157. Epithelial cells: role in health and disease

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Late-breaking abstract: Activation of the macrophage inflammasome by TLR3 ligation potentiates release of IL-8 and IP-10 from alveolar type II epithelial cells via a paracrine cytokine network

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Recognition of microbial ligands by Toll-like receptors is central to the innate immune response of the peripheral lung to infection.
This study investigated the effect of TLR3 ligation on cytokine release by primary human alveolar type II epithelial (ATII) cells and macrophages (AMs) alone and in co-culture. We hypothesised that TLR3 ligation elicits a distinct cytokine profile in both cell types which is enhanced by co-culture.
Monocultures and co-cultures of ATII cells and AMs were were exposed to the TLR3 ligand PolyI:C for 24h. Both cells released IL-8 and IP-10 in response to PolyI:C; co-culture significantly potentiated their release 4- and 5.7-fold respectively ($P<0.0001$). The potentiation was not ATII:AM cell contact dependent; addition of Poly I:C treated AM conditioned medium to ATII monocultures maintained the response whereas conditioned medium from ATII cells did not induce potentiated release from AMs. To elucidate which macrophage-derived cytokines were responsible for the potentiated response, neutralising antibodies were added to conditioned medium from AMs prior to incubation with ATII monocultures. Results demonstrated that neutralization of inflammasome-related cytokines, IL-1β and IL-18, significantly inhibited release of IL-8 and IP-10 (Table1).

Inhibition of cytokine release from ATII cells

Neutralising Ab	Inhibition of IL-8	Inhibition of IP-10
IL-1β	20% ($P<0.01$)	ns
IL-18	47% ($P<0.0001$)	40.3% ($P<0.0001$)

In conclusion, our study demonstrates that TLR3 ligands activate the AM inflammasome, inducing release of IL-1β and IL-18 which potentiates the innate immune response of the alveolar epithelium.

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Compressive forces stimulate release of TGFβ2 from differentiated bronchial epithelial cell cultures from asthmatic but not normal donors

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Bronchoconstriction induces mechanical stress in the airways and normal bronchial epithelial cells respond by inducing profibrogenic responses. No studies have examined whether these responses are similar in asthmatic and non asthmatic populations. We tested the hypothesis that primary bronchial epithelial cells from asthmatic and non asthmatic donors respond similarly to mechanical stress mimicking bronchoconstriction by release of TGFβ2.
Methods: Bronchial epithelial cells from healthy (n=9) and asthmatic (n=9) volunteers were obtained at bronchoscopy, expanded and grown at an air liquid interface (ALI). These were apically compressed with 5%CO_2 in air (30cm water pressure) for 1 hour, or sham compressed. 24 hours later total TGFβ2 was measured by ELISA in the basolateral medium.
Results: After sham compression, TGFβ2 release was similar in ALI cultures from healthy (median (IQR) 203.4 (143.8-271.7) pg/ml) or asthmatic (245.5 (162.3-276.6)) donors (p=0.67). After active compression, TGFβ2 significantly increased in the asthmatic ALI culture medium (median (IQR) of 299.4 (205.4-365.0)) (p=0.02) but there was no increase in healthy cultures (median (IQR) 254.0 (137.7-281.7)) (p=0.68). This change amounted to a median (IQR) difference of 65.7 (24.5-110.7) pg/ml in the asthmatic group and 11.6 (-35.6-48.7) in the healthy group (p=0.04).
Conclusions: Bronchial epithelial cells from asthmatic patients respond differently from normal cells to compressive force in vitro that mimics bronchoconstriction. This new finding suggests that there are at least two abnormalities in the asthmatic airway; the initial bronchoconstriction, and the epithelial response to it.

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SIRT6-induced activation of autophagy inhibits CSE-induced bronchial epithelial cell senescence

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Introduction: Senescence has been implicated in the pathogenesis of COPD, and tobacco smoke is known to induce cellular senescence. SIRT6, a class III histone deacetylase (HDAC), has been demonstrated to potentially antagonize cellular senescence. Autophagy, a lysosomal degradation pathway, is associated with celluar senescence.
Aim: To elucidate the regulatory role for SIRT6 in autophagy activation in terms of tobacco smoke-induced cellular senescence
Methods: Primary HBEC were used for the experiments. Senescence associated beta-galactosidase (SA-b-gal) staining and western blotting of p21 were performed to evaluate cellular senescence. SIRT6 expression vector and siRNA were transfected into HBEC. To characterize autophagy, fluorescence microscopic detection of LC3-EGFP dot formation and western blotting for LC3, p62 were performed.
Results: CSE-induced cellular senescence was inhibited by SIRT6 overexpression, while SIRT6 knock down increased the percentage of SA-b-gal positive cells. SIRT6 overexpression increased autophagy activation as shown by formation of LC3-EGFP dot and increased conversion from LC3-I to –II at basal level after CSE treatment. In contrast, SIRT6 knock down repressed autophagy activation. Furthermore, SIRT6-induced autophagy suppressed the accumulation of p62 expression after CSE treatment. SIRT6-induced autophagy regulated CSE-induced cellular senescence, because autophagy inhibition by knock down of LC3 diminished anti-senescent effect of SIRT6 overexpression.
Conclusion: These findings suggest the pivotal regulatory role of SIRT6 in autophagy activation and CSE-induced cellular senescence. SIRT6 might be a target molecule for the treatment of COPD.

