as well as in rat pulmonary artery and microvascular endothelial cells was determined by real time PCR. Ado receptor agonist treated and untreated endothelial cell (EC) proliferation was determined using CyQuant cell proliferation kit.
Results: All four Ado receptors were expressed in the lung tissue. The A2A receptor was the most abundant. The 1 week hypoxic exposure significantly upregulated A1 receptor expression indicating it's role in the adaptive response to hypoxia. The pulmonary arterial pressure (PAP) were significantly elevated after 3 weeks of hypoxia (33±2.3 versus 18±2.3 normoxia). Studies in vitro revealed that Ado receptors are differentially expressed in pulmonary vascular endothelial cells and that treatment with selective adenosine receptor A1 (N6-cyclopentyadenosine) and A3 (HEMADO) agonists affects EC proliferation.
Conclusions: The expression profile of the Ado receptors is regulated by hypoxia and targeting adenosine receptors might be promising approach to treat PAH.
Funded by AHA 0735388N, FAMRI CIA 072053, Emphysema Research Fund and Bixler Family Foundation.

P3333

Potential contribution of precursor cells to vascular remodeling in the AdTGF-β1 model of lung fibrosis and pulmonary hypertension

Laszlo Farkas[1], Daniela Farkas[1], Donatas Kraskauskas[1], Jack Gauldie[2], Martin Kolb[2], Norbert Voelkel[1]. *[1]Department of Internal Medicine, Victoria Johnson Center, Virginia Commonwealth University, Richmond, VA, United States; [2]Departments of Medicine, Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada*

Pulmonary hypertension (PH) is associated with increased mortality in patients with idiopathic pulmonary fibrosis (IPF). The interaction between the fibrotic process and the pulmonary vasculature is incompletely understood. There is evidence in human and experimental pulmonary fibrosis that precursor cells may play a role in fibrogenesis, and that precursor cells may also be important for vascular remodeling in chronic models of pulmonary hypertension (PH). But the contribution of precursor cells to vascular remodeling and PH in pulmonary fibrosis has not been investigated yet.
This study aimed to investigate the potential contribution of precursor cells to vascular remodeling in the AdTGF-β1 model of lung fibrosis and PH.
Female Sprague Dawley rats received AdTGF-β1 or AdDL70 and were sacrificed at different time points (7, 14 and 28 days). Immunofluorescence stainings were performed on 3 μm sections of the left lung after formalin fixation.
We detected cells expressing markers of endothelial progenitor cells (CD133/vWF, c-kit/vWF, CD133/VEGFR-2) in pulmonary arteries of AdTGF-β1 animals, mainly after 14 days. We also found cells expressing markers of fibrocytes (CXCR4/α-SMA, CXCR4/prolyl-4-hydroxylase, S100A4/CD34), around pulmonary arteries of AdTGF-β1 animals. In contrast, we did not find such cells in or around pulmonary arteries in AdDL70 treated animals.
In conclusion, our data support the concept that precursor cells may contribute to postapoptotic vascular repair and pulmonary artery muscularization in experimental lung fibrosis. The detailed mechanisms of precursor cell attraction and activation are currently under investigation.

P3334

Caspase inhibition reduces severe pulmonary hypertension in the AdTGF-1β/SU5416 model of angioproliferative pulmonary hypertension and lung fibrosis

Laszlo Farkas[1], Daniela Farkas[1], Donatas Kraskauskas[1], Jack Gauldie[2], Martin Kolb[2], Norbert Voelkel[1]. *[1]Department of Internal Medicine, Victoria Johnson Center, Virginia Commonwealth University, Richmond, VA, United States; [2]Departments of Medicine, Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada*

