392. Molecular biology of pro- and anti-inflammatory responses in the lung

P3707

Induction of inflammation, oxidative stress and autophagy in human alveolar type I epithelial cells following exposure to silver nanoparticles

Martina Zambianchi, Andrew Thorley, Teresa Tetley. *Lung Cell Biology, National Heart and Lung Institute, London, United Kingdom*

The use of silver nanoparticles (AgNP) in health products is increasing due to their antimicrobial activity. However, their impact on health is poorly understood. It is known that the particle reactivity increases as size decreases, thus AgNPs may induce inflammatory responses that their bulk sized counterparts do not. It is known that 50% of inhaled nano-sized (<100nm) particles preferentially deposit in the peripheral lung. Thus, inhalation of AgNPs might have adverse effects on the alveolar epithelium. We hypothesize that AgNPs induce an oxidative stress-dependent proinflammatory response in the human alveolar epithelium and activate autophagy.

Human alveolar type I epithelial cells (TT1) were exposed to 80nm AgNPs for up to 24h. IL-6 and IL-8 release was measured by ELISA and reactive oxygen species (ROS) production measured by dihydroethidium staining. n-acetylcysteine (NAC) was used to evaluate the role of ROS in mediator release. The plasmid assay was used to assess free radical-induced DNA damage and the autophagy markers LC3II/LC3I measured by immunoblotting.

AgNPs induced significant oxidative stress within 4h and IL-6 and IL-8 by 24h. NAC pre-treatment inhibited Ag50μg/ml-stimulation of IL-6 by 60% ($P<0.001$). AgNPs induced significant DNA damage (density of supercoiled fraction: Ag1μg/ml=0.31; Ag50μg/ml=0.073) and induced a significant increase in the LC3II/LC3I ratio ($P<0.01$; Ag 50% vs nt), suggesting that AgNPs activate autophagy.

Our study shows that AgNPs induce oxidative stress-dependent inflammation, DNA damage and a unique finding of autophagy responses in TT1 cells suggesting that AgNPs may have adverse effects on the lung.

P3708

Defective macrophage phagocytosis in COPD is associated with reduced STAT1 phosphorylation

Rebecca Holloway[1], Peter Fenwick[1], Iain Kilty[2], Peter Barnes[1], Louise Donnelly[1]. [1]*National Heart and Lung Institute, Imperial College, London, United Kingdom;* [2]*Inflammation and Remodelling Research Unit, Pfizer Inc., Cambridge, MA, United States*

Macrophages are professional phagocytes that maintain sterility and remove invading pathogens. Chronic obstructive pulmonary disease (COPD) is associated with increased lung macrophages but the lower airways are colonised with bacteria such as *Haemophilus influenzae* and *Streptococcus pneumoniae.* This is associated with a decreased phagocytic response of COPD macrophages to these bacteria. IFNγ is increased in COPD airways and is associated with decreased phagocytosis. IFNγ activates the JAK/STAT pathway via phosphorylation of STAT1 to initiate signal transduction. We hypothesise that reduced phagocytosis in COPD is linked to STAT1 phosphorylation and JAK/STAT activation. Monocyte derived macrophages (MDM) from non-smoker, smoker and COPD donors and COPD lung tissue macrophages (n=3-4) were challenged with fluorescently labelled inert beads or heat killed bacteria (*H. influenzae* or *S. pneumoniae*). IFNγ stimulation (10ng/ml, 10min) was used as a positive control. STAT1 phosphorylation was assessed by Western blotting.

STAT1 was phosphorylated in response to inert beads in both non-smoker and smoker MDM but not in those from COPD donors. In addition, none of the phagocytic prey initiated STAT1 phosphorylation in COPD lung tissue macrophages. However, IFNγ stimulation caused phosphorylation of STAT1 in all three donor groups.

To conclude, COPD MDM and lung tissue macrophages are capable of STAT1 phosphorylation although not in response to phagocytic prey. This is in contrast to non-smoker and smoker cells that show JAK/STAT activation in response to inert beads. Further investigation of this signalling pathway in phagocytosis may lead to increased knowledge of COPD pathogenesis.

P3709

The dissociated steroid receptor ligand from plant origin called compound A (CpdA) inhibits the production of steroid-resistant chemokines induced by TNFα/IFNγ in airway smooth muscle (ASM) cells in both asthma and healthy subjects

Latifa Chachi[1], Adelina Gavrila[1], Omar Tliba, Christopher Brightling, Yassine Amrani. [1]*Infection, Immunity and Inflammation, Institute for Lung Health, University of Leicester, England, United Kingdom;* [2]*Department of Pharmaceutical Sciences, Thomas Jefferson University, Jefferson School of Pharmacy, Philadelphia, United States*

Corticosteroids (GC) act by two molecular mechanisms known as transactivation or transrepression. Transrepression mediates the vast majority of the anti-inflammatory actions of corticosteroids, whereas transactivation has been associated with the unwanted side effects of corticosteroids. One unanswered question is whether compounds that can dissociate the transactivation from the beneficial transrepression function of GC could be used to treat severe patients who are refractory to steroid therapy. The purpose of the present study was to study the effect of CpdA, a dissociated steroid receptor ligand, on the production of steroid-resistant chemokines induced by TNFα/IFNγ in ASM cells. We found that TNFα/IFNγ combination stimulates the expression of CXCl10, CCl5 and CX3Cl1 in both asthmatic and healthy airway smooth muscle cells, with a greater production of CXCl10 and CCl5 by asthmatic cells (n=3 subjects). Pretreating cells with fluticasone (100 nM, 2 hr) did not affect cytokine-induced expression of CXCl10 while CX3Cl1 levels were significantly increased by 2.3 fold in the presence of fluticasone. Interestingly, CpdA (5 μM) suppressed cytokine-induced expression of CXCl10 by 50% while CCl5 and CX3Cl1 responses were completely inhibited in both asthmatic and healthy cells. These data show that CpdA can inhibit the expression of steroid-resistant inflammatory genes in airway smooth muscle cells. This study suggests that dissociated steroid receptor ligands could offer a novel therapeutic alternative to treat steroid resistance in severe asthmatics.

