**333. Epithelial cell biology**

**P3080**
The bronchial epithelium promotes B cell survival and immunoglobulin production in a coculture system  
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**Background:** Chronic obstructive pulmonary disease (COPD) is associated with chronic airway inflammation and structural remodelling, in particular of the epithelium, which is impaired in its capacity to transport immunoglobulin (Ig) A. Although peribronchial lymphoid follicles have been described in severe COPD, it remains unknown whether B-cell conditioning (e.g. for IgA production) is altered in this disease.

**Objectives:** In this study, we report on preliminary data using a model of coculture of B cells with human primary bronchial epithelium (re)differentiated in vitro in air-liquid interface.

**Methods:** IgA synthesis was studied in CD19+ B cells (purified by immunomagnetic sorting from healthy blood donors) following co-culture for 13 days with a bronchial epithelium. B cells were also assessed by flow cytometry for cell activation and survival (annexinV/propidium iodine staining).

**Results:** In two independent experiments, we observed that Ig production was upregulated in B cells cocultured with the bronchial epithelium, as compared to B cells cultured alone (2.2 fold increase in IgG, and modest 1.4 fold increase in IgA), whereas IgM was decreased (0.8 fold). In contrast to expression of CD69, CD80 and CD86 which did not change, B-cell survival also increased (55.3% vs 20.8% for B cells alone) following epithelial coculture.

**Conclusion:** These preliminary data using this model of coculture, which should reveal useful to investigate crosstalks between the epithelium and B cells with respect to COPD, confirm that the airway epithelium provides B cells with signals promoting both survival and Ig synthesis.

**P3081**
DUOX1-mediated hydrogen peroxide release regulates sodium transport in bronchiolar epithelial cells  
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Clara-like H441 cells grown on non porous support form epithelial domes in the presence of dexamethasone. This reflects an increase in expression and activity of amiloride inhibitable epithelial sodium channels, ENaC. Dexamethasone has been also shown to facilitate the expression of NADPH oxidase DUOX1 in lung fetal cells. Dexamethasone has been also shown to facilitate the expression of NADPH oxidase DUOX1 in lung fetal cells. We assessed the role of DUOX1 and submillimolar (<0.3 mM) amounts of H2O2 on both ENaC expression and activity in H441 cells. Cells forming epithelial domes induced by dexamethasone (0.1 μM, 24h) and by another differentiation agent 5-aza-2'-deoxytidine (5AZA, 1 μM, 48h) expressed higher amounts of DUOX1 as evidenced by RT-PCR and immunocytochemistry. Dome induction by dexamethasone and 5AZA can be inhibited by exogenous catalase in concentration-dependent manner (5-10 kU/ml) and by the NADPH oxidase inhibitor diphenyl iodonium (2.5 μM), thus suggesting the involvement of H2O2. Single application of 0.2 mM H2O2 induced transient dome formation. Dexamethasone stimulated mRNA expression of all the subunits of ENaC, however this expression was not inhibited by catalase, thus suggesting that H2O2 increased the channel activity. In patch-clamp experiments, H2O2 (0.1 mM) activated amiloride-sensitive whole-cell currents from 3.91±0.79 pA/pF to 4.76±0.98 pA/pF (p=0.01) as well as ENaC single-channel activity in cell-attached patches. These observations suggest that tonic production of H2O2 by DUOX1 maintains the level of vectorial sodium transport by lung alveolar cells. They also suggest that the upregulation of DUOX-mediated H2O2 release observed in cystic fibrosis (CF) may contribute to the hyperabsorptive epithelial cell phenotype observed in CF lung disease.

**P3082**
Increased expression of α1-hydroxylase (CYP27B1) in IL-13-exposed primary bronchial epithelial cells (PBEC): Consequences for activation of vitamin D and expression of the antimicrobial peptide hCAP-18/LL-37  
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Vitamin D3 (25(OH)D3) increases expression of hCAP-18/LL-37 in PBEC. This requires conversion of 25(OH)D3 by 1α-hydroxylase (CYP27B1) into its active form.
P3084 Epithelial mesenchymal interactions in asthmatic children
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The alteration of the mesenchymal layer underlying the bronchial epithelium, triggered by epithelial dysfunction, is a topic of emerging interest in asthma. Whether this dysfunction is already present in asthmatic children, and therefore in the early stages of the disease, has been scarcely investigated. We evaluated the degree of epithelial damage, E-cadherin (E-cad) and TGFβ expression, and their relationship with biologic markers (hBD-2 and -3 and BM) in asthmatic children. Bronchial specimens were obtained from 27 children undergoing bronchoscopy for appropriate indications: 16 asthmatics (age 2-10 yrs) and 11 nonasthmatic controls (4-9 yrs). Epithelial damage and BM thickness were quantified by histomorphometry and E-cad and TGFβ by IF and Western blot for hCAP-18/LL-37.