Pulmonary hypertension (PH) is associated with increased mortality in patients with idiopathic pulmonary fibrosis (IPF). The interaction between the fibrotic process and the pulmonary vasculature is incompletely understood.
The current study aimed to investigate whether broad spectrum caspase inhibition can reduce severe angioproliferative PH in the combined model of AdTGF-β1 lung fibrosis and the VEGF receptor inhibitor SU5416.
Female Sprague Dawley rats received AdTGF-β1 intratracheally at day 0, as well as one dose of SU5416 s.c. or CMC. Some AdTGF-β1/SU5416 animals received the caspase inhibitor Z-Asp-CH2-DCB or vehicle (DMSO) from day 6-28 At day 28, invasive pulmonary hemodynamics were assessed. The right lung was used for protein and RNA isolation, and the left lung was inflated with formalin and processed for histology.
We detected clusters of VWF+ endothelial cells occluding the lumen of small pulmonary arteries in AdTGF-β1/SU5416, together with severe PH in AdTGF-β1/SU5416 rats vs. AdTGF-β1/CMC. At the same time, lung fibrosis was increased, as indicated by elevated mRNA expression of profibrotic and matrix genes. Western blots showed a significant increase in caspase-3 cleavage in AdTGF-β1/SU5416 rats. Treatment with Z-Asp-CH2-DCB reduced right ventricular systolic pressures by 19.4 mmHg in average in AdTGF-β1/SU5416 animals (P<0.05 vs. DMSO).
In conclusion, our results indicate that angioproliferative pulmonary vasculopathy was induced in this new model, together with severe fibrosis, and that increased apoptosis contributes to both increased fibrosis and vascular pathology.

P3335

Effects of type I, II and III interferons on endothelin-1 release by human pulmonary artery smooth muscle cells

Peter M. George[1], Rekha Badiger[1], Hime Gashaw[1], Neil Galloway-Phillipps[1], Trevor T. Hansel[2], Jane A. Mitchell[1]. *[1]Department of Cardiothoracic Pharmacology, Imperial College, London, United Kingdom; [2]Imperial Clinical Respiratory Research Unit (ICRRU), Imperial College, London, United Kingdom*

The potent vasoconstrictor and mitogen peptide endothelin-1 (ET-1) is a therapeutic target for the treatment of pulmonary hypertension. Work from our group has shown that ET-1 release by human pulmonary artery smooth muscle cells (HPASMCs) is critically regulated by interferons (IFN) and TNF. We have shown that type I IFNα and IFNβ and type II IFNγ, but not type III IFNλ, all released in host responses to viral infection, induce ET-1. As viral infection and IFN therapy are increasingly associated with lung toxicity, including pulmonary hypertension, we have investigated the nature of any interaction between IFNs for ET-1 release by HPASMCs. Cells from 3 separate donors were stimulated in 96-well plates with IFNα, -β, -γ and -λ (all 10ng/ml). Supernatants were collected after 24 hours and ET-1 concentrations measured by sandwich ELISA. In the presence of TNFα (10ng/ml), type I IFNs (-α and -β) or type II IFNγ, but not type III IFNλ, induced ET-1 release. Additive release of ET-1 was seen with IFNα/γ and IFNβ/γ but not IFNα/β. IFNλ did not release ET-1 under any condition studied. Type I and II IFNs act independently to stimulate ET-1 from HPASMCs, which reflects what is known about their separate receptor pathways.

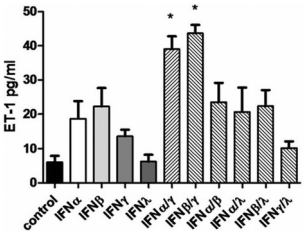

Figure 1. Data is mean ± SEM. *P<0.05 one-way ANOVA for combination of IFNs v IFNs alone.

Our finding that IFNλ is inactive in these cells may suggest that type III IFN spares the lung vasculature.

