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Late-breaking abstract: Differential expression profiles of genes involved in oxidative stress and inflammation in blood and sputum from healthy subjects and COPD patients

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Background: Environmental (mainly cigarette smoke) and genetic factors are known to be involved in the development of chronic obstructive pulmonary disease (COPD); however, a better understanding of the COPD genes expression dysregulation remains a major challenge.
Increased oxidative stress is thought to be central in COPD pathogenesis and directly involved in local and systemic inflammation.
Methods: We have investigated, by RT-PCR array, the mRNA expression profile of 95 genes involved both in inflammation and oxidative stress in sputum and blood from COPD patients (n = 18) and healthy controls (n = 17). We have used Ingenuity Pathway Analysis Software (IPA) to identify the networks of interactions, the biological processes and pathways in which genes showing a significant expression modification are involved.
Results: In the blood cells of COPD, around half of genes showed modifications (26 up- and 19 down-regulated) compared to healthy controls and these were essentially involved in inflammation. Using IPA, we found that the most important cellular function altered was the cellular movement. In sputum cells, only 13 genes showed modifications (6 up- and 7 down-regulated, five were common with blood), most of them being involved in free radical scavenging and cell death.
Conclusions: Compared to healthy subjects, there was a clear dysregulation in gene expression at systemic level, and to a lesser extent, at airway level. Therefore, gene expression profile shows differences between local and systemic compartments.

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Late-breaking abstract: Cycloxygenase- and lypoxigenase-dependent generation of omega-3 electrophilic fatty acid-derivatives with anti-inflammatory properties

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Chronic obstructive pulmonary disease (COPD) is characterized by persistent inflammation of the airways and extensive oxidative damage. Activated macrophages and neutrophils are elevated in the airways of COPD patients where they sustain the inflammatory response and contribute to tissue damage. During inflammatory reactions arachidonic acid (AA) is released from cell membranes and is converted into the pro-inflammatory prostaglandins and leukotrienes by the action of

cycloxygenase-2 (COX-2) and lypoxigenases (LO). It has been recently discovered that COX-2 and LO are able to convert alternative substrates, such as the omega-3 docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA), into mediators that actively repress the inflammatory reactions and promote the resolution of inflammation. Herein we report the formation of new omega-3 electrophilic fatty acid oxo-derivatives by the action of COX-2 and LO in activated human macrophages and stimulated neutrophils. These compounds displayed cytoprotective and anti-inflammatory actions measured as repression of pro-inflammatory enzymes and cytokines and activation of the Nrf2-dependent anti-oxidant response. Data presented herein strongly suggest that electrophilic derivatives of omega-3 fatty acids are generated in inflamed airways of COPD patients where they may contribute to limit tissue damage and inflammatory processes.

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Late-breaking abstract: Surfactant protein D (SP-D) as a biomarker for mortality: A study in elderly Danish twins

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Background: SP-D is synthesized and secreted into the airspaces of the lung by alveolar and bronchiolar epithelial cells. Systemic measurements of SP-D are previously demonstrated to predict survival in patients with idiopatic pulmonary fibrosis and ALI/ARDS. However, SP-D may be a general indicator of pulmonary health status and associate to mortality in the general population.
Aim: To investigate the association between higher serum SP-D levels and mortality in normal Danes.
Methods: A total of 689 twins (234 males and 455 females) provided blood samples during a six month period in a survey (LSADT) performed in 1997. During a 13-year follow-up period 181 (77%) men died and 292 (64%) women died. SP-D serum levels were measured by ELISA technique.
Results: Survival analysis was performed and SP-D levels were adjusted to age, smoking and BMI. SP-D levels were positively and significantly correlated to mortality in females. At the end of January 2010, there were 84 male twin pairs (39 MZ and 45 DZ) and 160 female twin pairs (73 MZ and 87 DZ) in which at least one twin had died. There was no evidence of an association between increasing intra-pair difference in log transformed SP-D and mortality in males. In contrast, intrapair analyses in females showed that the twin with the highest SP-D level had a higher risk of dying than the co-twin (OR=1.66, p=0.047). Adjusting this analysis with intrapair difference in smoking (packyear) and BMI did not affect this association (OR=1.75, p=0.040).
Conclusion: The study indicates that circulating SP-D levels may be a biomarker to track the pulmonary health status and to forecast mortality in the elderly women.