P3710

Neovascularisation in asthma: Altered angiogenic potential of ASM cells from asthmatic patients

Laura Keglowich[1], Michael Roth[1], Maria Philippova[2], Thérèse J. Resink[2], Michael Tamm[1], Peter Borger[1]. [1]*Pulmonary Cell Research & Pneumology, University Hospital, Basel, Switzerland;* [2]*Signal Transduction, Department Biomedicine, University Hospital, Basel, Switzerland*

Background: Airway remodeling is a key pathology in asthma with increasing changes of the airway wall structure, including thickening of airway smooth muscle (ASM) bundles and increased vascularisation. ASM cells require oxygen and nutritions which are delivered via blood vessels. Understanding the mechanism of neo-vascularisation may therefore lead to novel strategies to counteract remodeling.
Methods: The capacity of ASM cells of asthmatics and non-asthmatics to induce sprouting was determined with a sprouting assay. To get broad insight into the factors which may be involved we used an angiogenesis array. Some of the 43 analyzed proteins are of further interest and their concentration was measure by ELISA.
Results: Conditioned Medium (CM) from ASM cells was applied to a spheroid assay. In this assay the CM of ASM cells derived from asthmatics lead to more sprout outgrowth compared to CM derived from controls. Analyzing the CM with an commercial available angiogenesis array revealed that several angiogenic factors are elevated. The CXC-Chemokines ENA-78, GRO-alpha and IL-8 have been further analyzed by ELISA and were confirmed to be upregulated in asthmatics.
Conclusion: Our results indicate that the angiogenic potential of ASM cells from asthmatic patients is higher compared to that of control cells. The ELR-motif-containing chemokines ENA-78, GRO-alpha and IL-8 are upregulated in asthmatics and might therefore contribute to neo-vascularisation. Counteracting this process may lead to novel asthma therapies.
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P3711

TNFα-induced glucocorticoid resistance: Effect of steroidal and non-steroidal GR agonists, formoterol and inhibition of inflammatory signalling

Christopher Rider[1], Suharsh Shah[1], Anna Miller-Larsson[2], David Proud[1], Malcolm Johnson[3], Mark Giembycz[1], Robert Newton[1]. [1]*Airway Inflammation Research Group, University of Calgary, AB, Canada;* [2]*AstraZeneca R&D, AstraZeneca, Mölndal, Sweden;* [3]*Respiratory Science, GlaxoSmithKline, Uxbridge, Middlesex, United Kingdom*

Inhaled glucocorticoids (GCs) w/wo long-acting β_2-adrenoceptor agonists (LABA) are the most effective treatment for asthma. However, GC insensitivity is a facet

of severe asthma and COPD. We have examined TNFα-induced resistance to GC-dependent transcription by steroidal and non-steroidal GC receptor (GR) ligands in the absence and presence of a LABA, formoterol.
Methods: GC-dependent transcription was modeled by a simple 2×GRE-luciferase reporter in human bronchial epithelial BEAS-2B cells treated with TNFα (10 ng/ml) for 1 h prior to addition of GR ligands, and harvested after 6 h for luciferase assay.
Results: TNFα reduced by 43-54% the ability to drive 2×GRE-dependent transcription by dexamethasone, budesonide, fluticasone propionate or fluticasone furoate. The GCs des-ciclesonide, GW870086X, RU24858 and the non-steroidal GR agonist, GSK9027, all showed reduced maximal responses (E_{max}) with intrinsic activities 0.5-0.77 relative to dexamethasone. In each case, TNFα reduced E_{max} by a further 39-55%. Conversely, formoterol enhanced GRE-dependent transcription by each ligand and rescued the resistance induced by TNFα; these effects were proportional to the E_{max} of each drug. Statistically significant reversal of TNFα-induced resistance was observed with the c-jun N-terminal kinase inhibitor, JNK8, and PS-1145, an IκB kinase 2 (IKK2) inhibitor.
Conclusions: TNFα induces GC resistance to steroidal GR ligands that are both full and partial agonists, as well as to a non-steroidal GR agonist. This effect is rescued by the addition of formoterol. It is possible that inhibition of inflammatory signalling may also reduce GC resistance.