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A three miRNA signature regulates the CF transmembrane conductance regulator (CFTR) in cystic fibrosis airway epithelium

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Expression profiling studies have identified altered miRNA patterns in several human diseases, however little is known of the role miRNA play in cystic fibrosis (CF). Here we examined the impact of altered miRNA expression in CF bronchial epithelium on CFTR expression.
We performed miRNA expression profiling on bronchial brushings taken from five CF and five non-CF individuals by qRT-PCR using Taqman Low Density Arrays (TLDAs). Expression of altered miRNA was validated by qRT-PCR in additional brushings and *in vitro* in CF and non-CF bronchial epithelial cell lines. CFTR gene expression was also measured. miRNA inhibition and over-expression studies were performed in CFBE41o– and 16HBE14o– cell lines respectively *in vitro* and CFTR mRNA and protein was detected by qRT-PCR and western blot.
Of the 667 miRNA examined 56 were down-regulated and 36 up-regulated in CF. Using *in silico* analysis we identified miRNAs predicted to target the CFTR gene that were collectively up-regulated in the CF samples e.g. a three miRNA signature consisting of miR-145, miR-223 and miR-494. Knockdown of miR145, -223 and -494 in CFBE41o– cells led to an increase in CFTR protein production. Conversely a decrease in CFTR expression was observed following miRNA over-expression in 16HBE14o– cells. CFTR mRNA expression was significantly decreased in CF vs. non-CF bronchial brushings.
These data show that miRNA are differentially expressed in the CF airway epithelium, miRNA targeting CFTR are up-regulated and CFTR is reciprocally down-regulated. The regulation of CFTR by miRNA may represent an additional endogenous level of control over mutant CFTR in classes I, II or V CFTR mutations.

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IL-17A induces glucocorticoid insensitivity in airway epithelial cells

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Glucocorticoids (GC) are the cornerstone of asthma treatment. However, a subset of asthma patients is insensitive, which is a problem in the management of asthma. Previous studies suggest that GC-insensitivity is associated with Th17 cells. Th17

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cells act by producing inflammatory cytokines, including IL-17A. We aimed to assess whether IL-17A reduces GC sensitivity in airway epithelial cells and to elucidate the underlying mechanism.
We investigated the effect of IL-17A on the suppressive effect of budesonide (BUD) (10^{-10}-10^{-9}M) on TNF-α-induced IL-8 secretion and on Histone Deacetylase (HDAC) activity in the human bronchial epithelial cell line 16HBE with/without specific inhibitors for the ERK, p38 and PI3K pathways and upon overexpression of HDAC2.
We observed that IL-17A-induced IL-8 secretion is normally sensitive to GC in 16HBE, in contrast pre-treatment with IL-17A (2h) significantly reduces the sensitivity of TNF-α-induced IL-8 secretion to BUD. Immunodetection revealed that IL-17A activates the p38, ERK and PI3K pathways, but only inhibition of PI3K signaling reversed this GC-insensitivity. Our data suggest that IL-17A-induced GC-insensitivity is mediated by a reduction in HDAC2 activity, as IL-17A reduced HDAC activity, while overexpression of HDAC2 reversed IL-17A-induced GC-insensitivity. In contrast, IL-17A did not affect BUD-induced transcriptional activity, suggesting that IL-17A does not impair translocation of the ligated GC receptor. In conclusion, we show that IL-17A induces GC-insensitivity in bronchial epithelium which likely involves PI3K-dependent reduction in HDAC2 activity. IL-17A-induced PI3K signaling may thus serve as a new target for therapeutic intervention in GC-insensitive asthma.

