376. Cellular signalling mechanisms in the airways

P3402 Effect of TGF-β on FoxO activity in airway smooth muscle cells

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Background: TGF-β is a mediator of abnormal airway smooth muscle (ASM) function in asthma and COPD. TGF-β triggers ASM cell (ASM) hyperplasia and increases intracellular oxidants whilst reducing antioxidant enzyme expression. The O subfamily of forkhead box transcription factors (FoxO1, -3 and -4) activate antioxidant enzyme, cell cycle inhibitor and pro-apoptotic genes.

Aims & objectives: Determine whether TGF-β reduces FoxO activity in ASMCs leading to attenuated antioxidant enzyme expression and resistance to apoptosis.

Methods: mRNA and protein expression were determined by qRT-PCR and Western blotting, respectively. FoxO transcriptional activity was determined by a histone deacetylase (HDAC) activity by treatment with trichostatin A (TSA).

Results: TGF-β (1 ng/ml) reduced the mRNA levels of the FoxO target genes BinEL (∼75%; p<0.01), PGC-1α (∼90%; p<0.01) and Smad7 genes, and histone deacetylase (HDAC) activity by treatment with trichostatin A (TSA).

Conclusion: TGF-β is a mediator of abnormal airway smooth muscle (ASM) function in asthma and COPD. TGF-β triggers ASM cell (ASM) hyperplasia and increases intracellular oxidants whilst reducing antioxidant enzyme expression. The O subfamily of forkhead box transcription factors (FoxO1, -3 and -4) activate antioxidant enzyme, cell cycle inhibitor and pro-apoptotic genes.

P3403 Mast cells influence the physiological functions of fibroblasts

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Rationale: Mast cells (MC) play a role in allergic disease and anaphylaxis, by releasing their granules containing pro-inflammatory and pro-fibrotic mediators. Recent studies, suggest that mast cells may have a role in non-allergic chronic lung diseases. Since tissue remodelling and fibrosis is a key feature in these diseases our hypothesis is that the mast cell-fibroblast interplay is altered.

Methods: MC isolated from human peripheral blood and fibroblasts (HFL-1 fibroblasts and primary fibroblasts from lungs) were used in the experiments. The effects of the MC proteases, chymase and tryptase on fibroblasts were analyzed with western blot and qPCR. To investigate the impact of cell-cell interaction, co-cultures between MC and fibroblasts was established and differentiation and production of extracellular matrix proteins were investigated.

Results: Mast cell tryptase significantly increased HFL-1 cell migration while chymase had no effect. This effect was not due to increased cell proliferation as neither tryptase nor chymase influenced the cell number. Tryptase triggered increased expression of alpha-smooth muscle actin, a marker for myofibroblasts. This was accompanied by a change in the production of collagen and proteoglycans. MC adhered directly to HFL-1 and primary fibroblasts when using co-cultures. The cell-cell contact caused alterations in the fibroblast phenotype and in the extra cellular matrix production.

Conclusions: These data indicate that MC and their mediators influence fibroblast phenotype and the production of extra cellular matrix proteins. Our results suggest that mast cells may play an important role in tissue remodelling and fibrosis by modulating the physiological function of fibroblasts.

P3404 Ageing lung shows reduced expression and activation of the cyclic AMP response element binding protein

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Background: Lung ageing is associated with morphological and, therefore, physiological changes. While much attention has been paid to changes of the extracellular matrix, less is known about the effect of age on intracellular transcription factors influencing transcription factors. As the transcription factor cyclic AMP response element binding protein (CREB) is involved in various cellular mechanisms, including glucose metabolism and growth factor-dependent cell survival, our study aimed at effect of lung ageing on CREB.

Methods: Lung tissues of young, adult and old mice were studied by PCR, immunoblot and immunohistochemistry. Moreover, we studied pre-senescent and senescent primary human lung fibroblasts, and the effect of known age-related factors.

Results: Lung tissue was characterized by an age-dependent reduction of activated (phosphorylated) and total CREB protein, whereas ageing did not influence the mRNA level of CREB. The mRNA of the phosphoephrinpyruvate carboxykinase 1, which is a target of active CREB, was also reduced in old lung tissue. This in regard, the protein but not mRNA level of CREB was lower in senescent than pre-senescent lung fibroblasts. Down-regulation of CREB by siRNA transfection caused a lower maximal population doubling and higher cell death of human fibroblasts in vitro. Among several age-related factors we identified the increased non-enzymatic modification of the extracellular matrix with advanced glycation end-products as one reason for the reduced level of CREB protein in old lung tissues.

