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. We assessed the role of DUOX1 and submillimolar (<0.3 mM) amounts of H_2O_2 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 H_2O_2. Single application of 0.2 mM H_2O_2 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 H_2O_2 increased only the channel activity. In patch-clamp experiments, H_2O_2 (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 H_2O_2 by DUOX1 maintains the level of vectoral sodium transport by lung alveolar cells. They also suggest that the upregulation of DUOX-mediated H_2O_2 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)D_3) increases expression of hCAP-18/LL-37 in PBEC. This requires conversion of 25(OH)D3 by 1α-hydroxylase (CYP27B1) into its active

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metabolite 1,25$(OH)_2D_3$, that increases expression of both hCAP-18/LL-37 and the vitamin D degrading enzyme (CYP24A1). IL-13 is highly expressed in the lungs of asthmatics and low serum 25$(OH)D_3$ levels were associated with lower lung function in asthmatics.
The aim of the present study was to investigate the role of IL-13 in vitamin D metabolism and to assess its effect on expression of hCAP-18/LL-37.
Well-differentiated PBEC cultured at the air-liquid interface from 5 different donors were pre-incubated with IL-13 for 24 hours and subsequently exposed to 25$(OH)D_3$ for 48 hours to investigate CYP27B1, CYP24A1, vitamin D receptor (VDR), human β-defensin (hBD)-2 and -3 by qPCR. hCAP-18/LL-37 expression was determined by qPCR, immune fluorescence (IF) and Western blot.
Exposure of PBEC to IL-13 alone increased CYP27B1 and hBD-3 mRNA (fold increases of 5.2 and 3.13 resp.; $p<0.05$), whereas addition of 25$(OH)D_3$ to IL-13 treated cells resulted in higher expression ($p<0.05$) of hCAP-18/LL-37 and CYP24A1 mRNA (fold increase: 72.18 and 29.9, resp.) when compared to 25$(OH)D_3$ alone (fold increase: 32.6 and 13.9, resp.). These results were confirmed by IF and Western blot for hCAP-18/LL-37.
The present results show that IL-13 increases vitamin D metabolism in bronchial epithelial cells resulting in increased expression of hCAP-18/LL-37. Whether IL-13 contributes to host defence against infection through this mechanism requires further studies.

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 TGFβ1 expression, and their relation with basement membrane (BM) thickness in asthmatic children. Bronchial biopsies were obtained from 27 children undergoing bronchoscopy for appropriate indications: 16 asthmatics (age2-10yrs) and 11 nonasthmatic controls (4-9yrs). Epithelial damage and BM thickness were quantified by histochemistry and E-cad and TGFβ1 by immunohistochemistry. Asthmatic children had evidence of epithelial damage compared to controls (median;range:52;20-91 vs 33;0-83% $p<0.05$), and E-cad, evaluated by a semiquantitative score was increased in areas of damaged epithelium, but not in those of intact epithelium (61;33-100 vs 39;28-52% $p<0.05$). Furthermore, asthmatic children had increased epithelial expression of TGFβ1 (10;0-70 vs 1;0-13cell/mm p=0.01) and a thickened BM compared to controls (5;4-12 vs 4;2-5μm p=0.003). Of interest the expression of E-cad in damaged epithelium was related to both TGFβ1 expression (r=0.56,p=0.003) and BM thickening (r=0.42,p=0.04). In conclusion this study shows that, in children with asthma, damaged epithelial cells may promote a fibrotic reaction and thickening of the underlying mesenchymal layer through the expression of stress signals (E-cadherin), and growth factors (TGFβ). Epithelial damage is therefore an early event in the natural history of the disease which may profoundly affect airway remodeling.

P3085

Increased expression of IL-19 in metaplastic epithelium of patients with chronic rhinosinusitis and nasal polyps

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Chronic rhinosinusitis (CRS) (including nasal polyps) is an inflammation of the nose and of the paranasal sinuses. Nasal polyposis (NP) is common in many patients with severe asthma. The pathogenesis of NP is poorly understood.
The aim of the present study was to identify biomarkers for NP. We collected nasal biopsies from normal subjects without CRS (n=12), from subjects with CRS but without NP (n=10) and from patients with CRS and NP (n=10). We used Human Asthma Gene Array and real time PCR to evaluate gene expression and western blot analysis and immunohistochemistry for protein expression.
Human Asthma Gene Array showed an evident increase in IL-19 gene expression in NP from patients with CRS and NP in comparison to mucosa from inferior turbinate of normal subjects. Real time PCR confirmed the IL-19 mRNA up-regulation in patients with CRS and NP and showed an up-regulation of IL-19, at lower extent, also in the mucosa from inferior turbinate of patients with CRS in comparison to normal subjects. Western blot analysis confirmed that IL-19 is increased also at protein level in patients with CRS and NP in comparison to normal subjects. Immunohistochemistry showed that in NP IL-19 is highly expressed in the metaplastic nasal epithelium (score 4-9) when compared to normal or hyperplastic epithelium (score 1-3). When patients with CRS and NP were classified on the basis of the presence or of the absence of allergy, no differences were observed in terms of IL-19 expression.
In conclusion, the results of the present study provide compelling evidence on the putative use of IL-19 as remodelling biomarker for identifying patients with chronic rhinosinusitis and NP.