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Corticosteroid-dependent transcription is reduced by inflammatory stimuli in human airway epithelial cells: Rescue by long-acting β_2-adrenoceptor agonists

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Rationale: Inhaled corticosteroids (glucocorticoids) are the most effective treatment for inflammatory diseases such as asthma. However, in some patients with severe disease, or who smoke, or suffer from COPD, these drugs are less effective. While many investigators focus on the repression of inflammatory gene expression, corticosteroids also induce the expression (transactivation) of numerous genes to elicit anti-inflammatory effects.
Results: Using human bronchial airway epithelial, BEAS-2B, and pulmonary, A549, cells, we show that tumour necrosis factor (TNF) α, interleukin (IL)-1β, fetal calf serum (FCS), phorbol ester, cigarette smoke extract and a G_q-linked G-protein coupled receptor agonist, all attenuate simple glucocorticoid response element (GRE)-dependent transcription. With TNFα and FCS, this was not overcome by increasing concentrations of dexamethasone, budesonide or fluticasone propionate. Thus, maximal GRE-dependent transcription was reduced and this was confirmed for the glucocorticoid-induced gene, p57KIP2. Long-acting β_2-adrenoceptor agonists (LABAs), formoterol fumarate and salmeterol xinafoate, enhanced simple GRE-dependent transcription to a level that could not be achieved by glucocorticoid alone. In the presence of TNFα or FCS, which repressed corticosteroid responsiveness, LABAs restored corticosteroid-dependent transcription to that achieved by corticosteroid alone.
Conclusions: The repression of transactivation represents a mechanism to explain corticosteroid resistance and its reversal may explain the clinical benefit of LABAs as an add-on therapy in asthma and COPD.
Funded by AstraZeneca.

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Azithromycin fails to restore apoptosis in cystic fibrosis airway epithelium post viral infection

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Rationale: Airway epithelium is primary target for respiratory viruses, especially human rhinovirus (HRV). Apoptosis as early host defense mechanisms is induced in response to viral infection. We have previously observed damped apoptotic & increased viral replication in cystic fibrosis (CF) airway epithelium compared to healthy controls. Azithromycin induces innate immune responses in non-CF epithelium. We hypothesize that addition of azithromycin to HRV-infected CF cells may restore/elevate their current apoptotic response to viral insult.
Methods: Airway epithelial cells (AEC) from healthy children (HNA) & those with CF were infected with HRV1b at various multiplicities of infection (MOI: 3-25). AECs were either pre-treated for 24hr or co-treated with azithromycin (1-10μM). Viral replication was determined using qPCR, apoptosis assessed using ssDNA apoptosis kit & IL-8 measured via ELISA.
Results: HRV1b replication was 10-fold greater in CF compared to HNA despite the expression of HRV1b-specific receptor remaining the same between the two cohorts. Furthermore, CF exhibited a dampened apoptotic (~2 fold) compared to the healthy counterparts. Pre- or co-treatment of CF AEC with azithromycin had little effect in restoring apoptosis to levels seen in healthy cells. Azithromycin also did not reduce the level of IL-8 in CF in response to HRV infection.
Conclusion: Although CF are more susceptible to HRV1b infection, the dampened apoptotic response seen in CF epithelium after HRV infection cannot be restored with pre or co-treatment with azithromycin. The increased production of IL-8 observed post viral infection was not ameliorated following macrolide treatment.
Funding: NHMRC, ARC, ACFRT.

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Transcription factor p63 regulates differentiation- and repair-related genes in primary human bronchial epithelial cells: Implications for airway remodeling in asthma

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Rationale: We have previously shown increased expression of the transcription factor p63 in the asthmatic epithelium, both in vivo and in vitro (Hackett et al. AJRCCM 2009 180(2):122-33). p63 is a crucial regulator of development and maintenance of a stratified epithelium in tissues such as the skin and kidney. Whether p63 has a similar role in regulating genes involved in airway epithelial barrier function, and could thus contribute to the airway remodeling seen in asthma, is unknown.
Objective: To determine the role of p63 in regulating expression of genes involved in airway epithelial adhesion, differentiation, structural integrity and cell cycle progression.
Methods: Monolayer cultures of primary human bronchial epithelial cells (n=5) were treated with 50nM custom Stealth siRNA (Invitrogen) targeting various p63 isoforms. RNA was collected at 48h and real-time qPCR for 21 epithelial genes was performed (SA Biosciences, USA). Results were normalized to GAPDH expression. One-way ANOVA with Dunnett's post-test were employed to assess statistical significance.
Results: Loss of p63 expression was found to significantly ($p<0.05$) decrease expression of several genes, most notably the differentiation-associated genes β-catenin and Jagged 1, the proliferation and repair-related gene Epidermal Growth Factor Receptor (EGFR) and the extracellular matrix component Lamininγ2.
Conclusions: In primary human airway epithelial cells, p63 loss results in decreased expression of key genes involved in epithelial differentiation and repair. This may have an impact on the phenotype observed in the asthmatic epithelium.

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