P3712
The effects of human lung mast cell products on the synthetic functions of lung fibroblasts
Hatem Alkhouri[1], Michael Wai-Shing[1], David Krimmer[2], Brian Oliver[2], Carol Armour[2], J. Margaret Hughes[1]. *[1]Faculty of Pharmacy, University of Sydney, NSW, Australia; [2]Woolcock Institute of Medical Research, University of Sydney, NSW, Australia*

Activated mast cell numbers are generally increased in the bronchial wall and alveolar parenchyma of people with asthma. Mast cells release a wide variety of cytokines and mediators that may modulate the activities of structural cells such as fibroblasts and contribute to airway remodelling in asthma.
Objective: To examine the effects of human lung mast cell (HLMC) products on the synthetic functions of airway and parenchymal fibroblasts.
Methods: HLMC were stimulated with IgE/anti-IgE and their supernatants (SN) collected after 2 and 24h. The SN were added to serum-deprived airway and parenchymal fibroblasts for up to 48h. Fibroblast and mast cell cytokine release and extracellular matrix (ECM) deposition were measured using ELISAs.
Results: Both 2 and 24h HLMC SN significantly increased the synthetic functions of parenchymal and airway fibroblasts in a concentration-related manner. Release of CXCL8 was increased up to 3.9 and 3.1-fold from parenchymal fibroblasts by the 2 and 24h SN respectively. The 2 and 24h SN respectively also increased IL-6 release from parenchymal [5.8 and 6.6-fold] and airway [8.7 and 22.6-fold] fibroblasts in a similar manner. Interestingly, although fibronectin deposition was unchanged, both the 2 and 24h SN significantly increased collagen IV deposition by airway, but not parenchymal, fibroblasts up to 1.5- fold, whereas only the 24h SN increased tenascin-C deposition [1.8-fold] by the airway cells.
Conclusions: HLMC products increase lung fibroblast cytokine release and differentially regulate airway and parenchymal fibroblast ECM deposition. Thus HLMC may promote further inflammation and airway remodelling in asthma.

P3713
TNFα stimulates the expression different chemokines including CXCl10, CCL5 and CXCL8 in developing airway smooth muscle (ASM) cells: Modulation by fluticasone and TNFα receptors
Hitesh Pandya[1], Abubaccar Gassama[1], Latifa Chachi[1], Helen Pearson[1], Y.S. Prakash[2], Erol Gaillard[1], Yassine Amrani[1]. *[1]Infection, Immunity and Inflammation, Institute for Lung Health, University of Leicester, England, United Kingdom; [2]Department of Anesthesiology, Mayo Clinic, Rochester, MN, United States*

Corticosteroids are only partially effective in treating children with Chronic Lung Disease (CLD) or chronic asthma. 'Low to moderate' doses of steroids are generally effective in limiting symptoms, but higher doses are lead to serious side effects with little extra effect on symptom control. In this study, we used fetal ASM cells from human airways to determine their responsiveness to inflammatory chemokines associated with CLD. Fetal ASM cells exposed to TNFα secrete different chemokines including CXCl10, CCL5 and CXCL8. Pre-treating cells with fluticasone (0.001-100nM) led to a dose-dependent suppression of all chemokines although the magnitude of inhibition was greater on CCL5 (over 95%) when compared to CXCl10 or CXCL8 (less than 60%) at 100 nM fluticasone. Using rtPCR, we also found that expression of cytokine genes was only partially repressed by fluticasone (100nm). Neutralizing antibodies against TNFα receptors revealed that engagement of both TNFR1 and TNFR2 mediates TNFα-induced chemokines expression. Our data suggest that generation of pro-inflammatory chemokines by fetal lung cells is only partially responsive to corticosteroid therapy. The mechanisms underlying this resistance are unclear.

P3714
p38 MAPK inhibitors in COPD
Patricia Macedo[1], Joann Rhodes[2], Iain Kilty[2], Peter Barnes[1], Louise Donnelly[1]. *[1]Airway Diseases, National Heart and Lung Institute, Imperial College, London, United Kingdom; [2]Pfizer, Pfizer Inc, Cambridge, MA, United States*

p38 MAPK signalling upregulates inflammation and is known to be increased in COPD. This study investigated whether p38 inhibitors PF75 and PF32 suppressed release of inflammatory cytokines from macrophage-lineage cells.
Peripheral blood mononuclear cells (PBMC) and monocyte-derived macrophages (MDM) were isolated from non-smokers (NS), smokers (S) and COPD patients. Cells were pre-treated with either p38 inhibitor or dexamethasone (DEX) prior to stimulation with LPS. Cell media was harvested at 24h and cytokine release (IL-6, CXCL8, IL-10 and TNF) measured by ELISA.
Both p38 inhibitors and DEX suppressed cytokine release in a concentration-dependent manner in PBMC and MDM. There were no differences between subject groups.

EC50 values (nM) for DEX, PF75 and PF32 on cytokine release

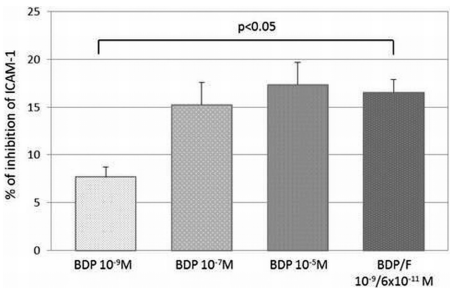
	cline5-7	PBMC			MDM		
		NS	S	COPD	NS	S	COPD
DEX		n=10	n=11	n=13	n=8	n=11	n=12
	TNF	358±273	46.8±8.23	53.2±17.6	13.6±6.98	16.9±10.2	62.3±3.01
	IL-6	33.5±13.3	24.9±10.5	43.4±14.1	27±11.2	3.14±3	21.9±11.7
	CXCL8	32.4±16.8	16.0±5.33	22.2±9.63	21.9±11.7	3.45±1.88	19.3±8.27
	IL-10	25.8±10.1	240±158	29.5±12.8	16.1±7.43	23.8±10.4	126±101
PF75		n=6	n=10	n=8	n=6	n=9	n=7
	TNF	9.23±2.87	12.4±4.12	10.6±4.14	5.47±2.74	3.31±1.48	3.4±0.99
	IL-6	21.4±9.62	15.1±7.84	43.1±20.3	4.82±2.74	1.03±0.96	1.91±1.5
	CXCL8	30.8±15.5	1.3±1.06	38.4±34.3	5.96±3.84	1.71±1.41	0.07±0.03
	IL-10	4.72±4.59	23.4±17.8	514±327	34.6±30	3.98±3.83	20.8±12.9
PF32		n=8	n=9	n=7	n=6	n=7	n=6
	TNF	1.04±0.38	1.96±0.57	1.57±0.64	0.76±0.59	0.50±0.34	2.31±0.85
	IL-6	0.65±0.53	0.10±0.02	10.2±8.57	4.97±4.51	6.22±6.00	234±232
	CXCL8	3.37±1.83	1.72±1.05	0.99±0.51	2.48±2.4	3.7±3.02	1.66±1.01
	IL-10	1.49±0.68	229±228	2.01±0.87	4.94±4.86	1.93±1.41	0.65±0.46