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Late-breaking abstract: Lung regional differences in tissue adaptation to chronic hypoxia (CH)

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Hypoxia (H) impacts to all body tissues which have to adapt to a decrease in O_2 delivery. In CH (10% O_2 for 3 weeks) two lung regions from rats, the upper and the lower lobe, showed differences in interstitial tissue structure (Rivolta, I *et al*. ERJ 2011; 37:943-9). On these samples, where the metabolic needs are related to a matrix remodeling, we evaluate how H effects mitochondrial activity. We estimated: mRNA level of KGF, that controls tissue remodeling and matrix components deposition; activity of mitochondrial citrate synthase (CS), index of oxidative activity; expression of the PGC-1a, strong O_2 sensor, and of HIF1a, key transcription factor of the O_2-response system. 50% decrease in KGF mRNA level in lower lobe in normoxia, revealed a topographical difference in the two lobes matrix reorganization; H exposure had a little effect on its expression (0.8±0.3 and 0.7±0.4 hypoxic upper and lower lobe, respectively, relative to normoxic upper lobe). CS activity slightly but significantly decreased in upper lobe while it increased in hypoxic lower lobe. In H, HIF1a doubled but did not show regional differences, while PGC-1a increase was almost 66% greater in the lower compared to the upper lobe. We conclude that variances in tissue structure organization of the two lobes can be accounted for a topographic difference in the level of KGF present in normoxia. On this background, differences in the adaptive response to H might be mediated by differential expression of PGC-1a since HIF1a response was equally affected in both lobes. These results represent a breakthrough in the molecular mechanisms of H adaptation understanding, furthermore, this is the first study considering PGC-1a in the hypoxic lung.

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Engineered nanoparticles induce apoptosis of human bronchial epithelial cells

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Engineered nanoparticles are widely used by the industry, however, it is not clear whether they posses a risk on respiratory health. The objectives of our study were to investigate effects of Titanium dioxide (TiO_2) and multi walled carbon nanotubes (MWCNT) on bronchial epithelial cell (BEC) viability and death. BEAS-2B cells and primary BEC obtained from both smokers and patients with COPD were incubated with 0-300μg/ml TiO_2 and MWCNT for 24-48hrs. Cell viability was assessed by MTT, and apoptosis was analyzed by flow cytometry using Annexin V-PE and 7AAD dyes. TiO_2 significantly decreased the viability of BEAS-2B cells at 100 (optical density [OD]=0.65; $p<0.0001$) and 300μg/ml (OD=0.45; $p<0.0001$) concentrations after 24hrs as compared control cells (OD=0.85). Similarly, 100 and 300μg/ml MWCNT decreased viability of these cells following 24 and 48hrs' incubation. Although 30μg/ml TiO2 induced the viability of primary BEC of smokers (OD=2.15 vs OD=1.52; $p<0.0001$), 300μg/ml suppressed cell viability (OD=1.02; $p<0.0001$) after 24hrs. TiO2 did not change the viability of BEC of COPD patients after 24hrs, whereas 300μg/ml decreased viability of these cells (OD=0.40 vs 1.72; $p<0.001$) following 48hrs. Flow cytometry studies of BEAS-2B cells demonstrated that TiO_2 (300μg/ml) decreases percentage of viable cells (90.66% vs 94.01%; p= 0.0009), while inducing the percentage of late apoptotic (0.72% vs 0.42%; p=0.017) and necrotic (6.63% vs 4.03%; p=0.0009) cells. MWCNT also showed similar effects on apoptosis of BEAS-2B cells. These findings suggest that engineered nanoparticles may possess a risk on respiratory health by modifying viability and apoptosis of bronchial epithelial cells.

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IL-17 enhances IL-8 production by attenuating both the ARE-mediated and the microRNA-mediated degradation of IL-8 mRNA