Conclusion: The reduction of active CREB might contribute to morphological and physiological changes in the ageing lung by a lower transcription of CREB target genes.

P3405 Notch signaling negatively regulate the expression of MUC5AC in airway goblet cells

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Introduction and background: Goblet cell metaplasia and airway mucus hypersecretion contributed to the pathogenesis of asthma and COPD. Evidence has shown that Notch signaling modulates the development of lung epithelial and the differentiation of intestinal goblet cells. Whether Notch signaling can regulate the expression of MUC5AC, a major component of airway mucus, is still unknown.

Aim and objective: To investigate whether Notch signaling can regulate MUC5AC expression in airway goblet cells.

Methods: Expression of Notch receptors and downstream molecules in airway goblet cells was examined. Then, transfected with pEFBOS-NIC (upregulating Notch signaling) or treated with γ-secretase inhibitor (downregulating Notch signaling), the MUC5AC expression in airway goblet cells was examined. Finally, the transcriptional regulatory mechanisms were analyzed.

Results: Airway goblet cells express Notch1, Notch3 and downstream transcription factor hes1. Notch signaling can modulate the MUC5AC expression both in vivo and in vitro. Two N-box sites (hesi binding site), -1439 and -493, were identified within mouse MUC5AC promoter. One N-box site (-293) was identified within human MUC5AC promoter. The Notch intracellular domain NIC, the active form of Notch receptors, activates the reporters driven by mouse or human MUC5AC promoters. Site-directed mutagenesis reporter assays confirmed that mouse N-box (-893) and human N-box (-293) negatively regulated MUC5AC promoter activity. Chip assays showed that Hes1 bound to the MUC5AC promoter in both mouse and human airway goblet cells.

Conclusions: Notch signaling negatively regulates the MUC5AC expression through Hes1-dependent mechanism in airway goblet cells.
Study: 25-hydroxide-vitamin D induces TSLP expression in human bronchial epithelial cells via the VDUP1 pathway

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Background: Airway epithelial cells (AEC) express 1α-hydroxylase and are able to convert 25-hydroxide-vitamin D (25(OH)D) to an active form that plays a role in mucosal immunity. Thymic stromal lymphopoeitin (TSLP), a cytokine mainly produced by AECs during allergic asthma reactions, plays a critical role in the activation of Th2 inflammatory responses. Therefore, we hypothesized that 25(OH)D would enhance the expression of TSLP in airway epithelia (16HBE cell line) and that vitamin D3 upregulated protein 1 (VDUP1) could be involved in this process.

Methods: 16HBE cells were cultured with 25(OH)D, and TSLP and VDUP1 mRNA and protein expression were then determined by means of quantitative PCR, ELISA, and Western blot analysis, as appropriate. The role of VDUP1 on TSLP expression was assessed in untreated and 25(OH)D-treated 16HBE cells, where VDUP1 levels were manipulated via overexpression or siRNA-mediated silencing. The effect of 10 M IL-1α, interleukin-1α, on the expression of TSLP was also determined.

Results: 25(OH)D significantly induced TSLP and VDUP1 mRNA expression in 16HBE cells. Silencing of VDUP1 dramatically reduced 25(OH)D-mediated induction of TSLP, and overexpression of VDUP1 upregulated baseline TSLP expression and 25(OH)D-induced TSLP expression in 16HBE cells. Inhibition of 1α-hydroxylase reduced 25(OH)D-induced TSLP expression in 16HBE cells.

Conclusion: These data demonstrated that vitamin D increased TSLP expression in 16HBE cells via upregulation of VDUP1.

P3407 Acetylcholine leads to STAT-1 mediated oxidative/nitrosative stress in human bronchial epithelial cells

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The induction of nitric oxide synthase (iNOS) expression via the signal transducer and activator of transcription 1 (STAT1) is involved in the mechanism of oxidative/nitrosative stress. Acetylcholine (ACh) or TLR4 are implicated in the activation of the bronchial epithelial cells in COPD. We aimed to investigate whether ACh generates oxidative/nitrosative stress in bronchial epithelial cells during airway inflammation of COPD and to evaluate the effects of anticholinergic drugs and long-acting β-agonists in this mechanism.