P3086

Increased epithelial production of LPLUNC1 in cystic fibrosis lung disease

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Members of the PLUNC family of secreted proteins have been implicated in innate defence of the upper airways and nasopharynx. Although array data suggest that they are differentially expressed in some lung diseases validation has not been forthcoming. We previously showed that SPLUNC1 is increased in severe cystic fibrosis (CF). In the present study we have investigated the expression of LPLUNC1 in severe CF and studied both proteins in mouse models of CF lung disease. There was marked epithelial staining of LPLUNC1 in diseased small airways and submucosal glands. in CF, similar to our previous data with SPLUNC1. The two proteins are not co-expressed in the CF lung as LPLUNC1 is co-localised with MUC5A/C in goblet cells, whereas SPLUNC1 is present in a non-ciliated, non-goblet cell population. Expression of both proteins was unchanged (and very limited), in CFTR knockout mice. However, in CCSP-betaENaC transgenic mice, a model for CF lung disease, there was very strong staining of both proteins in the airways and in the luminal contents. This was most marked for lplunc1 and was noted within 2 weeks of birth. As in CF, the two proteins are present in distinct cells within the epithelium. Lplunc1 was absent from the lavage fluid of non-transgenics, in keeping with the lack of goblet cells, but was readily detected in transgenics. Our results suggest that alterations in expression of these putative innate immune molecules is associated with CF lung disease in both humans and mice. It remains unclear if this elevation of protein production, which results from phenotypic alteration of the cells within the diseased epithelium, plays a role in the pathogenesis of the disease.

P3087

Co-expression of LPLUNC1 and MUC5B in the bronchiolarized epithelium of usual interstitial pneumonia

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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 "Usual Interstitial Pneumonia" (UIP) 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 array 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 MUC5A/C positive goblet cells in the upper airways. Immunohistochemical analysis of lung tissue from patients with UIP revealed strong staining of LPLUNC1 within the bronchiolarized epithelium lining the honeycomb cysts as well as in the mucosubstance filling these regions and dispersed throughout the peripheral lung. The related protein, SPLUNC1 was not co-expressed. MUC5B was localized to the same cells as LPLUNC1, whereas MUC5A/C was found in goblet cells within the airways. The same pattern of staining was not seen in other chronic lung diseases, suggesting a degree of specificity for IPF. Our data support the idea that in UIP, airway epithelial cells develop characteristics of SMGs and suggest that LPLUNC1 may be a useful marker for the disease.

P3088

LSC 2011 Abstract: Differential inflammatory responses of nasal and bronchial epithelial cells to cigarette smoke extract

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Few studies compare the function of primary bronchial (PBEC) and nasal (PNEC) epithelial cells. Our aim was to compare the responses of paired PNEC and PBEC

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cultures to LPS stimulation and any modulatory effects of exposure to cigarette smoke extract (CSE).
Cells, from subjects with COPD, were obtained by nasal or bronchial brushing and used at passage 3. They were stimulated for 24 h with LPS [0–25 μg/ml] ± pre-treatment with CSE. CSE was prepared by combusting a 12 mg tar Marlboro cigarette through 25 ml of media. Supernatants were collected and IL-8 and IL-6 measured by ELISA. The localization of TLR-4 was established by FACS.
For the PNEC cultures, a brief incubation with CSE (4h) significantly inhibited LPS-induced IL-6 and IL-8 release (IL-8: 24h treatment with 25 μg/ml LPS alone 5457±424 pg/ml and with 4h CSE pre-treatment 3772±432 pg/ml). A more prolonged incubation with CSE (24h) was pro-inflammatory (IL-8: 25 μg/ml LPS alone 5485±562 pg/ml and with 24h CSE pre-treatment 7757±449 pg/ml). Although a brief incubation with CSE resulted in a lower percentage of surface and intracellular TLR4, a prolonged incubation was without effect. In contrast, both a brief and a prolonged exposure of PBEC cultures to CSE reduced LPS induced IL-8 release (IL-8: 24h treatment with 25 μg/ml LPS alone 5107±797 pg/ml, with 4h CSE pre-treatment 3345±650 pg/ml, and with 24h CSE pre-treatment 3010±328 pg/ml), and both lead to a reduced percentage of surface and intracellular TLR4. There was minimal IL-6 release from the PBEC cultures.
In conclusion, our data indicate that PNEC cultures are not a suitable surrogate for PBEC cultures in terms of their response to CSE/LPS combination treatment.