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Background: Interleukin (IL)-17 is a cytokine implicated in chronic inflammation. Enhanced amounts of IL-17 have been found in the airways of patients with asthma and COPD. Previously we have reported that IL-17 potentiates TNF-α or IL-1β-induced IL-8 and IL-6 production in human lung epithelial cells, predominantly by stabilizing IL-6 and IL-8 mRNA.
Aim: IL-6 and IL-8 mRNA contain AU-rich elements (ARE) in the 3'UTR, targeting these mRNAs for facilitated degradation. This decay pathway may be mediated by AU-binding proteins (AU-bps) and possibly microRNAs (miRs). We have assessed whether AUBps (TTP, KHSRP, AUF1) and/or miRs are involved in IL-8 mRNA degradation. And, if so, whether IL-17 modulates these pathways.
Results: Inhibition of miR16, or knock-down of TTP or KHSRP resulted in a marked increase of TNF-α-induced IL-8 production, which was paralleled by stabilizing IL-8 mRNA. Conversely, knock-down of AUF-1 reduced IL-8 production and promoted degradation of IL-8 mRNA. IL-17 halts IL-8 mRNA degradation by enhancing the role of AUF1 which protects IL-8 mRNA from degradation. Furthermore, IL-17 reverses the TNF-α-induced production of miR16.
Discussion: These findings strongly indicate that IL-17 enhances IL-8 production by attenuating two pathways that degrade IL-8 mRNA. We have obtained similar data for several other inflammatory mediators. Since airway epithelial cells from patients with asthma display enhanced IL-8 production we are assessing whether there is an intrinsic disturbance in these mRNA decay pathways.
This work is supported by the Netherlands Asthma Foundation (3.2.06.031)

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A quantitative method for the detection of spliced X-box binding protein-1 (XBP1) mRNA in primary bronchial epithelial cells (PBEC) as a measure of endoplasmatic reticulum (ER) stress

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Accumulation of unfolded or misfolded proteins in the ER can cause ER stress, which is increasingly seen in diseases such as cystic fibrosis, alpha-1 antitrypsin deficiency and Alzheimer disease. ER stress leads to the activation of the unfolded protein response (UPR). UPR signaling involves splicing of XBP1 mRNA and leads to the induction of the chaperone protein BiP and the transcription factor CHOP, which is a key-signaling component of ER-stress induced apoptosis. In present studies, XBP1 splicing is frequently used as an important marker for ER-stress and is visualized by gel electrophoresis which is laborious and difficult to quantify. The aim of this study was to develop and validate a quantitative RT-PCR (qPCR) which detects only the spliced form of XBP1 mRNA. We stimulated PBEC with thapsigargin and tunicamycin, both known ER-stress inducers, and performed a qPCR with primers that were designed to recognize only the spliced form of XBP1. We also performed qPCR for CHOP and BiP and correlated this with

the spliced XBP1 mRNA to validate the results. The spliced XBP1 PCR product was also confirmed by DNA sequencing. The correlation of XBP1 splicing with the induction of CHOP and BiP was r=0.962 (p<0.000) and r=0.884 (p<0.000), respectively. We compared the new method with the visualization of the spliced XBP1 mRNA by gel electrophoresis and we obtained similar results. In conclusion, we have developed a simple and quantitative method for the detection of spliced XBP1 mRNA.

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Pulmonary apoptosis in fetal Down syndrome

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Background: Increased levels of apoptosis have been implied in various non-pulmonary conditions frequently found in Down syndrome (DS). Children with DS are at increased risk for acute lung injury and fetal lung development is disrupted in DS. In both processes, apoptosis plays a key role. Nevertheless, pulmonary apoptosis has not been studied in DS.
Aim: We hypothesized that the amount of apoptotic epithelial cells in fetal lungs of DS is increased compared to controls.
Methods: We compared lung tissue sections from autopsies of 21 fetuses with DS and 12 controls (16-24 weeks gestational age (GA)). Sections were double stained with antibodies against pan-cytokeratin (CK) and activated caspase-3 (C3), markers for epithelium and apoptosis. Per section, 7 random photographs were taken at 200x magnification. Spectral imaging software was used to quantify the mean number of pixels that showed colocalization of CK and C3. All sections were H&E stained to determine the presence of canalicular or saccular morphology.
Results: The mean (SD) percentage of CK-positive pixels was equal between DS and controls (27.2% (4.7) versus 27.1% (6.2), p=0.97). The median percentage (IQR) of CK-positive pixels that showed colocalization of C3 was 0.16% (0.18) in DS compared to 0.27% (0.24) in controls (p=0.45). This was independent of GA. The mean (SD) number of CK-positive pixels increased from 22.5% (5.2) to 30.4% (4.6) with the appearance of saccular morphology in controls but not in DS (p=0.01).
Conclusion: The number of apoptotic epithelial cells in lungs of DS fetuses does not differ from controls. We did find a difference in the development of epithelial structures between DS and controls. This might explain anomalies in alveolar development found at birth in DS.