Human bronchial epithelial cells (16HBE) were stimulated (4 hrs, 37°C) with induced sputum supernatants (ISSs) from healthy controls (HC) (n=6), healthy smokers (HS) (n=6), healthy smokers (HS) patients (n=6), respectively, as well as with ACh (1 μM). STAT-1 activation (Ser727 and Tyr701) and iNOS were evaluated in the cell lysates using Western blot analysis, while reactive oxygen species (ROS) and nitric oxide (NO) in the cells and nitrotyrosine in the supernatants were evaluated by flowcytometry and by ELISA, respectively. The effect of Tiotropium (Spiriva®) (20μM), alone, alone in combination with Oleodolater (10μM), was tested. ISSs from COPD patients and ACh significantly increased the phosphorylation of STAT-1Ser727 and STAT-1Tyr701, the expression of iNOS and the production of ROS/nitrotyrosine in stimulated 16HBE when compared with ISSs from HC or HS subjects. The use of Tiotropium and Oleodolater alone well controlled these events. These results support the use of Tiotropium and Oleodolater to reduce the oxidative/nitrosative stress generated by ACh during airway inflammation of COPD via the STAT-1 pathway activation in human bronchial epithelial cells.

P3408 TLR2 and TLR4 induced tolerance in alveolar macrophages; differential effect on TNFα and IL-8 release

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Alveolar macrophages (AM) play a key role in the pathogenesis of COPD. Bacterial stimulation of toll-like receptors (TLR) on AM causes secretion of inflammatory mediators such as IL-8, promoting neutrophil migration to the lung. Repeat stimulation of TLR results in a reduced inflammatory response, known as tolerance.

To emulate events during bacterial colonisation of the lung, we investigated the effects of repeated TLR stimulation in AM from COPD subjects. AM from healthy subjects and COPD (n=9) patients were isolated. For tolerance induction, treatment with media, LPS (1μg/ml) or Pam3CSK4 (0.1μg/ml) was performed over 24 hour period. Cells were then washed before a repeat stimulation. Levels of IL-8 and TNFα were measured post-treatment.

Repeat LPS exposure reduced TNFα production but had no effect on IL-8 release from AM (table 1). Differences between healthy and COPD AM responses were negligible. Pre-incubation with LPS before Pam3CSK4 stimulation appeared to prime cells for further IL-8 production compared to levels secreted after two consecutive Pam3CSK stimulations.

Table 1: TNFα and IL-8 Release Following LPS-induced Tolerance in COPD AM

<table>
<thead>
<tr>
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<th>1st 24 hr</th>
<th>Media</th>
<th>Media</th>
<th>LPS</th>
<th>LPS</th>
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<tbody>
<tr>
<td>TNFα ng/ml (x.e.m)</td>
<td>0.01 (0.007)</td>
<td>11.2 (2.9)</td>
<td>3.6 (1.8)*</td>
<td>352.7 (64.5)</td>
<td></td>
</tr>
<tr>
<td>IL-8 ng/ml (x.e.m)</td>
<td>8.6 (2.0)</td>
<td>299.4 (42.7)</td>
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Med/LPS vs LPS/LPS *p<0.05. Chronic LPS exposure in COPD lungs results in diminished TNFα production but enhanced IL-8 release. This may cause neutrophil recruitment and inflammation, further contributing to disease pathogenesis.

P3409 TLR4 signaling potentiates airway secretion from the swine submucosal gland via NO/cGMP/cGK pathway

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Rationale: Airway secretion plays an important role in the airway defense as a part of innate immunity. A major fraction of the airway fluids appears to be derived from submucosal gland in response to ACh. Toll-like receptor 4 (TLR4) recognizes gram-negative bacteria and activates the innate immune systems. However, the biological role of TLR4 in the airway secretion is not well understood.

Methods: Freshly isolated swine tracheal submucosal gland cells were investigated their secretory activities as ion currents by applying a patch-clamp technique. LPS was used as a potent TLR4 ligand. The expression of TLR4 was estimated by an intracellular NO indicator, DAF-2DA.

Results: LPS significantly potentiated the ACh-evoked ion currents. This potentiating effect was completely abolished by the pretreatment of anti-TLR4 antibody. To investigate whether TLR4 generates oxidative/nitrosative stress in human bronchial epithelial cells via the VDUP1 pathway, we focused on the TLR5 signaling as other candidate for a potentiator of airway secretion.

Conclusions: Our studies revealed that TLR4 signaling could potentiate the electrolyte and water secretions from tracheal submucosal glands via the activation of NO/cGMP/cGK pathway. These findings suggest that TLR4 takes part in the airway mucosal innate immune systems as one of important pathogen-specific secretagogues.