P3336

Roles of sex hormones on bone morphogenetic protein signaling in pulmonary artery differed between testosterone and estrogen

Hiroaki Ichimori, Shigetoyo Kogaki, Kunihiko Takahashi, Keiichi Ozono.
Pediatrics, Osaka University Graduate School of Medicine, Suita, Japan

Background: Epidemiologic studies have revealed the female predominance in the morbidity of idiopathic PAH (pulmonary arterial hypertension) in the world,

suggesting involvement of sex hormones in the pathogenesis of PAH. Recent studies have identified a role of bone morphogenetic protein (BMP) signaling in the pathogenesis of PAH and we reported that BMP signaling in pulmonary arterial endothelial cells (PAEC) was attenuated under hypoxic condition *in vitro* and *in vivo*.
Purpose: The aim is to investigate effects of estradiol (E) and testosterone (T) on the BMP signaling in PAEC and analyze their mechanisms.
Materials and methods: PAEC were cultured and incubated with β-estradiol (10^{-7}M), testosterone (10^{-8}M), or vehicle under 1%O_2 (hypoxia) and 21%O_2 (normoxia). BMP signaling including Smad1/5/8, phosphorylated (p-) Smad1/5/8, and Id1 was examined by western blotting and quantitative RT-PCR. The effects of HIF (hypoxia-inducible factor) -1α expression on the BMP signaling were also examined.
Results: Under normoxia, p-Smad1/5/8 protein and Id1 mRNA were augmented 1.6 and 1.5-fold by E, but suppressed 0.3 and 0.4 -fold by T. Under hypoxia, conversely, p-Smad1/5/8 protein and Id1 mRNA were suppressed 0.5 and 0.4-fold by E, but augmented 3.2 and 2.4 -fold by T. HIF-1α accumulation led to alterlation of BMP signaling similar to hypoxia, whereas HIF-1α inhibitor altered the signaling similar to normoxia.
Discussion: Sex hormones could change BMP signaling in PAEC depending on oxygen concentration. Our observations provide the new mechanism how sex hormone affects on BMP signaling, and sex hormones may be novel therapeutic targets in the treatment of PAH.

P3337
Pulmonary hypertension in the newborn GTP-cyclohydrolase 1 deficient mouse is unrelated to endothelium-dependent vasorelaxation potential
Jaques Belik[1], Brendan McIntyre[1], Jingyi Pan[1], Jeannette Vasquez-Vivar[2]. *[1]Pediatrics, The Hospital for Sick Children, Toronto, ON, Canada; [2]Biophysics, Medical College of Wisconsin, Milwaukee, WI, United States*

Background: Tetrahydrobiopterin (BH4) is an endothelial nitric oxide (NO) synthase (eNOS) cofactor. Its absence results in eNOS uncoupling and a shift from NO to reactive oxygen species (ROS) generation. The hph-1 mouse is deficient in GTP-cyclohydrolase 1 (GCH1) production, resulting in lowered BH4 tissue content. The adult hph-1 mouse has pulmonary hypertension secondary to eNOS uncoupling, yet whether similar changes are evident in the newborn is presently unknown. Thus, we evaluated 1-3 day old newborn hph-1 pups and compared them to wild-type mice (C57BL/6xCBA).
Methods and results: Lung morphometry, BH4 and its oxidized metabolite BH2 content were measured and near-resistance pulmonary arteries were studied. In control and hph-1 mice, the BH4 lung content is inversely proportional with age, but significantly lower in the mutated animals ($P<0.01$). Pulmonary hypertension is evident in the newborn hph-1 as an increase in the right ventricle-to-left-ventricle+septum ratio, compared with wild-type mice ($P<0.05$). In response to the thromboxane A2 analogue (U46619), the pulmonary arteries of hph-1 mice generate less force ($P<0.01$), when compared with controls, but show a similar vasorelaxant response to eNOS-dependent and -independent stimulation. As compared with wild-type, a significant increase in medial thickness of small pulmonary arteries is evident in the newborn hph-1 mice ($P<0.05$).
Conclusion: Pulmonary hypertension is present from birth in the GCH1 deficient mice, not as a result of vasoconstriction, but secondary to pulmonary vascular remodeling.