P3089

Cigarette smoke alters the expression of the pro-inflammatory LTB4 receptor and increases the neutrophil adhesion in bronchial epithelial cells

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LTB4 is importantly involved in the inflammatory responses of chronic obstructive pulmonary disease (COPD). In COPD an increased expression of LTB4 receptors, BLT1 and of PPAR-a, was observed. Since LTB4 is reduced upon TLR4 mutation and since cigarette smoke extracts (CSE) increase the expression of TLR4 in bronchial epithelial cells, the aims of this study were to explore whether CSE with/without LPS (a ligand of TLR4) or mini-BAL supernatants from smokers alter, in bronchial epithelial cells, the expression (evaluated by flow-cytometry analysis) of pro (BLT2) and anti-inflammatory (PPAR-a) LTB4 receptors. Moreover, we evaluated the effects of CSE on the expression of ICAM-1 (by flow-cytometry analysis), on the binding of STAT-1 to ICAM-1 promoter (by ChiP analysis), and on the adhesiveness of bronchial epithelial cells to neutrophils (by fluorimetry).
CSE alone increased BLT2 while decreased PPARa expression. The addition of LPS did not modify CSE effects. mini-BAL from smokers increased BLT2 but not PPARa expression. A neutralizing TLR4 antibody reduced the expression of BLT2 but it had no effects in PPAR-a expression. CSE increased the binding of STAT-1 to ICAM-1 promoter and increased the expression of ICAM-1 in bronchial epithelial cells. CSE and mini-BAL from smokers increased the adhesiveness of bronchial epithelial cells toward neutrophils more than mini-BAL from non-smokers. These findings suggest that, in bronchial epithelial cells, CSE promote a prevalent induction of pro-inflammatory BLT2 receptors and activate mechanisms leading to increase neutrophil adhesion, a mechanism contributing to airway neutrophilia.

P3090

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

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Background: Activation of the coagulation cascade is involved in the pathogenesis of pulmonary fibrosis. Although, according to the traditional view, coagulation factors present in the lung are derived from the general circulation, recent data indicate that factor (F) X is locally increased in fibrotic lung tissue in its active form (FXa), where it is capable of signaling a fibrotic response via proteinase activated receptor1 (PAR1) (Scotton, 2009). While oxidative stress has been shown to induce FX synthesis by A549 alveolar cells, the mechanisms of FX activation are not clear. Microparticles (MP) are procoagulant and proinflammatory vesicles shed by eukariotic cells. It is not known whether alveolar cells generate microparticles capable of FX activation.
Aim: To investigate whether oxidative stress can also induce the generation of procoagulant microparticles by A549 cells.
Methods: The generation of MP by A549 was investigated through a prothrombinase assay, that measures the concentration of negative charges on the cell membrane, expressed as phosphatidylserine (PS) concentration, and a one-stage clotting assay, that investigates the procoagulant activity mediated by tissue factor (TF).
Results: Incubation of A549 cells with H_2O_2 (100 μM) for 20 hours caused a significant increase in PS-containing MP (731±173 vs. 170±31 pM; mean±SEM; $p<.05$); N acetyl cysteine (1 μM) inhibited the effect (260±54 pM). MP-associated TF activity was also increased (197±98 vs. 19±14 arbitrary U).
Conclusions: Oxidative stress induces the generation of functionally active, MP-associated TF by A549 alveolar cells, demonstrating a possible mechanism of local FX activation.

P3091

Effect of cigarette smoke on proteasome function in alveolar epithelial cells

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Cigarette smoke is the major risk factor for chronic obstructive pulmonary disease (COPD), which accounts for several million deaths annually world-wide. The generation of reactive oxygen species (ROS) is considered to be the major contribution 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 proteasome function as ROS can directly modify the proteasome complex. In the present study, we have investigated 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 proteolytic activities of the proteasome were assessed using peptide substrates. 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 treatment. The altered proteasomal activity corresponded to accumulation of oxidatively modified and polyubiquitinated proteins. The effect of CSE on the ratio and composition of 26S and 20S proteasomes was analysed by native gel electrophoresis, immunoprecipitation and subsequent mass-spec analysis. Preliminary data reveal posttranslational modifications of proteasomal subunits which may be responsible for the alteration of proteasome activity.