mean ± SEM; n = number of subjects.

p38 inhibitors suppress release of inflammatory cytokines and may have a therapeutic role in COPD.

P3715
Effect of beclomethasone dipropionate, formoterol and their combination on TNF-α-induced ICAM-1 and IL-8 expression in human bronchial epithelial cells
Silvana Cianchetti[1], Cristina Cardini[1], Gabriele Nicolini[2], Alessandro Celi[1], Pierluigi Paggiaro[1]. *[1]Cardiothoracic and Vascular Department, University of Pisa, Italy; [2]Medical Affairs Department, Chiesi Farmaceutici S.p.A., Parma, Italy*

Background: The adhesion molecule and chemokine expression in the airways has been implicated in inflammatory lung diseases.
Aim: To investigate the effect of beclomethasone dipropionate (BDP) and formoterol (F) alone and in combination (BDP/F) on TNF-α-induced ICAM-1 expression and IL-8 release in human bronchial epithelial cell line Calu-1.
Methods: Calu-1 cells were treated (30') with BDP (10^{-11}–10^{-5}M) or F (6/100 with respect to BDP) or BDP/F (10^{-9}/6x10^{-11}M) or drug diluent (control cells: CC) and then with TNF-α (200 U/ml; 4 hrs). Surface ICAM-1 expression and IL-8 release were measured by ELISA.
Results: BDP inhibited ICAM-1 expression (mean % decrement±SEM, n=11) only at the highest concentrations (10^{-7}M, 10^{-5}M: 15.3±2.3%, 17.4±2.3%, p<0.05 vs CC) while BDP/F was also effective at lower doses (10^{-9}/6x10^{-11}M:16.6±1.6% vs BDP 10^{-9}M:7.7±1.3%, p<0.05), achieving an effect comparable to that observed with the highest concentrations of BDP; BDP/F also decreased IL-8 release (10^{-9}/6x10^{-11}M:20.4±6.5% vs BDP 10^{-9}M:12±4%; p=0.05, n=7). F did not affect ICAM-1 and IL-8 expression.



Conclusions: BDP in combination with F provided greater inhibition of ICAM-1 and IL-8 expression as compared to each drug. These observations may imply

that BDP/F exerts anti-inflammatory effects in airways by modulating adhesion molecule and chemokine expression in bronchial epithelial cells.

P3716

Anti-inflammatory and cytoprotective actions of the endogenous docosahexaenoic acid (DHA) electrophilic derivative 17-oxo-DHA

Chiara Cipollina[1,2], Stefania Gerbino[1], Serena Di Vincenzo[1], Rosalia Curto[1], Mark Gjomarkaj[1], Bruce A. Freeman[3], Francisco J. Schopfer[3], Elisabetta Pace[1]. *[1]Istituto di Biomedicina e Immunologia Molecolare (IBIM), Consiglio Nazionale delle Ricerche (CNR), Palermo, Italy; [2]Fondazione Ri.MED, Fondazione Ri.MED, Palermo, Italy; [3]Department of Pharmacology & Chemical Biology, University of Pittsburgh - School of Medicine, Pittsburgh, PA, United States*

Inflammation of the airways is a hallmark of chronic obstructive pulmonary disease (COPD), one of the leading mortality cause worldwide. Bronchial epithelial cells and resident macrophages represent the first barrier in the lung against pathogens and external insults such as cigarette smoke, which is a major risk factor for COPD. Although the activation of the innate inflammatory response is required for defence purposes, uncontrolled activation, typical of COPD, leads to chronic inflammation causing tissue damage and enhanced risk of infection. Currently there is no therapy able to revert disease progression in COPD and therefore the search for new drugs is highly active.
Omega-3 derived electrophilic fatty acids have been recently discovered as endogenous anti-inflammatory molecules produced by activated macrophages by the action of cyclooxygenase-2.
In the present work, the anti-inflammatory and cytoprotective actions of the electrophilic DHA-derivative 17-oxo-DHA were evaluated in lipopolysaccharide-activated macrophages and in bronchial epithelial cells. We report that 17-oxo-DHA suppresses LPS-induced TNFalpha production in macrophages and increases intracellular glutathione and the expression of heme-oxygenase 1 in both cell types, thus providing protection against oxidative stress caused by cigarette smoke and inflammatory reactions.
Although the molecular mechanisms are still under investigation, overall the present results support a role for the electrophilic, omega-3 derived 17-oxo-DHA in limiting inflammatory reactions and modulating the antioxidant response, thus reducing cellular damage and promoting the resolution of inflammation.