P3338
Autoimmunity and pulmonary arterial hypertension: The role of leptin
Alice Huertas[1,2,3], David Montani[1,2,3], Natalia Gambaryan[1,2,3], Frédéric Perros[1,2,3], Barbara Girerd[1,2,3], Sylvia Cohen-Kaminsky[1,2,3], Marc Humbert[1,2,3]. *[1]U999 Pulmonary Hypertension: Pathophysiology and Novel Therapies, INSERM, Le Plessis-Robinson, France; [2]Hôpital Antoine Béclère, Service de Pneumologie et Réanimation Respiratoire, Université Paris-Sud 11, Clamart, France; [3]Centre National de Référence de l'Hypertension Pulmonaire Sévère, Hôpital Antoine Béclère, Clamart, France*

It is suggested that immune mechanisms could play a significant role in pulmonary arterial hypertension (PAH) genesis or progression, but the pathophysiology is still unclear. Recent evidence has demonstrated a detrimental involvement of leptin in promoting various autoimmune diseases by controlling regulatory T cells (Treg). Despite this knowledge, the role of leptin in PAH is unknown. Here, we considered whether leptin promotes the immunopathogenesis of PAH by regulating Treg. To test this hypothesis, we withdrew blood samples from PAH patients (idiopathic (I), heritable (H) and connective tissue disease-related (CTD)) and controls. To detect circulating Treg and those expressing leptin receptor (ObR), we stained the peripheral blood mononuclear cells with surface antibodies and selected Treg expressing ObR as CD4+CD25+CD127lowObR+ cells by flow cytometry (FACS). Finally, we tested TregObR+ cell activation by FACS using an intracellular antibody anti-STAT3. Serum levels of leptin were higher in all PAH patients compared to controls and even higher in IPAH and CTD-PAH compared to HPAH patients. Treg number was comparable in all PAH patients and controls. However, the percentage of TregObR+ was higher in PAH patients compared to controls and even higher in IPAH and CTD-PAH compared to HPAH patients. Interestingly, STAT3 expression was lower in all PAH groups compared to controls. Our findings show for the first time that leptin and its receptor are increased in PAH patients, whereas Treg function is inhibited. Interestingly, IPAH and CTD-PAH are comparable. Therefore, leptin and its receptor could play an important role in the immunopathogenesis of PAH. Support: Medical Research Foundation Josso Award 2010.

P3339
Risk factors for elevated liver function tests (eLFTs) in patients with pulmonary arterial hypertension (PAH) treated with sitaxentan and followed in a European safety registry
Lie-Ju Hwang[1], John Teeter[2], Michael Louie[2], Henrik Toft Sørensen[3], Cynthia de Luise[4]. *[1]Department of Statistics, Specialty Care, Pfizer Inc, New York, NY, United States; [2]Department of Clinical Development and Medical Affairs, Pulmonary Vascular Disease, Pfizer Inc, New York, NY, United States; [3]Department of Clinical Epidemiology, Aarhus University Hospital, Aarhus, Denmark; [4]Department of Epidemiology, Worldwide Safety Strategy, Pfizer Inc, New York, NY, United States*

Background: Endothelin receptor antagonists, including sitaxentan (Thelin®), treat PAH but may cause hepatoxocity. The Thelin Outcomes for Patient Surveillance (TOPS) safety registry monitored prespecified events in European patients (pts).
Aim: Describe risk factors for eLFTs in TOPS pts.
Methods: Sites entered data for eligible pts monthly from baseline to discontinuation; eLFTs were defined as ALT or AST levels >3xULN. Logistic regression assessed effects on subsequent eLFTs of: treatment exposure (mo), age (by 10-y periods), functional class (III/IV vs I/II), etiology (idiopathic vs associated, and presence or absence of each variable: female sex; Caucasian race; prior PAH therapy failure; previous bosentan or ambrisentan; concomitant sildenafil or tadalafil; or baseline LFT elevation. Cox regression assessed time to eLFTs using the same factors.
Results: Of 932 pts, AST or ALT was <3xULN in 97.6% (1.1% missing) at baseline and 83.7% (11.5% missing) during followup. The 46 pts (4.9%) with eLFTs during followup were younger (54 vs 61 y), had shorter treatment (9 vs 14 mo), and had higher baseline ALT/ULN and AST/ULN ratios vs pts without eLFTs during followup (all $P<0.01$). Age (odds ratio [OR], 0.74; 95% CI, 0.60–0.91), exposure length (OR 0.91; 95% CI, 0.87–0.96), and baseline LFT elevation (OR 9.4; 95% CI, 1.5–60.3) were associated with eLFTs at followup (all $P<0.02$). These factors also predicted time to eLFTs (all $P<0.005$).
Conclusions: 4.9% of pts receiving sitaxentan had e LFTs during followup; age, treatment exposure, and baseline eLFTs were significantly associated with eLFTs during followup.