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Airway epithelial protocadherin-1 expression is regulated by house-dust mite and cigarette smoke exposure in mice

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Recently, we identified Protocadherin-1 (*PCDH1*) as a novel susceptibility gene for Bronchial Hyperresponsiveness (BHR). *PCDH1* is expressed in airway epithelial cells and encodes two isoforms of a protocadherin transmembrane protein. We aim to get insight into PCDH1 function in airway epithelial cells in relation to BHR. Therefore, we analyzed *in vivo* regulation of *Pcdh1* isoforms in lung under basal conditions and in mouse models of short-term cigarette smoke (CS) exposure and house-dust mite (HDM) driven experimental asthma. *Pcdh1* gene-structure was investigated by Rapid Amplification of cDNA Ends (RACE). Pcdh1 expression was investigated by qRT-PCR, western blotting and immunohistochemistry using isoform-specific antibodies.
We identified a novel isoform of Pcdh1 lacking the transmembrane domain but retaining the intracellular signalling motifs, indicating a novel function as signalling adapter molecule. Bronchial epithelial cells expressed all isoforms of Pcdh1, while airway smooth muscle only expressed the isoforms encoding the signal transduction domains. Surprisingly, Pcdh1 expression was unaffected during HDM-exposures, but increased after termination of the treatment, indicating a putative role in epithelial repair. In strong contrast, *Pcdh1* mRNA expression was markedly reduced by CS exposure, as soon as 6 hours after a single exposure. These latter data are especially of interest given the initial identification of linkage to the *PCDH1* region in CS-exposed families. We conclude that CS-induced changes in airway epithelium directly affect Pcdh1 expression levels, and hypothesize that PCDH1 regulation contributes to the epithelial response to CS-induced injury.

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Fluticasone furoate restores leptin/leptin receptor pathway in nasal epithelial cells

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Leptin/leptin receptor pathway has been shown to be involved in the epithelial homeostasis and in tissue repair. Allergic rhinitis (AR) is characterized by a IgE-mediated inflammation induced by the allergen exposure, leading to a chronic inflammation with consequential structural abnormalities in the nasal epithelium. Topical corticosteroids are recommended as first-line therapy in AR. The role of the leptin/leptin receptor pathway and the specific effect of fluticasone furoate (FF), a new topical corticosteroid, in the homeostasis of nasal epithelial cells are largely unknown. We aimed to determine whether a nasal epithelial dysfunction of leptin/leptin receptor pathway contributes to AR pathogenesis and to investigate the effect of FF on this pathway. The human nasal epithelial cell line RPMI 2650 was first examined for leptin/leptin receptor expression by immunocytochemistry and by flow-cytometry. Then, the RPMI 2650 cells were cultured in the presence or absence of the allergen extract *parietaria judaica* (PARJ1), of the fibrogenic cytokine TGF-β1 and of FF and analyzed for leptin receptor by flow-citometry and for cell proliferation by clonogenic assay. The RPMI 2650 cells express leptin and leptin receptor. PARJ1 and TGF-β1 significantly decreases the leptin receptor expression and cell proliferation and FF completely abolishes and reverts the effects of both PARJ1 and TGF-β1. In conclusion, allergen exposure and TGF-β1 alter the homeostasis of nasal epithelium by down-regulating leptin/leptin receptor pathway whereas FF is able to restore both this pathway and nasal epithelial homeostasis.

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S-CMC-Lys reduces oxidative stress of respiratory cells and increases GSH intracellular content

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The treatment of human respiratory cells with S-carbocysteine lysine salt monohydrate (S-CMC-Lys) determines a significant increase in GSH secretion by stimulating glutathione (GSH) efflux from cells.The aim of this study was to evaluate the S-CMC-Lys effects on the GSH intracellular content and methabolism after an oxidative stress or after exposure to Cigarette Smoke Extract (CSE).
Methods: GSH and Reactive Oxygen Species (ROS) content in response to S-CMC-Lys,H_2O_2 and CSE treatments was evaluated with fluorimetric or luminescence based essays in respiratory cell lines.The expression of GSH system enzymes was detected by real-time PCR and western blot experiments.
Results: S-CMC-Lys induces in respiratory cells lines a significant increase of the GSH intracellular content and a significant increase in the expression of the catalytic subunit of γ-GCS (γ-Glutamyl Cysteine Synthase), a key enzyme for the synthesis of GSH. S-CMC-Lys pre-treatment of cells prior H_2O_2 exposure was able to reduce ROS.The co-subministration of S-CMC-Lys with CSE for a prolonged period (24 hours)resulted in an increase in the GSH content and a significantly increased level of γ-GCS and GR (glutathione reductase) mRNA.
Conclusions: S-CMC-Lys increases the GSH intracellular content of respiratory cell lines by enhancing the expression of γ-GCS catalytic subunit.The pre-treatment of respiratory cells with S-CMC-Lys exert a protective function during oxidative stress,reducing the ROS-mediated inflammatory response.The cosubministration of S-CMC-Lys potentiates the cell adaptive response to a CSE exposure,counteracting the CSE negative effects on the GSH system and ROS production.