P3717

Comparative anti-oxidant effects of carbocysteine and fluticasone propionate in cigarette smoke stimulated airway epithelial cells

Elisabetta Pace[1], Maria Ferraro[1], Serena Di Vincenzo[1], Rossella Balsamo[2], Luigi Lanata[2], Andreina Bruno[1], Luana Lipari[3], Mark Gjomarkaj[1]. *[1]Institute of Biomedicine and Molecular Immunology, National Research Council, Palermo, Italy; [2]Medical Affair, Dompè, Milan, Italy; [3]Istologia ed Embriologia del Dipartimento di Medicina Sperimentale e Scienze Neurologiche (BioNec), Università degli Studi di Palermo, Italy*

Cigarette smoke extracts (CSE) induce oxidative stress, an important feature in chronic obstructive pulmonary disease (COPD). Oxidative stress contributes to the poor clinical efficacy of corticosteroids. Carbocysteine, an anti-oxidant and mucolytic agent, is effective in reducing the severity and the rate of exacerbations in COPD patients. The effects of carbocysteine on CSE induced oxidative stress in nasal and in bronchial epithelial cells as well as the comparison of these anti-oxidant effects of carbocysteine with those of fluticasone propionate are largely unknown. The present study was aimed to assess: the effects of carbocysteine (10^{-4} M) in cell survival and intracellular reactive oxygen species (ROS) production in CSE stimulated bronchial (16-HBE) and in CSE stimulated nasal (RPMI 2650) epithelial cells and to compare these effects with those of fluticasone propionate (10^{-8} M). Carbocysteine or fluticasone propionate did not induce cell necrosis (propidium positive cells) or cell apoptosis (Annexin V positive/propidium negative cells) in 16 HBE and in RPMI 2650 at the tested concentrations. CSE increased intracellular ROS production in 16HBE and in RPMI 2650. Fluticasone propionate was not able to significantly reduce intracellular ROS production in both 16HBE and in RPMI 2650. Carbocysteine was able to significantly reduce intracellular ROS production and was more effective than fluticasone propionate in reducing these CSE mediated effects in both cell lines.
In conclusion, the present study provides compelling evidences that carbocysteine may be considered a promising strategy in diseases associated to corticosteroid resistance.

P3718

Effect of hypoxia and cigarette smoke on hypoxia-inducible factor 1α (HIF-1α) and heat shock protein 72 (HSP72) system of alveolar epithelial cells

Balazs Odler[1], Katalin Szabo[1], Krisztina Gal[1,2], Erna Sziksz[3], Adam Vannay[3], Attila Szabo[2], Gyorgy Losonczy[1], Veronika Muller[1]. *[1]Department of Pulmonology, Semmelweis University, Budapest, Hungary; [2]1st Department of Pediatrics, Semmelweis University, Budapest, Hungary; [3]Research Laboratory of Pediatrics and Nephrology, Hungarian Academy of Sciences, Budapest, Hungary*

Smoking is the main risk factor of alvolar destruction in emphyseama/COPD. In COPD airway obstruction causes hypoventilated areas in the lung leading to alveolar hypoxia. HIF-1 α plays a key role in the defense against hypoxic cellular damage. The inducible HSP72 has a central role in the maintenance of cell integrity, apoptosis and cellular immunity. It is unknown, what role HIF-1α and HSP72 play in cell damage caused by hypoxia and cigarette smoke.
HIF1α and HSP72 mRNA expression using RT-PCR of immortalized alveolar epithelial cells (A549) was analyzed using hypoxia (FiO2 20,9% (control); 13±1%; 6±1%; 1±1%) and treatment with cigarette smoke extract (CSE).
Under hypoxic conditions cell count grew significantly (FiO2 13±1%: 13,33x105; FiO2 6±1%:13,2x105; FiO2 1±1%: 12,86x105 vs. normoxia FiO2 20,9%: 7,73x105; $p<0.01$), whereas treatment with CSE decreased cell number compared to control. Mild hypoxia significantly increased HIF-1α mRNA, while atmospheric oxygen tension resulted in unchanged expression. Severe hypoxia and CSE treatment significantly lowered HIF-1α mRNA expression in A549 cells (CSE: 4,30±0,60, FiO2 1±1%: 3,24±1,03 vs. control: 11,671±0,70 arbitrary unit, $p<0,05$). HSP72 mRNA expression increased using FiO2 20,9%, in contrast decreased in all hypoxic and CSE treated cells. Dexamethasone treatment of CSE treated cells dose-dependently increased HIF-1 α and HSP72 mRNA expression.
Hypoxia increases, whereas CSE treatment decreases alveolar epithelial cell count. HSP72 mRNA decreases following hypoxic or CSE induced cellular stress, while severe hypoxia and CSE treatment decreases HIF-1α mRNA expression of A549 cells.