P3340
Increased $p130^{Cas}$ activity induces smooth muscle cell proliferation and migration in idiopathic pulmonary arterial hypertension
Ly Tu[1], Frances De Man[2], Florence Lecerf[1], Charlene François[1], Philippe Dartevelle[1], Gerald Simonneau[1], Elie Fadel[1], Marc Humbert[1], Saadia Eddahibi[1], Christophe Guignabert[1]. *[1]Cardiovascular Research Department, INSERM U999, Le Plessis-Robinson, France; [2]Department of Pulmonology, VU University Medical Center, Amsterdam, Netherlands*

Introduction: $p130^{Cas}$ is a docking protein integrating and regulating extracellular cues and intracellular signaling pathways that controls cell proliferation and motility. Therefore, we hypothesize that $p130^{Cas}$ contributes to excessive migration and proliferation of PA-SMCs in idiopathic Pulmonary Arterial Hypertension (iPAH).
Methods: Protein and phosphorylation levels of $p130^{Cas}$ were quantified by Western blot in surgically resected lung specimens from iPAH-patients and normal subjects. To assess the functional role of $p130^{Cas}$ in iPAH and controls, we evaluated migration and proliferation of cultured PA-SMCs with and without $p130^{Cas}$ inhibition by siRNA.
Results: We observed significantly elevated $p130^{Cas}$ protein expression and activity in iPAH-patients compared to controls. In addition, decreasing $p130^{Cas}$ signaling by RNA-interference reduced to similar levels both the migration and proliferative potentials of IPAH and control PA-SMCs. The decreased migration and proliferation are respectively mediated by decreased matrix metalloproteinase (MMP)-2 release and by decreased mitogen-activated protein kinase (MAPK) activation.
Conclusions: These results demonstrate that $p130^{Cas}$ protein expression and activity induces PA-SMC proliferation and migration in iPAH, suggesting $p130^{Cas}$ as an attractive drug target for PAH therapy.

P3341
Ghrelin effects on local renin angiotensin from pulmonary vessels
Irina Luciana Dumitriu[1], Elena Cojocaru[1], Luminita Gina Vata[1], Marcel Costuleanu[2], Bogdan Gurzu[1], Simona Mihaela Slatineanu[1]. *[1]Department of Functional Sciences, "Gr. T. Popa" University of Medicine and Pharmacy, Iasi, Romania; [2]Department of General and Oro-Maxillo-Facial Pathology, "Gr. T. Popa" University of Medicine and Pharmacy, Iasi, Romania*

Background: Published data sustain the participation of vascular renin angiotensin system (RAS) on alteration of pulmonary vessels reactivity during the allergic airway inflammation. Ghrelin is a growth hormone-releasing peptide involved in modulation of immune function.

Objective: This study aims to investigate the interaction between ghrelin and local RAS from rat pulmonary vessels during ovalbumin – induced allergic airway disease.
Methods: The angiotensinogen (AGT) – induced contractions were assessed on isolated pulmonary artery and veins from ovalbumin sensitized rats receiving either saline (OSR) or ghrelin (OSG) by endotracheal instillation. Experiments were performed in the absence or the presence of losartan, D-ALA7, chymostatin and N-nitro-L-arginine methyl ester (L-NAME).
Results: The angiotensinogen (AGT) contractile effects mediated by AT1 receptors were lower with at least 25% on vessels from OSG than from OSR. The D-ALA7 and LNAME significantly increases the AGT - induced contraction on OSG. The amount of nitric oxide released after stimulation with angiotensinogen (AGT) is higher on OSG and it is blocked by D-ALA7.
Conclusion: Our results suggested that pulmonary delivery of ghrelin could modulate the local RAS from pulmonary vessels, probably by promoting the angiotensin 1-7 mediated effects. These data sustained the existence of another possible way for ghrelin's beneficial effects on the lung.