Increased epithelial production of LPLUNC1 in cystic fibrosis lung disease
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Idiopathic Pulmonary Fibrosis (IPF) is an irreversible and progressive lung disease with limited life expectancy after diagnosis. The cause of the condition remains elusive but it has both familial and environmental associations. Histopathological studies of IPF lungs reveal the typical features of the so-called desquamative interstitial pneumonia (DIP) pattern with epithelial hyperplasia, areas of scarring with fibroblast foci and characteristic morphological abnormalities, including bronchiolization of alveolar ducts, cysts and alveoli. Although it seems likely that bronchiolar abnormalities are caused by changes in epithelial cell differentiation, specific markers of this process remain elusive. By analysis of published raw data sets from IPF patients, we identified LPLUNC1 as a potential candidate marker for the disease. This putative innate defence protein is normally expressed in submucosal glands (SMGs) and in a population of MUC5AC/positive goblet cells in the upper airways. Immunohistochemical analysis of lung tissue from patients with IPF revealed strong staining of LPLUNC1 within the bronchiolized epithelium lining the honeycomb cysts as well as in the mucousoblast filling these regions and dispersed throughout the peripheral lung. The related protein, SPLUNC1 was not expressed. LPLUNC1 was co-localized to the same cells as LPLUNC1, whereas MUC5AC was found in goblet cells within the airways. The same pattern of staining was not observed in other chronic lung diseases, suggesting a degree of specificity for IPF. Our data support the idea that in IPF, airway epithelial cells develop characteristics of SMGs and suggest that LPLUNC1 may be a useful marker for the disease.
cultures to LPS stimulation and any modulatory effects of exposure to cigarette smoke. Two of the three main proteasome activities, namely the chymotrypsin-like and caspase-like activity, showed a time and dose-dependent alteration in response to CSE. The altered proteasomal activity corresponded to accumulation of oxidatively modified and polyubiquitinated proteins. The effect of CSE on the ratio of LPS- and CSE-induced increase in proteasome activity was observed in both in vitro and in vivo settings. A neutralizing TLR4 antibody reduced the expression of BLT2 and PPARα expression. In conclusion, our findings suggest that, in bronchial epithelial cells, CSE promote a prevalent inductive action of proinflammatory BLT2 receptors and active mechanisms leading to increased neutrophil adhesion, a mechanism contributing to airway neutrophilia.

**P3089**

Cigarette smoke alters the expression of the pro-inflammatory LTβR receptor and increases the neutrophil adhesion in bronchial epithelial cells

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**Background:** Oxidative stress is a major risk factor for chronic obstructive pulmonary disease (COPD) and is involved in the pathogenesis of inflammatory lung diseases. Oxidative stress can lead to the formation of reactive oxygen species (ROS) that can cause damage to cellular proteins and DNA. In this study, we investigated the effects of cigarette smoke on the expression of the pro-inflammatory leukocyte beta receptor (LTβR) and its role in neutrophil adhesion in bronchial epithelial cells.

**Methods:** Primary bronchial epithelial cells (PBEC) were isolated from healthy donors and cultured in a defined medium. The cells were treated with cigarette smoke extract (CSE) and LPS to determine the effects of oxidative stress on the expression of LTβR and neutrophil adhesion.

**Results:** Treatment with CSE alone or combined with LPS resulted in a significant increase in LTβR expression compared to control samples. Neutrophil adhesion to CSE-treated cells was also increased compared to control cells. The effects of CSE were reversed by the addition of a TLR4 antagonist to the culture medium.

**Conclusions:** Our findings suggest that cigarette smoke exposure leads to an increase in LTβR expression and enhances neutrophil adhesion in bronchial epithelial cells, potentially contributing to inflammation in the airways.

**P3090**

Oxidative stress induces the generation of tissue factor bearing microparticles by airway epithelial cells in vitro

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**Background:** Oxidative stress is a major contributor to the pathogenesis of pulmonary fibrosis. It is known that exposure to cigarette smoke leads to increased oxidative stress in the lungs. In this study, we investigated the effects of cigarette smoke on the generation of procoagulant microparticles by airway epithelial cells.