P3410 Flagellin potentiates the Ca2+-dependent electrolytes secretion from airway submucosal gland in a TLR4-independent, TLR5-dependent and cGK-dependent manner

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Background: Airway surface fluids are mainly secreted from submucosal glands and play important roles in the airway defense via the upregulation of mucociliary transport. Although Toll-like receptors (TLRs) recognize distinct pathogen-associated molecular patterns, it is not well known whether TLRs directly upregulate the airway secretions. Recently, we have reported that TLR4 works as one of important secretagogues in the airways (AJRCMB 2011). In the present study, we focused on the TLR5 signaling as another candidate for a potentiator of airway secretion.

Methods: Freshly isolated swine tracheal submucosal gland cells were prepared and measured their secretory activities as ion currents by applying a patch-clamp technique. The expression of TLR5 was estimated by both the immunofluorescent staining and RT-PCR. The intracellular mechanisms were investigated by applying some inhibitors of NO or cGMP-dependent protein kinase (cGK).

Results: Flagellin, a TLR5 ligand, showed significant potentiating effects on ACh-evoked ion currents. These effects were abolished by the pretreatment with anti-TLR5 antibody, but not by specific TLR4 antagonist. Two different inhibitors of each NOs and cGK abolished these effects. We also revealed that the expression of TLR5 on the tracheal submucosal gland cells.

Conclusion: Our study showed that flagellin caused the potentiating effects on airway submucosal gland via NO/cGMP/cGK pathway. This finding suggest that TLR5 also works as a potentiator of airway secretion in the airway mucosal immune systems.
**Conclusion:** The concentration-sensitive Na⁺ channel (Na₇.3), a member of the family of voltage-dependent Na⁺ channels, opens in response to increased Na⁺ concentration in the extracellular fluid ([Na⁺]₀). Although the expression of Na₇ in airway epithelial type I (AT I) cells has been reported, the physiological role of Na₇ in lung tissue has not yet been established. Various ion channels in the alveolar epithelium are involved in maintaining the alveolar fluid balance; therefore, we hypothesized that Na₇-mediated Na⁺ transport contributes to the clearance of Na⁺ from the alveolar fluid. In this study, we examined Na₇ distribution in mouse lung tissue and the Na⁺-dependent influx of Na⁺ into mouse alveolar epithelium.

**Methods:** We used immunohistochemistry and immunofluorescence to study mouse lung tissue using antibodies against Na⁺ and other ion transport proteins. In situ hybridization was performed using a digoxigenin-labeled antisense probe for Na⁺, mRNAs. Na⁺ dynamics in mouse alveolar epithelium were analyzed using sodium-binding benzofuran isophthalate and an image analyzer Argus-50.

**Results:** The alveolar epithelial type I (AT I) cells showed positive staining with anti-Na⁺ antibodies. Similarly, Na⁺ mRNA signals were detected in the AT I cells by in situ hybridization. Confocal laser scanning microscopy showed the presence of Na⁺ in the cell membrane of the AT I cells. Na⁺ was partially colocalized with y epithelial sodium channels (ENaC). Results of image analysis showed that Na⁺ influx into alveolar epithelium was dependent on elevation of [Na⁺]₀.

**Conclusions:** These findings suggest that Na₇ expressed in AT I cells, is involved in Na⁺ transport in the alveolar epithelium.

**P3412**

**Characterisation of endogenous IL-33 using a model of Alternaria-induced pulmonary inflammation**

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**Background:** IL-33 is a potential mediator of chronic inflammatory diseases including asthma. However, endogenous forms of IL-33 are poorly understood. Here we have used a model of Alternaria alternata (ALT) challenge to investigate in vivo mechanisms of IL-33 processing and release.

**Methods:** Cytokines were quantified in bronchoalveolar lavage (BAL) from BALB/c mice by MSD assay. Mast cells were differentiated from bone marrow over 5 weeks and stimulated with BAL or recombinant IL-33 for 24 hours.

**Results:** Intranasal challenge with 50,000 ALT/μl for 24 hours resulted in increased cytokine release, including IL-4, IL-5, IL-13, and IL-17. However, no significant increase in IL-33 was observed. In contrast, in vitro studies using mast cells, respiratory epithelial cells, and human bronchial epithelial cells demonstrated increased IL-33 expression following ALT exposure.

**Conclusions:** IL-33 has not been identified as a major contributor to the inflammatory response in chronic respiratory diseases. Further studies are needed to determine the role of IL-33 in the pathogenesis of asthma and other chronic inflammatory diseases.