P3342
Nestin expressing progenitor cells in pulmonary vasculature
Farhan Saboor[1], Claudia Berndt[1], Norbert Weissmann[2], Ralph Schermuly[2], Dieter Müller[1], Ralf Middendorff[1]. *[1]AG Signal Transduction, Institute of Anatomy and Cell Biology, Giessen, Germany; [2]Department of Internal Medicine, Medical Clinic II, Giessen, Germany*

Vascular smooth muscle cells (VSMCs) and pericytes (PCs), distinguished by the expression of neuronal stem cell marker "Nestin", may represent stem cell-like progenitor cells for tissues in various organs. In one of our previous studies, we found that nestin-expressing VSMCs and PCs in testicular blood vessels are the progenitors of testosterone producing Leydig cells.
To analyze the expression pattern of nestin and its role as marker for proliferating progenitor cells in the lung, nestin expression and localization was investigated during postnatal development in nestin-GFP mice. To investigate nestin expression during vascular remodelling, samples from two models of pulmonary hypertension (PH) [monocrotaline (MCT) rat model and hypoxic mouse model] as well as human samples from patients of PH were analyzed. Nestin data was compared with expression of proliferation markers (PCNA, Ki67) and PDGF receptors.
Nestin was found in a subpopulation of VSMCs and PCs of lung vasculature. As compared to adult normoxic controls significantly higher nestin expression was observed in pulmonary vasculature of postnatal tissues and in adult lungs between day 3-7 of hypoxic exposure but not at later time points when PH became evident. Increase of nestin correlated well with an increase of cell proliferation. In hypoxic lungs peak of phosphorylated (activated) PDGF receptor β correlated with nestin one. Increase of nestin-immunoreactive VSMCs and PCs was also found in MCT rat and human lung samples.
Certain contractile cells capable of proliferation could be identified by Nestin expression in lungs and may be used as prognostic marker and new target for therapeutic interventions of diseases like PH.

P3343
Endothelial cell mechanics are altered in pulmonary arterial hypertension (PAH)
Corey Hardin[1], Ramaswamy Krishnan[2], Dhananjay Tambe[2], Sharif Sheik[3], Greeshma Manomohan[2], Jeffrey Fredberg[2], Aaron Waxman[3]. *[1]Pulmonary and Critical Care Unit, Massachusetts General Hospital, Boston, MA, United States; [2]Molecular and Integrative Physiology Program, Harvard School of Public Health, Boston, MA, United States; [3]Pulmonary and Critical Care Medicine, Brigham and Women's Hospital/Harvard Medical School, Boston, MA, United States*

Idiopathic pulmonary arterial hypertension (IPAH) and collagen vascular disease associated PAH (APAH) are associated with a significant elevation of inflammasome activation and release of IL-1β and IL-6 in patient plasma [1]. We hypothesized that these biochemical changes will affect intercellular force distribution in the constituent endothelial cells. To test this hypothesis, we applied to cultured Human Lung Microvascular Endothelial Cell (HLMVEC), serum derived from IPAH and APAH patients, and measured subsequent changes in HLMVEC intercellular forces [2]. In comparison to time-matched controls (n=4), HLMVEC monolayers exposed to patient serum (IPAH, n=7, APAH, n=8) were significantly more contractile (average contractile moment per monolayer; control cells = 153.5 + 13.5 pNm, IPAH cells = 222.3 + 9.6 pNm, and APAH cells = 223.8 + 22.8 pNm) and exhibited greater number of intercellular stress hot-spots. Accordingly, we suggest that inflammasome mediated enhancements in endothelial intercellular forces may play an important role in decreased vascular compliance observed in PAH.
References:
[1] Shaik, R.S., Kolliputi, N., Waxman, A.B., Activation of Inflammasome in Pulmonary Arterial Hypertension. American Journal of Respiratory and Critical Care Medicine, 2010(May);181:A4865.
[2] Tambe, D.T., Hardin, C., Angelini, T.E., Rajendran, K.,Serra-Picamal, X., Zama, M.,Butler, J.P., Weitz, D.A., Fredberg, J.J.,Trepat, X., Collective Cell Guidance by Cooperative Intercellular Forces. Nature Precedings, 2011. doi: 10.1038/npre.2010.5405.1.2010.