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Mitochondrial dysfunction in airway epithelium increases pro-inflammatory IL-8, impairs barrier function and reduces glucocorticoid responsiveness

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Introduction: Despite the broad anti-inflammatory effects of glucocorticoids (GC), they provide little therapeutic benefit in COPD. Mitochondrial dysfunction has been described in COPD patients and can be induced in airway epithelial cells in vitro by cigarette smoke. We hypothesize that impaired mitochondrial function induces a phenotypic shift in airway epithelial cells leading to increased pro-inflammatory responses, impaired barrier function and reduced GC sensitivity.
Methods: We determined IL-8 secretion (ELISA) and epithelial barrier function (ECIS) in the alveolar epithelial cell line A549 and the mitochondrial impaired cell line A549.B2 and their sensitivity to the GC budesonide (10-8M).

Results: Basal production of IL-8 by mitochondrially impaired A549.B2 cells is increased compared to A549 control cells. Moreover, suppression of IL-8 by the GC budesonide (10-8M) is significantly less (24%±9%, $p<0.02$) in A549.B2 cells compared to A549 cells. Furthermore, while budesonide was able to efficiently increase barrier function in control A549 cells ($p<0.001$), there was not significant effect in A549.B2 cells. Finally, we observed that A549.B2 cells were less capable to recover from wounding than control A549 cells.
Conclusion/Discussion: In conclusion, our data indicate that mitochondrial dysfunction leads to increased pro-inflammatory activity, inefficient wound healing and reduced responsiveness to GCs. We speculate that mitochondrial dysfunction as observed in COPD may contribute to the GC-insensitive chronic airway inflammation in this disease.

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Effect of phosphodiesterase IV inhibitors on eotaxin expression in bronchial epithelial cells – Comparison between immortalized and primary line
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Eotaxins are an important agents of the patophysiology in the obstructive airway diseases. They are responsible for eosinophil recrutation into respiratory tract. Phosphodiesterases (PDEs) are a huge and diversified family of enzymes decomposing cAMP. PDE4 inhibitors as drugs, act through cAMP elevation and can inhibit inflammation in many ways.
The aim of this work was to evaluate the effect of PDE4 inhibitors (rolipram and RO-20-1724) on eotaxin (CCL11, CCL24 and CCL26) expression in human bronchial epithelial cells: immortalized - BEAS-2B (ATCC) and primary (ATCC). Cells were preincubated with PDE4 inhibitors for 1 h and stimulated with IL-4+TNF-α or IL-13+TNF-α for 48h. Protein levels were measured using ELISA kits, changes in genes expression were measured using real time PCR.
Results: The both of cell lines produced different eotaxins: BEAS-2B synthesized CCL11 and CCL26, PBEC - CCL24 and CCL26. Distinct effects of PDE4 inhibitors in immortalized as compared to primary cell line were observed. PDE4 inhibitors decreased the mean level of eotaxin gene and protein expression in BEAS-2B usually in statistically significant manner. PDE4 inhibitors stimulated eotaxin gene expression; specially rolipram significantly increased the mean levels of eotaxin gene expression, but did not change their protein synthesis.
Conclusions: Our results do not permit to predict the effects of PDE4 inhibitors on bronchial epithelial cells because of the differences in the biology of the both used cell lines. Unequivocal resolution of the problem needs more experimental trials using more primary cell lines or experimental animal model.

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ciliaFA: A free research tool for accurate, automated, high-throughput measurement of ciliary beat frequency (CBF)
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Aim: To develop a program that allows batch processing of multiple movie (.AVI) files for measurement of CBF.
Introduction: A program was developed that converts average pixel intensities within a region of interest to a waveform. The waveform is analysed by fast fourier transformation (FFT) to extract the dominant frequency. The code runs on freeware (ImageJ) coupled to Microsoft Excel. It can be used with.AVI from any source, and has been developed for research use with cilia from the respiratory, fallopian or brain epithelia.
Methods: Image files of 115 ciliated epithelial cell cultures were captured using a digital high-speed video recorder. In order to capture a range of CBF measurements, the bacterial toxin, pneumolysin, was used to inhibit CBF. Mean CBF measurements made by conventional frame by frame counting of ciliary beat cycles by slow motion playback were compared with those obtained using the automated ciliaFA system.
Results: The mean (sd) difference between the ciliaFA and conventional methods was -0.4 (1.6) Hz, the correlation coefficient was shown to be 0.8 and the Bland-Altman limits of agreement were -3.5 to 2.7 Hz.