**P3414**

**Inhibition of A. fumigatus growth by human respiratory cells**

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The innate immune response to fungi mainly involves macrophages and neutrophils. By contrast, the possible participation of respiratory epithelial cells (HECs) has been poorly studied. In the present study we observed that REC display the property of inhibiting mycelium development of Aspergillus fumigatus (A.f) and that this activity is linked to PI3 kinase activation.

In the present study we observed that REC display the property of inhibiting mycelium development of Aspergillus fumigatus (A.f) and that this activity is linked to PI3 kinase activation. Neutrophils (BEAS-2B cell line) were incubated with A.f and the growth of the fungus was estimated by optical microscopy observation and measurement of galactomannan concentrations in the extracellular media. The role of PI3 kinase was evidenced by the use of the specific inhibitor LY294002 (30 μM). Sipore internalization in REC was followed by epifluorescence. FITC-labeled spores (green) were observed in REC with green fluorescence indicating spore internalization in REC with green fluorescence indicating spore internalization.

**Conclusions:** REC contribute to the innate immune response to fungi mainly involving macrophages and neutrophils. By contrast, the possible participation of respiratory epithelial cells (HECs) has been poorly studied. In the present study we observed that REC display the property of inhibiting mycelium development of Aspergillus fumigatus (A.f) and that this activity is linked to PI3 kinase activation. Neutrophils (BEAS-2B cell line) were incubated with A.f and the growth of the fungus was estimated by optical microscopy observation and measurement of galactomannan concentrations in the extracellular media. The role of PI3 kinase was evidenced by the use of the specific inhibitor LY294002 (30 μM). Sipore internalization in REC was followed by epifluorescence. FITC-labeled spores (green) were observed in REC with green fluorescence indicating spore internalization in REC with green fluorescence indicating spore internalization.

**Acknowledgements:** Work supported in part by a grant from Vaincre la Mucoviscidose.
P3416

Activation of both transcription factors STATA and IRF-1 is insensitive to corticosteroids in asthmatic bronchial smooth muscle cells exposed to TNFa/IFNγ

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We have previously developed a cellular model in healthy airway smooth muscle (ASM) cells where corticosteroids (CS) lose their anti-inflammatory action when cells are exposed to TNFα in combination with IFNγ. The molecular mechanisms by which TNFα/IFNγ promote CS insensitivity has not been completely investigated although an increased activation of transcription factors such as STATA has been implicated in other cell types. It is also not known whether TNFα/IFNγ also interfere with CS in ASM cells derived from asthmatic patients. In the present study, we found that TNFα/IFNγ-induced expression of different steroid-resistant chemokines CX3C11, CCL5 and CCL11 in asthmatic ASM cells (n=3). We also found that TNFα/IFNγ (0-6hr) increased phosphorylation of STATA in a time-dependent fashion reaching a maximum at 50 min following by a lower activated state that was sustained up to 6 hr. Prior treatment with fluticasone (0.1-100 nM) did not affect cytokine-induced STATA activation but dose-dependently increased STATA phosphorylation. Similarly, the sustained activation of the transcription factor IRF-1 by TNFα/IFNγ (up to 6 hr) was also unaffected by fluticasone (01-100 nM). Together, these data show for the first time that TNFα/IFNγ induced a sustained activation of corticosteroid-resistant STATA and IRF-1 in asthmatic ASM cells (n=3). Because STATA and IRF-1 have been associated with reduced CS action in other cell types, our findings suggest the possible involvement of these transcription factors in driving CS insensitivity in asthmatic airway smooth muscle cells.

P3417

Formoterol restores oxidative stress-induced corticosteroid insensitivity via activation of protein phosphatase PP2A

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Introduction: Oxidative stress induces corticosteroid (CS) insensitivity via the activation of phosphoinositide-3-kinase (PI3K) and reduced HDAC2 activity. We have demonstrated that the long-acting δ2-agonist formoterol (FORM) restores HDAC2 activity and Akt signaling in asthmatic airway smooth muscle cells where corticosteroids (CS) lose their anti-inflammatory action when cells are exposed to cigarette smoke. FORM also counteracted H2O2-induced increase of Akt phosphorylation, decrease of HDAC2 activation and PP2A activity. Pretreatment with OA phosphorylation status at 2 hr showed no difference between groups.