P3344
Involvement of cytoskeletal protein paxillin in the pathogenesis of pulmonary hypertension
Christine Veith, Werner Seeger, Norbert Weissmann, Grazyna Kwapiszewska. *University of Giessen Lung Center, ECCPS, Giessen, Germany*

Pulmonary arterial hypertension (PAH) is a fatal disease characterised by a pronounced remodelling of the pulmonary vasculature. The remodelling process entails deposition of the extracellular matrix (ECM) proteins, proliferation of pulmonary arterial smooth muscle cells (PASMC), and changes in the composition of cytoskeletal proteins. Paxillin is one of the most important cytoskeletal proteins, mediating protein-protein interactions and consequently modulating cell signalling. In this study we have investigated the contribution of Paxillin in vascular remodelling.
In lungs of IPAH patients we detected enhanced Paxillin expression compared to controls on both mRNA and protein levels. Immunohistochemical analysis demonstrated expression of Paxillin in pulmonary vasculature and PASMC. Similarly, in the hypoxia mouse model of pulmonary hypertension, expression of Paxillin was localised to the vessels. Laser microdissection of intrapulmonary arteries revealed elevated Paxillin expression in hypoxic lung vessels. Functional measurements were performed by silencing Paxillin expression. Paxillin knockdown caused changes in the phosphorylation status of Akt, and Erk1/2 leading to decreased cell viability, proliferation as well as increased apoptosis of human primary PASMC. Furthermore, immunofluorescence of PASMC revealed that Paxillin knockdown led to cytoskeletal alterations and impaired cell adhesion.
Paxillin has previously been documented to be involved in cell spreading and migration, features characteristic of vascular remodelling. This is however the first report that indicates the involvement of Paxillin in vascular remodelling in the lung, and its association with human PAH disease.

P3345
Determination of cell-derived microparticles in patients with pulmonary hypertension and connective-tissue diseases using flow cytometry
Xhylsime Kqiku[1], Gabor Kovacs[1], Eva Rohde[3], Dirk Strunk[2], Horst Olschewski[1]. *[1]Division of Pulmonology, Medicial University of Graz, Graz, Austria; [2]Division of Hematology, Medicial University of Graz, Graz, Austria; [3]University Hospital for Blood Group Serology and Transfusion Medicine, Paracelsus Medical University/Salzburger Landeskliniken, Salzburg, Austria*

Background: Microparticles (MPs) are small plasma membrane vesicles released from different cell types during activation or apoptosis.Increased MPs levels have been associated with cardiovascular diseases,thrombotic disorders and systemic inflammatory conditions.In pulmonary hypertension (PH) the role of MPs is poorly understood.
Aims and objectives: The aim of this study was to analyze the MPs fractions in PH-and connective-tissue disease (CTD) patients which represent a risk factor for PH development.
Patients and methods: Plasma samples derived from PH and CTD patients were tested using flow cytometry (FC). Circulating MPs from platelets (CD61+), endothelial cells (CD31+) as well as Annexin V+ were measured by FC in 28 PH patients,36 CTD patients and 41 healthy controls.The levels of MPs were compared in these three groups.
Results: The overall fraction of MPs was higher in PH patients (57±26% of total events) and CTD patients (51±21%)as compared to controls (42±18%) ($p<0,05$). Platelet derived CD61+ MPs were tendentially increased in PH patients comparing to controls (p=0,096) whereas CD31 expression was not found to be different on MPs from patients and controls.The expression of Annexin V on MPs from PH patients were significantly higher as compared with controls (mean fluorescence intensities: 56±26%vs.38±17%,p=0,003).There were no significant differences between Annexin V expression levels on MPs in PH vs. CTD patients (p=0,384) and not between CTD vs.controls (p=0,179).
Conclusions: According to these preliminary data, the plasma levels of AnnexinV+MPs are increased in PH patients and may be related to an activated pro-coagulatory and inflammatory vascular status.