**Methods:** Primary bronchial epithelial cells from healthy donors were cultured in a defined medium. The cells were treated with cigarette smoke extract (CSE) and LPS to determine the effects of oxidative stress on the generation of procoagulant microparticles. Microparticles were detected by flow cytometry and their procoagulant activity was assessed using a tissue factor (TF) assay.

**Results:** Treatment with CSE alone or combined with LPS resulted in a significant increase in the generation of procoagulant microparticles compared to control samples. The effects of CSE were reversed by the addition of a TLR4 antagonist to the culture medium.

**Conclusions:** Our findings suggest that cigarette smoke exposure leads to an increase in the generation of procoagulant microparticles by airway epithelial cells, potentially contributing to inflammation and coagulation in the airways.

**P3091**

Effect of cigarette smoke on protease function in alveolar epithelial cells

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**Background:** Oxidative stress is a major risk factor for chronic obstructive pulmonary disease (COPD), which accounts for several million deaths annually worldwide. The generation of reactive oxygen species (ROS) is considered to be the major contributor to smoke-induced inflammation, DNA damage and posttranslational modifications resulting in subsequent misfolding of cellular proteins. The ubiquitin proteasome system is essential for the turnover of the majority of cellular proteins. The proteasome also plays a vital role in protein quality control as it degrades abnormal and misfolded proteins, among them oxidatively-modified proteins. Conditions of massive oxidative stress, however, may result in impairment of protease function as ROS can directly modify the proteasome complex.

**Methods:** In a recent study, we have investigated the proteasome function in response to cigarette smoke extract (CSE) of human and mouse epithelial cells. Treatment of these cells with CSE resulted in a time and dose-dependent increase in ROS levels. The proteasome activities of the proteasome were measured using peptide substrates.

**Conclusions:** Our findings suggest that cigarette smoke exposure leads to an increase in ROS levels and impairment of proteasome function, potentially contributing to inflammation and tissue damage in the airways.

**P3092**

Variations in organic cation transporter (OCT) expression in bronchial epithelial cell layers after environmental insults

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**Background:** Organic cation transporters (OCTs) are transmembrane carriers that mediate the transport of endogenous amines in both the lung and liver. They are essential for the preservation of several organs and have been associated with chronic inflammatory disorders like Crohn’s disease and rheumatoid arthritis. They are also dysregulated in the lung of allergic rodents after an acute ovalbumin challenge (Lips, K.S., et al. Life Sciences, 2007; 80:2263-2269).

**Methods:** In this study, we investigated the proteasome function in response to cigarette smoke extract (CSE) of human and mouse epithelial cells. Treatment of these cells with CSE resulted in a time and dose-dependent increase in ROS levels. The proteasome activities of the proteasome were measured using peptide substrates.

**Conclusions:** Our findings suggest that cigarette smoke exposure leads to an increase in ROS levels and impairment of proteasome function, potentially contributing to inflammation and tissue damage in the airways.

**P3093**

Human 3D airway models to explore in vivo inhalation

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**Background:** Human 3D airway models are promising tools for safety and efficacy evaluation of compounds targeting the airways. The two most important reasons are (1) the models are fully differentiated and functional (incl. metabolism activity, mucous production and cilia beating) and (2) they are cultured at air-liquid interface, allowing exposure to gasses, vapours, aerosols and particles via air (relevant exposure). Healthy and diseased airway tissue (asthma, COPD) are available. Some models include cells from the upper respiratory tract (nasal tissues, larynx and trachea), where the highest impact of inhaled compounds is expected.

**Methods:** We used the 3D airway models, including MucAir™, for toxicity testing, e.g. the COMET assay, cell viability and tissue and cell membrane integrity. Some substances were applied in droplets, and a one-stage cloting assay, that investigates the procoagulant activity mediated by tissue factor (TF).

**Results:** Incubation of AS49 cells with H2O2 (100 μM) for 20 hours caused a significant increase in PS-containing MPs (73%±17% vs. 120±31% pM; mean±SEM; p<0.5), N-acetyl cysteine (1 μM) inhibited the effect (260±54% MP-associated TF activity was also increased (197±98 vs. 19±14 arbitrary U). In this study, our findings suggest that cigarette smoke exposure leads to an increase in ROS levels and impairment of proteasome function, potentially contributing to inflammation and tissue damage in the airways.

**Conclusions:** In conclusion, our findings suggest that cigarette smoke exposure leads to an increase in ROS levels and impairment of proteasome function, potentially contributing to inflammation and tissue damage in the airways.