P3719

Role of ICAM-2 in neutrophil transepithelial migration in the lung

Carine Rebeyrol[1], Joanna C. Porter[1,2]. *[1]Centre for Respiratory Research, University College London, United Kingdom; [2]Respiratory Medicine Service, University College London Hospitals NHS Trust, London, United Kingdom*

Many inflammatory diseases of airways including COPD and cystic fibrosis are characterized by migration of neutrophils from the interstitium across the respiratory epithelium into the airspaces. Neutrophil transepithelial migration (egression) across the bronchial epithelial cell (BEC) barrier promotes resolution of inflammation, whereas movement across the alveolar epithelial cell (AEC) barrier, often required for pathogen control, may prove detrimental to the human and resulting lung injury.
We have shown that intercellular adhesion molecule (ICAM)-2 is required for leukocyte migration across the bronchial epithelium. We have then used immunohistochemistry (IHC), to show that although ICAM-2 is expressed on the bronchial epithelium there is very little expression on the alveolar epithelium in normal human lung. Our hypothesis is that egression of neutrophils across the bronchial and alveolar epithelia are differentially regulated, and that understanding these processes is essential to develop effective ways to promote safe resolution of inflammation.
Primary human (h)BECs and AECs are grown at air liquid interface on Transwell™ collagen coated polyester membranes. Then, the cells are stimulated with pro-inflammatory mediators (TNF-α, TGF-β, LPS) for 4h or 24h. IHC, qRT-PCR, western blot and flow cytometry assays of ICAM-2 are performed. The role of ICAM-2 in neutrophil migration across hBEC and hAEC monolayers in the physiological basal to apical direction is observed.
Our results establish the role of ICAM-2 in regulating neutrophil-epithelial interactions in the lung and the central role of this molecule in the resolution of inflammation.

P3720

Anti-inflammatory effects of adiponectin in A549 cells exposed to TNFα and IL-1β

Ersilia Nigro[1,6], Olga Scudiero[1,2], Daniela Sarnataro[1], Maria Ludovica Monaco[1,6], Nicolina De Rosa[3], Matteo Sofia[4], Andrea Bianco[5], Aurora Daniele[1,6]. *[1]CEINGE – Biotecnologie Avanzate S.c.a.r.l., SUN, Seconda Università di Napoli, Naples, Italy; [2]Dipartimento di Biochimica e Biotecnologie Mediche, Università degli Studi di Napoli "Federico II", Naples, Italy; [3]Unità Operativa di Anatomia Patologica, AORN "Monaldi", Naples, Italy; [4]Unità Operativa Complessa di Malattie dell' Apparato Respiratorio, Università degli Studi di Napoli "Federico II", Naples, Italy; [5]Dipartimento di Scienze per la Salute, Università del Molise, Campobasso, Italy; [6]Dipartimento di Scienze Ambientali, SUN, Seconda Università di Napoli, Caserta, Italy*

Adiponectin (Acrp30) is an insulin-sensitizing hormone with beneficial effects on a number of biological and metabolic processes by two widely expressed receptors, AdipoR1 and AdipoR2. To date, the role of Acrp30 in lung is not completely assessed but altered levels of Acrp30 and modulated expression of both AdipoRs have been described in Chronic Obstructive Pulmonary disease (COPD) (1). Lung inflammation, a crucial component of COPD progression, is mainly sustained by two potent pro-inflammatory cytokines: Tumor Necrosis Factor α (TNFα) and Interleukin 1β (IL-1β). These mediators in turn propagate and perpetuate the inflammation also through NF-κB, a transcriptional factor for many genes involved in inflammation, injury and stress in lung. In this study we analyzed the effects of Acrp30 on human alveolar epithelial cell line (A549) selected as an in vitro model of lung epithelia. We treated A549 cells with TNFα or IL-1β and visualized the NF-κB nuclear transactivation by confocal microscopy. Successively, we evaluated the role of Acrp30 in this process: we treated the cells with Acrp30 and subsequently with TNFα or IL-1β and analyzed NF-κB nuclear transactivation. Finally, we investigated by western blotting the phosphorylation status of ERK1/2 kinase, one of the main molecules involved in Acrp30 signal pathway. A better elucidation

of the Acrp30 role in the control of local lung inflammatory state may contribute to develop new therapeutic approaches in inflammatory diseases as COPD, a major cause of morbidity and death worldwide.
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Reference:
[1] Daniele A et al. Int J Biochem Cell Biol. 2012 Mar;44(3):563-9.

P3721
Size-dependent particle uptake and trafficking by antigen presenting cells in different anatomical respiratory tract compartments
Fabian Blank[1], Deborah Strickland[2], Philip Stumbles[2,3,4], Patrick Holt[2], Christophe von Garnier[1]. *[1]Respiratory Medicine, Berne University Hospital, Berne, Switzerland; [2]Cell Biology, Telethon Institute for Child Health Research, Perth, WA, Australia; [3]School of Veterinary and Biomedical Sciences, Faculty of Health Sciences, Murdoch University, Perth, WA, Australia; [4]Centre for Child Health Research, University of Western Australia, Perth, WA, Australia*