P3346
The role of the accessory type III transforming growth factor-β receptors in the regulation of pulmonary vascular development
Gero Niess, Matthias Michiels-Corsten, Werner Seeger, Rory Morty. *Lung Development and Remodelling, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany*

Pulmonary artery smooth muscle cell behaviour, including proliferation, apoptosis, and matrix production, is controlled by transforming growth factor (TGF)-β, acting via two type I (Acvrl1 and Tgbfr1) and two type III [endoglin (Eng) and betaglycan (Tgfbr3)] TGF-β receptors. Knockdown of TGFBR3 by siRNA in primary human pulmonary artery smooth muscle cells (PASMC) increased PASMC proliferation (3-fold; assessed by BrdU incorporation) *in vitro*, in a TGF-β-independent manner. However, apoptosis rates of the PASMC were not affected by siRNA knockdown of TGFBR3. The siRNA knockdown of other TGF-β receptors, ACVRL1, TGFBR1, TGFBR2 and ENG, did not impact TGF-β-independent proliferation or apoptosis of PASMC. These data point to a novel, TGF-β-independent role for TGFBR3 in regulating PASMC growth. This idea assumes importance considering that we

have also observed perturbed expression of TGFBR3 in the lungs of neonatal mice with hyperoxia (85% O_2)-induced lung injury, which results in bronchopulmonary dysplasia (BPD). The mRNA levels (assessed by quantitative real-time RT-PCR) for *tgfbr3* were downregulated (4.4-fold, p=0.003), while TGFBR3 protein levels were downregulated by 70%. Laser capture microdissection confirmed dysregulated expression of TGFBR3 in the pulmonary vasculature of the developing mouse lung. Taken together, these data suggest a role for TGFBR3 in vascular smooth muscle cell function which could lead to a dysregulation of TGF-β signalling in the pulmonary vasculature, which in turn could contribute to the impaired pulmonary vascular growth and development associated with the lung hypoplasia observed in patients with BPD.

P3347

Elevated levels of adenosine in the lungs lead to chronic lung injury and pulmonary hypertension

Harry Karmouty-Quintana[1], Hongyan Zhong[2], Dewan Zeng[2], Luiz Belardinelli[2], Michael Blackburn[1]. *[1]Biochemistry and Molecular Biology, University of Texas - Medical School, Houston, TX, United States; [2]Cardiovascular Disease, Gilead Sciences, Inc, Palo Alto, CA, United States*

Pulmonary Hypertension (PH) is characterized by increased pulmonary vascular tone and remodeling of the pulmonary vasculature including muscularization of vessels. PH is often associated with underlying chronic lung diseases (CLD) such as chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF). The adenosine (Ado) A2B receptor (R) expression is increased in patients with COPD and IPF. Activation of the A2BR by Ado has been shown to regulate fibrosis through its action in inflammatory and structural cells. However, the role of Ado and the A2BR in the pathogenesis of PH is not known.
Hypothesis: Ado acting on the A2BR modulates the development of PH in CLD. Ado deaminase (ADA)-deficient mice have increased levels of Ado in the lung tissue that lead to CLD. On day 30, once lung injury was established, mice were provided with chow containing placebo or GS-6201, an A2BR antagonist, for the next 10 days. On day 41, right ventricle systolic pressure (RVSP), systemic blood pressure, heart rate and lung function measurements were performed.
ADA-deficient mice had increased RVSP compared to control mice. Lung function measurements revealed increased airway resistance and a reduction in airway and tissue compliance in ADA-/- mice. Blockade of the A2BR by GS-6201 inhibited the increased RVSP and restored lung function. No change in systemic systolic blood pressure or heart rate was observed in mice treated with placebo or GS-6201. These results highlight the role of the A2BR in the pathogenesis of PH associated with elevated tissue Ado and CLD. The results suggest that targeting the A2BR could be a potential target for the treatment of PH secondary to CLD.