Aims: Baseline GR is decreased in ASM cells of asthma, while induced GR translocation is impaired only in SA. Although TNFα induces greater p65 expression in SA, nuclear translocation and Dexamethasone (Dex) suppression of p65 expression are similar between all groups. Impaired nuclear translocation may underlie the mechanism of CS insensitivity in SA.

Results: TNFα induced greater p65 in SA, whereas baseline and TNFα-induced nuclear abundance, and Dexamethasone-induced translocation of p65 in SA, were similar between all groups. GR expression was increased in SA (p=0.001), with no difference between NSA and SA. Dexamethasone-induced nuclear translocation of GR in SA was approximately 60% of that in the healthy in SA at 30 min-2 hr, whereas baseline levels were similar. In healthy subjects, Dexamethasone (Dex) maximal induced GR phosphorylation at 2 hr (5.2-fold vs baseline, p<0.001), which was maintained over 4 hr, phosphorylation status at 2 hr showed no difference between groups.

Conclusion: Baseline GR is decreased in ASM cells of asthma, while induced GR translocation is impaired only in SA. Although TNFα induces greater p65 expression in SA, nuclear translocation and Dexamethasone suppression of p65 expression are similar between all groups. Impaired nuclear translocation may underlie the mechanism of CS insensitivity in SA.

P3419

A role of mTOR in cigarette smoke-induced corticosteroid insensitivity

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Introduction: The corticosteroid insensitivity is a major barrier to the treatment of severe asthma and COPD. It has been revealed that cigarette smoke induces inactivation of HDAC2 through activation of PI3K/Akt pathway, leading to corticosteroid insensitivity. Mammalian target of rapamycin (mTOR) is one of the most important effectors of PI3K/Akt pathway. It is a serine/threonine kinase, and implicated in disease states where growth is deregulated and homeostasis is compromised.

Aim: To determine the role of mTOR on the development of corticosteroid insensitivity in COPD and under oxidative stress.

Methods: mTOR activity was calculated by the phospho-p70s6k band intensity in Western blot analysis. Corticosteroid sensitivity was determined as the 50% inhibitory concentration of dexamethasone on TNFα-induced interleukin-8 release in U937 cells. Results: mTOR activity was increased within 5 minutes after cigarette smoke stimulation in U937 cells, then decreased gradually until below the baseline. mTOR inhibitor, rapamycin, totally suppressed this increase. The EC50 value of dexamethasone was 5.6nm and the Emax value was 62.8±6.4% (n=4) in the intact cells, but after incubation with cigarette smoke extract, EC50 was increased to 47.9nm and Emax was decreased to 42.4±9.1%, suggesting cigarette smoke decreased dexamethasone sensitivity almost by one-tenth. EC50-Dex was 12.9nm and Emax was 55.9±6.9% in the presence of Rapamycin (20nM, 2hr) and rapamycin partially reversed this insensitivity.

Conclusions: Cigarette smoke extract treatments increased mTORC1 activity in U937 cells, and rapamycin partially reversed corticosteroid insensitivity caused by cigarette smoke exposure.

P3418

Corticosteroid insensitivity in severe asthma: Impaired nuclear translocation of glucocorticoid receptor in airway smooth muscle cells

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Background: Patients with severe asthma respond poorly to corticosteroids (CS). Airway smooth muscle cells (ASMC) of severe asthma display CS insensitivity. CS mediate their effects through activation of the glucocorticoid receptor (GR) and suppression of NF-κB activity.

Aims and objectives: Compare protein/mRNA, phosphorylation, and nuclear translocation of GR and NF-κB (p65).

Methods: ASMC of the healthy (9, non-severe (NSA); 8) and severe asthmatics (SA; 8) were obtained from endobronchial biopsies, cultured at passage 4-5. Cells were treated with TNFα (10 ng/ml/dexamethasone (Dex; 10-7 or 10-6 M). Whole cell protein or nuclear extracts were assessed by Western Blot, mRNA by qRT-PCR.

Results: NF-κB activity was increased in SA, whereas baseline and TNFα-induced nuclear abundance, and Dex suppression of p65 expression, were similar between groups. GR expression was increased in SA (p=0.001), with no difference between NSA and SA. Dexamethasone-induced nuclear translocation of GR in SA was approximately 60% of that in the healthy in SA at 30 min-2 hr, whereas baseline levels were similar. In healthy subjects, Dexamethasone (Dex) maximal induced GR phosphorylation at 2 hr (5.2-fold vs baseline, p<0.001), which was maintained over 4 hr, phosphorylation status at 2 hr showed no difference between groups.