P3092

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

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OCTs are transmembrane carriers that mediate the transport of endogenous amines in both directions. 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). The airway epithelium is the primary protection barrier in lung defence and its role in the pathogenesis of chronic inflammatory respiratory diseases is becoming evident. Variations in OCT expression in response to environmental insults were quantified in bronchial epithelial cell layers in vitro. Layers of the human cell line Calu-3 and normal human bronchial epithelial (NHBE) cells showed similar OCT gene expression pattern after differentiation at an air-liquid interface for 21days. Calu-3 layers were exposed to the aeroallergen house dust mite or they were physically injured in a scrape wound model. Cells were harvested after recovery for real-time PCR analysis. Significant fold-increases in the expression of OCT1, OCT3, OCTN2 and the pro-inflammatory markers CCL17 and COX-2 were measured. "In-cell" western blotting confirmed OCT up-regulation at the protein level. It was shown OCT expression in bronchial epithelial cells is enhanced after allergen abuse and physical damage. Although further investigation into OCT functions in bronchial epithelial cells is needed, this study suggests a possible role of the transporters in the protection and regeneration of the bronchial epithelium.

P3093

Human 3D airway models to explore *in vivo* inhalation

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Human 3D airway models are promising models 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, mucus 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.
We explored the use of 3D airway models, including MucilAir™, for toxicity testing, e.g. the COMET assay, cell viability and tissue and cell membrane integrity. Substances were applied to the models in droplets. Tissue and membrane integrity, cell viability and ciliary beating were affected by exposure to chemical compounds. A dose-related response was found in the COMET assay with methyl methane sulfonate and we found a low and reproducible background level. Exposure of the MucilAir™ model to benzo[a]pyrene, silicon dioxide or ceriumdioxide nanoparticles did not show an increase in COMET formation. For benzo[a]pyrene, this is likely to be related to the exposure concentration and duration. Our preliminary results indicate that the MucilAir™ model may be a suitable model for safety testing, including genotoxicity and acute toxicity. In the future, we will further

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assess the applicability of these models for safety and efficacy testing by exposure through the air and comparison with *in vivo* inhalation data. Ultimately, these models may be useful in the safety evaluation of pharmaceuticals and chemicals.

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-1β. 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-14o cells) were involved in cigarette smoke-induced cytokine production.
The 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-14o cells to TLR4 or TLR9 ligands resulted in the release of CXCL8 and IL1β. 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 CXCL-8 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-14o cells via TLR4 and TLR9 and inflammasome activation. This signal transductions 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 A549 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 PI3-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±100, 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 6.5-fold increase in NTHi-induced IL-8 release by 48±3% and 75±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 chemoattractant 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^{-10}M) on TNF-α-induced CCL20 and IL-8 production (ELISA and qPCR) in 16HBE human bronchial epithelial cells and primary bronchial asthma epithelium. We used 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 by 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 in 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.

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Effects of JAK-STAT inhibitors on glucocorticosteroid resistant release of CXCR3 chemokines from human bronchial epithelial cells

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COPD is associated with glucocorticosteroid insensitive inflammation. CXCR3 chemokines are elevated in COPD and may drive recruitment of $CD8^+$ lymphocytes. Expression of CXCR3 chemokines is regulated by IFNγ. This study investigated the effect of JAK-STAT inhibitors PF95 and PF13 on CXCR3 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-1 and STAT DNA binding using Trans-AM kits.
DEX had no effect on the release of CXCL9, 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 STAT-1 in a concentration dependent manner. DNA binding of STAT-1 and STAT-3was inhibited but not of STAT5a or STAT5b.

Table 1. EC_{50} values for PF13 (nM) and PF95 (μM) on CXCR3 chemokine release from bronchial epithelial cells. Mean ± SEM, n=4

	Drug	Beas-2B		Primary	
		IFNγ	IFNγ+TNFα	IFNγ	IFNγ+TNFα
CXCL9	PF13	6.7±1.8	9.8±2.5	8.2±2.9	18.7.±6.2
	PF95	2.1±1.5	0.4±0.2	0.4±0.1	0.8±0.3
CXCL10	PF13	9.5±4.2	44±9.1	14.6±3.1	28.8±6.4
	PF95	0.5±0.4	1.3±0.4	1.0±0.2	2.2±0.8
CXCL11	PF13	13.4±3.2	11.9±3.6	5.0.±1.3	10.8±4.2
	PF95	0.6±0.2	0.7±0.2	0.5±0.2	0.7±0.2

JAK-STAT inhibitors therefore attenuate release of CXCR3 chemokines from airway epithelial cells under conditions of glucocorticosteroid insensitivity and have potential as a new anti-inflammatory treatment in COPD patients.

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