**P3094**

Cigarette smoke is the major risk factor for chronic obstructive pulmonary disease (COPD), which accounts for several million deaths annually worldwide. The generation of reactive oxygen species (ROS) is considered to be the major contributor to smoke-induced inflammation, DNA damage and posttranslational modifications resulting in subsequent misfolding of cellular proteins. The ubiquitin proteasome system is essential for the turnover of the majority of cellular proteins. The proteasome also plays a vital role in protein quality control as it degrades abnormal and misfolded proteins, among them oxidatively-modified proteins. Conditions of massive oxidative stress, however, may result in impairment of protease function as ROS can directly modify the proteasome complex. In the present study, we have investigated the proteasome function in response to cigarette smoke extract (CSE) of human and mouse epithelial cells. Treatment of these cells with CSE resulted in a time and dose-dependent increase in ROS levels. The proteasome activities of the proteasome were measured using peptide substrates.

**Conclusions:** Our findings suggest that cigarette smoke exposure leads to an increase in ROS levels and impairment of proteasome function, potentially contributing to inflammation and tissue damage in the airways.

**P3095**

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POSTER DISCUSSION

**P3094**
Cigarette smoke induces the release of CXCL-8 from human bronchial epithelial cells via TLRs and the induction of inflammasome

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COPD is a chronic airway diseases associated with inflammation and cigarette smoking. Airway epithelial cells are the first cells that will be exposed to cigarette smoke and are able to release CXCL-8 and IL-8. These cytokines are involved in the acute and chronic character of inflammatory processes in COPD. The aim of this study was to investigate whether Toll Like Receptors (TLRs) on human bronchial epithelial cells (HBE-140 cells) were involved in cigarette smoke-induced cytokine production.

Cigarette smoke-induced CXCL-8 production was inhibited by an antibody against TLR4 and by inhibitory ODN without CpGODN motif suggesting the involvement of TLR4 and TLR9. In addition, exposure of HBE-140 cells to TLR4 or TLR9 ligands resulted in the release of CXCL8 and IL-1β. TLR4 and also TLR9 were present on the cell surface and the expression of both receptors decreased after cigarette smoke exposure. The molecular mechanism was further investigated.

It was found that the purinergic P2X7 receptors and reactive oxygen species were involved. Interestingly, the inflammasome activator monosodium urate crystals (MSU) mimicked the release of CXCL8 and IL-1β and the caspase-1 inhibitor Z-VADDCB suppressed the cigarette smoke-induced release of CXCL-8. In addition, cigarette smoke, CpGODN, LPS and MSU all increased the expression of caspase-1 and IL-1β. In conclusion, cigarette smoke releases CXCL-8 from HBE-140 cells via TLR4 and TLR9 and inflammasome activation. This signal transduction pathways may contribute to cigarette smoke related diseases such as COPD.

**P3095**
Effect of roflumilast N-oxide on non-typable haemophilus influenzae (NTHI) interaction with human airway epithelial A459 cells

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Background/Aim: NTHI is associated with lung colonisation and exacerbations in COPD. Previously we found that NTHI invasion of airway epithelial cells is PI-3 kinase (PI3K)-dependent. Furthermore NTHI survives intracellularly in vacuoles with late endosome features, explaining airway colonisation despite NTHI-negative sputum. Here we explored whether the PDE4 inhibitor roflumilast N-oxide (RNO), the active metabolite of roflumilast (approved in EU for severe COPD), dexamethasone (DEX) and salmeterol (SAL) affect NTHI invasion of A549 cells and NTHI-induced IL-8 release.

Methods: For invasion, cells were infected with NTHI (100 bacteria/cell, 2h), incubated with gentamycin (1h) to kill extracellular bacteria, and lysed to determine intracellular bacteria by plating. Intracellular lifestyle was monitored by immunofluorescence with antibodies to late endosome markers (lamp1, CD63). PI3K-dependent Akt phosphorylation was assessed by Western. IL-8 was measured by ELISA.

Results: RNO (1µM) reduced NTHI invasion by 50% (c.f.u./well; Control: 5000±1000, RNO: 2439±122, n=3, p<0.05) while DEX (1µM) and SAL (1µM) failed. Forskolin (10µM) reduced NTHI invasion by 70%, supporting a role of cAMP. RNO (1µM) curbed NTHI-induced Akt phosphorylation by 40%. Neither drug affected NTHI intracellular lifestyle: RNO (1µM) or DEX (1µM) reversed a 5.5-fold increase in NTHI-induced IL-8 release by 48±3% and 76±3% respectively and NF-κB activation.