Effects of biomedical particles on lung antigen presenting cells (APC) such as dendritic cells (DC) and alveolar macrophages (AM) remain poorly understood to date.
BALB/c mice intra-nasally received different sized (20nm, 50nm, 100nm, 200nm, 1000nm) fluorescent polystyrene particles. Two and 24h after instillation, FACS and confocal microscopy were performed on main conducting airways lung parenchyma, lung draining lymph nodes (LDLN) and BALF to analyse particle uptake in CD11b-/CD103+ DC and AM.
In airways, frequencies of particle+ DC 24h after exposure were higher for 20nm particles compared to 1000nm particles (12.0±3.2% vs. 1.220±0.3%; p=0.0283). At 24 hours, in lung parenchyma DC, preferential uptake of 20nm (43.5±3.5%, p=0.0001) and 50nm particles (36.2±0.4%, p<0.0001) occurred, compared to 1000nm particles (9.700±0.9%). These changes mirrored LDLN, where DC preferentially trafficked 20nm (17.8±2.3%, p=0.0038) and 50nm particles (20.4±1.2%, p< 0.0001) compared to 1000nm particles (3.477±0.8%). AM ingested all sizes with a preference for, smaller particles (20nm: 88.0±1.8%, p=0.0001; 50nm: 8.6±0.5 79.5±1.2%, p<0.0001; 100nm: 69.2±2.1%, p=0.0041; 200nm: 61.5±2.1%, p=0.0361; 1000nm: 53.72±1.5%) 24h after exposure. Following uptake, DC up regulated expression of CD40 and CD86 independently of particle size. Confocal microscopy confirmed uptake and size distribution in AM and DC in situ.
Particle size is a key factor determining uptake and trafficking from the lung to LDLN. This has important consequences on downstream immunological effects of both ambient inhaled particles and novel carriers for pulmonary delivery of drugs or vaccines.

P3722
Airway region-specific effects of carbon black nanoparticles (CBNP)
Sandra Schlick, Heinz Fehrenbach. *Experimental Pneumology, Research Center, Borstel, Germany*

CBNP are present in industrially produced soot used for reinforcement of elastomers (e.g. in tyres) but also for paints, toner and batteries. CBNP may have lung cytotoxic and pro-inflammatory effects.
The commercially available CBNP Printex 90 (P90) and the quartz DQ12 were shown to possess cytotoxic and pro-inflammatory properties in mice and human epithelial cell lines. We sought to investigate whether these particles exert similar effects in distal versus proximal airway explants of mice.
The impact of CBNP on different airway regions was assessed by microdissection of proximal and distal airways from mouse lungs and ex vivo stimulation. Cytotoxicity was measured by LDH release and cytokine mRNA expression by qRT-PCR.
In contrast to previous findings in mice and epithelial cell lines our study could not show pro-inflammatory effects of P90 or DQ12 on microdissected airways. IL-1 β, KC and TNF α mRNA expression was unchanged. There was only a trend towards slightly upregulated IL-6 expression in proximal airways incubated with P90. Incubation with DQ12 did not result in increased cytotoxicity in distal and proximal airways which is also different from findings in cell culture. A slight increase in cytotoxicity was indicated with increasing P90 concentrations.
Although our results do not show significant cytotoxic or pro-inflammatory effects of P90 and DQ12 on proximal and distal airways, this does not exclude that oxidative stress, apoptosis or proliferation of airway cells are affected by P90 or DQ12. In the future, chemically modified CBNP will be analysed to reveal the impact of functional surface groups on the particle's toxicological properties.
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P3723
Targeting miRNA-based medicines to cystic fibrosis airway epithelial cells using nanotechnology
Paul J. McKiernan[1], Orla Cunningham[1,2], Noel G. McElvaney[1], Sally Ann Cryan[2], Catherine M. Greene[1]. *[1]Department Medicine, [2]School of Pharmacy, Royal College of Surgeons in Ireland, Dublin, Ireland*

Cystic Fibrosis (CF) is characterised by chronic pulmonary inflammation. microRNAs (miRs) are regulatory RNAs which inhibit gene expression. miR-126, a regulator of TOM1, is decreased in CF bronchial brushings; TOM1 is reciprocally increased. Polymeric nanoparticles of Polyethylenimine (PEI) and Chitosan-TPP can be used to deliver nucleic acids into bronchial epithelial cells. Here a proof-of-concept study was performed testing their efficacy at delivery of premiR-126 into non-CF and CF bronchial epithelial cells lines. PremiR-126-nanoparticles were prepared and characterized using a Zetasizer and used to transfect CFBE41o- and 16HBE14o- cell lines. RNA extraction was carried out 24h post transfection, cDNA was prepared and miR-126 and TOM1 expression was assessed using qRT-PCR. Toxicity was measured by high content analysis (HCA). Over-expression of miR-126 resulted in down-regulation of TOM1 in both cell types. The use of polymeric, cationic nanoparticles was shown to efficiently deliver premiR-126 into cells in order to achieve this knockdown. PremiR-126 encapsulated in PEI at a nanoparticle/premiR (N/P) ratio of 1:1 resulted in knockdown of TOM1 in CFBE41o- cells, with a reduction of ~47% in TOM1 expression in comparison to a scrambled negative control complexed with commercial transfection reagent ($p \leq 0.05$). HCA showed no significant difference in cell counts between untreated cells and cells treated with PEI- and chitosan-TPP-premiR-126 nanoparticles, suggesting they are relatively non-toxic. miRs are potential novel targets for respiratory gene therapy and could be targeted to CF bronchial epithelial cells in the lungs using PEI nanoparticles.
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P3724
Effect of anti-IgE therapy on microRNAs in the lungs of mice with allergen-driven airway remodeling
William Henderson[1], Jun Xue[1], Federico Farin[2], Richard Beyer[2], Theo Bammler[2]. *[1]Medicine, University of Washington, Seattle, WA, United States; [2]Environmental Health, University of Washington, Seattle, WA, United States*