Conclusions: PDE4 inhibition (1µM RNO) reduced NTHI internalisation in A549 cells by interfering with PI3K signalling. RNO inhibited IL-8 release. DEX, while reducing NTHI-induced IL-8, did not affect internalisation.

**P3096**
Glucocorticoids enhance CCL20 release in bronchial epithelial cells in a metalloprotease-dependent manner

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Glucocorticoid (GC) insensitivity is a major clinical problem in the management of asthma. Although a potential role for Th17 cells has been described in GC insensitive asthma, it is still unknown why GCs are unable to efficiently suppress Th17-mediated inflammation. CCL20 acts as a potent chemotractant for Th17 cells. To determine the effect of GCs on CCL20 secretion and to unravel the underlying regulatory mechanisms, we examined the effect of budesonide and fluticasone (10−7-10−15M) on TNF α-induced CCL20 and IL-8 production (ELISA and qPCR) in 16HBE human bronchial epithelial cells and primary bronchial epithelial cells (Human epithelial cells). We screened specific inhibitors for the ERK, p38, STAT3 and PI3K pathways, the GC receptor (GR), protein synthesis and a general metalloprotease inhibitor.

Surprisingly, we observed that GCs do not suppress, but enhance the release of CCL20 in 16HBE cells, under conditions where IL-8 was efficiently suppressed. Importantly, GCs also induced a substantial increase in the TNF α-induced release of CCL20 in asthma epithelium. Although the TNF α-induced CCL20 release was dependent on the ERK, p38 and STAT3 pathways, the upregulation by GCs was not blocked by their inhibition. Furthermore, our data demonstrate that the effect of GC is mediated by GR activation, which is likely mediated at posttranslational level by a metalloprotease-dependent manner.

Thus, we show for the first time that GCs enhance metalloprotease-dependent release of CCL20, which may constitute a novel mechanism of Th17-mediated GC insensitive neutrophilic airway inflammation in asthma and provide new opportunities for therapeutic intervention.

**P3097**
Effects of JAK-STAT inhibitors on glucocorticosteroid resistant release of CXC chemokines from human bronchial epithelial cells

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COPD is associated with glucocorticosteroid insensitivity inflammation. CXC chemokines are elevated in COPD and may drive recruitment of CD8+ lymphocytes. Expression of CXC chemokines is regulated by IFNγ. This study investigated the effect of JAK inhibitors PF95 and PF13 on CXC chemokine production from human bronchial epithelial cells. Beas-2B and primary human bronchial epithelial cells (HBEC) were pre-treated with PF95, PF13, or Dexamethasone (DEX) then stimulated with IFNγ or IFNγ+TNFα. After 24h, cytokines were measured by ELISA. Inhibition of the JAK-STAT pathway was measured by immunoblotting for phosphorylated and total STAT and STAT DNA binding using Trans-AM kits.

Dex had no effect on the release of CXC9, 10 or 11, however all were inhibited by the JAK-STAT inhibitors stimulated with either IFNγ or IFNγ+TNFα (Table 1). HBEC responded similarly to Beas-2B. Both inhibitors attenuated phosphorylation of STAT1 in a concentration dependent manner. DNA binding of STAT1 and STAT3 was inhibited but not of STAT5α or STAT5β.

Table 1. EC50 values for PF13 (nM) and PF95 (µM) on CXC chemokine release from bronchial epithelial cells. Mean ± SEM, n=4

<table>
<thead>
<tr>
<th>CXC</th>
<th>IFNγ</th>
<th>IFNγ+TNFα</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXC9</td>
<td>PF13</td>
<td>6.7±1.8</td>
</tr>
<tr>
<td>DMSO</td>
<td>PF13</td>
<td>2.1±1.5</td>
</tr>
<tr>
<td>CXC10</td>
<td>PF13</td>
<td>9.5±4.2</td>
</tr>
<tr>
<td>DMSO</td>
<td>PF13</td>
<td>5.5±0.4</td>
</tr>
<tr>
<td>CXC11</td>
<td>PF13</td>
<td>13.4±3.2</td>
</tr>
<tr>
<td>DMSO</td>
<td>PF13</td>
<td>0.6±0.2</td>
</tr>
</tbody>
</table>

JAK-STAT inhibitors therefore attenuate release of CXC chemokines from airway epithelial cells under conditions of glucocorticosteroid insensitivity and have potential as a new anti-inflammatory treatment in COPD patients.