Rationale: Current therapies have had limited effect on structural airway changes in patients with asthma. We recently found that anti-IgE therapy significantly decreased established airway hyperresponsiveness and subepithelial fibrosis in a mouse asthma model (Henderson et al., AJRCCM 183: A4066, 2011). MicroRNA (miRNA or miR)s, small non-coding RNAs, are key regulators of gene expression that may serve as novel biomarkers and therapeutic targets in disease states.
Study Aim: Determine the effect of anti-IgE therapy on miRNA expression in a mouse asthma model with airway remodeling.
Methods: Mice periodically given OVA (days 14-163) were treated with 100 μg monoclonal rat IgG1 anti-IgE (R35-92, Pharmingen) (OVA/anti-IgE group), rat IgG1 isotype control antibody (OVA/IgG1 group), or saline (OVA/Saline group) days 73-75, and then once weekly until day 163 when lung miRNA was isolated. MiRNA transcriptional profiling was carried out using Affymetrix GeneChip miR 2.0 arrays with data analysis by Bioconductor limma package. MiRNAs whose expression was changed >1.5-fold ($p<0.05$) were considered differentially expressed.
Results: 21 miRNAs were significantly changed in the OVA/Saline group vs saline-treated controls including upregulation of profibrotic miR-21 and miR-155. MiRs 467e, 511, and 744* were downregulated and the antifibrotic miR-16* upregulated in the OVA/anti-IgE group vs the OVA/IgG1 group.
Conclusions: The ameliorating effect of anti-IgE treatment on established airway remodeling in this asthma model is likely mediated by its differential effects on gene expression in the lung. Our data direct attention to key miRNAs that may serve as biomarkers for this remodeling process.

P3725
microRNA profiling of murine alveolar epithelial type II cells
Stefan Dehmel, Katharina Singer, Rabea Imker, Oliver Eickelberg, Melanie Koenigshoff, Susanne Krauss-Etschmann. *Comprehensive Pneumology Center, Klinikum der Universitaet Muenchen, Helmholtz Zentrum Muenchen, Germany*

Rationale: Alveolar epithelial type II cells (ATII) have key roles in innate immune response, surfactant production and as precursors for ATI cells (Mason RJ, Respirology, 2006). To obtain insight in gene regulatory networks specific for ATII we established a method for negative isolation of ATII cells and investigated their microRNA (miR) profiles.
Methods: Single cell suspensions were prepared from whole lungs of female C57BL/6 mice (6-12w) using dispase and mechanical dissociation. ATII were obtained by sorting (BD FACS Aria II) CD45-/CD31-/autofluorescence-high cells (sATII). Sorted cells were characterized by flow cytometry, immunocytochemistry (ICC) and RT-qPCR. MiR profiling was performed using TaqMan® Array Cards (ABI). MiR profiles of sATII were compared to cells obtained by panning (pATII) according to a standard method (Koenigshoff M *et al.*, JCI, 2009) using negative selection in antibody-coated (CD45, CD16/32) petri dishes.
Results: Up to 99% of sATII cells were CD45-/CD31-/CD74+. sATII expressed higher mRNA levels for SP-C while mRNAs for CD31, CD45, ZO-1 and α-SMA were expressed at lower levels compared to pATII. mRNA levels for CD74, AQP5 and SP-A were similar in both populations. ICC confirmed proSP-C expression on sATII. MiR profiling revealed 117 miRs expressed at equal abundance (-1.5x to 1.5x) in sATII and pATII. 128 miRs were up- (>1.5x) and 64 miRs were down-regulated (<-1.5x) in sATII compared to pATII. In addition, 3 miRs were only detectable in sATII while 14 miRs were unique to pATII.

Conclusion: This study presents novel data on microRNAs expressed in an important pulmonary cell type. Further analysis by *in silico* enrichment analysis may reveal ATII-specific mRNA targets and signalling networks.

P3726

The lung in a dish – A new tool to study interactions of inhaled (nano)materials with lung cells

Corinne Jud[1], Silvia Angeloni[2], Loretta Müller[3], Martha Liley[2], Alke Petri-Fink[1], Barbara Rothen-Rutishauser[1]. *[1]Adolphe Merkle Institute, University of Fribourg, Marly, Switzerland; [2]Centre Suisse d'Electronique et de Microtechnique, CSEM SA, Neuchâtel, Switzerland; [3]Center for Environmental Medicine, Asthma and Lung Biology, University of North Carolina, Chapel Hill, United States*

The human lung is exposed to a huge number of nanosized materials with each breath. To study the interaction of those materials with the lung, biomimetic cell culture models are needed.

The human air-blood tissue barrier consisting of epithelial cells, the basal lamina and endothelial cells is less than 1μm thick. Conventional cell culture inserts have a thickness of ~10μm which has been a major drawback to establish realistic models. Therefore, a novel ultrathin porous membrane with a thinness of 500nm was developed to refine an established human air-blood tissue barrier model consisting of epithelial cells (A549), monocyte-derived macrophages and dendritic cells by replacing the conventional inserts with the novel membranes but also complement it with endothelial cells (EA.hy926).

Epithelial cells seeded on the upper side of the membrane and endothelial cells on the lower side grow to confluence and form a tight bilayer. Immune fluorescence stainings revealed the typical characteristics of epithelial/endothelial cells and specific markers (von Willebrand factor and PECAM) were exclusively detected in the endothelial cell layer. Experiments with all four cell types have been performed and showed that all cell types can be cultured together as a quadruple model on the new ultrathin membrane.

Once fully established and characterized, this new system will offer an advanced 3D cell culture model of the human airway barrier and an excellent tool to study the effects of inhalable substances (e.g. nanomaterials) with a realistic lung model. This will significantly reduce the number of animals used to perform inhalation risk assessments and will offer a controlled system for different studies.