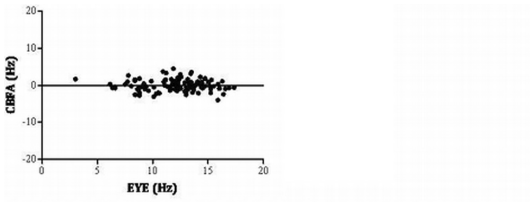

Fig. 1. Comparison of the mean CBF measurements by conventional frame by frame counting of individual ciliary beat cycles (horizontal axis) and the automated ciliaFA method (vertical axis). The line represents the linear regression of the correlation.

Conclusion: The data showed that the ciliaFA software calculated consistent CBF measurements. The advantages of this system include automated, high throughput CBF analysis; whole field and individual region of interest measurements, and elimination of selection bias.

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Interactions between epithelial cells and neutrophils during pro-inflammatory conditions
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Inter-cellular communication is essential for defense and survival of the organism. The aim of the study was to find out whether there is an active cross-talk between cells constituting the first line of defense; alveolar epithelial cells (A549) and neutrophils, following activation with pro-inflammatory stimuli *in vitro.* Further, to explore whether this communication is altered in chronic obstructive pulmonary disease (COPD), a condition characterized by chronic airway and lung inflammation.
Blood neutrophils from healthy subjects and COPD-patients were co-cultured with A549 cells in medium and in medium containing lipopolysaccharide (LPS), peptidoglycan (PGN) or tumor necrosis factor (TNF). The expression of TLR2, TLR4 and CD14 on the cell surface of neutrophils was assessed by flow cytometry and CXCL8 (IL-8) and soluble CD14 (sCD14) in the supernatant were measured with ELISA.
On neutrophils, the surface expression of TLR2 was diminished following activation with all three pro-inflammatory stimuli and membrane bound (mCD14) and TLR4 expression were increased in co-cultures compared to single cell cultures, irrespective of pro-inflammatory stimulation. A strong correlation between CXCL8 and sCD14 was observed in LPS-stimulated co-cultured cells.
These data showed a down regulation of TLR2 on neutrophils induced by pro-inflammatory stimuli and is strongly suggesting an active cross-talk between A549 cells and blood neutrophils, both in unstimulated cells and following activation with pro-inflammatory stimuli, *in vitro.*

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Effect of cigarette smoke extract or TGF-β1 on hyaluronan production and hyaluronan modulating enzymes in primary murine lung fibroblasts
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Hyaluronan (HA) is a component of the extracellular matrix and low molecular weight (LMW) HA fragments have pro-inflammatory capacities. Exposing mice to cigarette smoke (CS) for 1 or 6 months results in enhanced deposition of LMW HA in lung parenchyma and airway walls and in altered expression of HA synthases and hyaluronidases (Bracke *et al*., Am J Respir Cell Mol Biol. 2010;42(6):753-61). To pinpoint a source of HA, we studied HA-production and expression of HA modulating enzymes in primary murine pulmonary fibroblasts stimulated with cigarette smoke extract (CSE) or TGF-β1.
Fibroblasts were isolated from lungs of C57BL/6 mice and cultured *in vitro*. At passage 6, cells were stimulated for 24h or 48h with control medium, 5% CSE or 2ng/ml TGF-β1. mRNA expression of HA synthases (Has1, Has2, Has3) and hyaluronidases (Hyal1, Hyal2) was evaluated by RT-PCR. HA production was measured in supernatant by ELISA.
In vitro stimulation of pulmonary fibroblasts with CSE significantly decreased the mRNA expression of Has1 (synthesizing high molecular weight (HMW) HA) and significantly increased the expression of Hyal2 (degrading HMW HA to LMW HA fragments). Stimulation with TGF-β1 resulted in significantly increased mRNA expression of Has2 (synthesizing HMW HA). Accordingly, HA-levels in the fibroblast supernatant decreased significantly upon 48h stimulation with CSE, while they were significantly increased upon 24h or 48h stimulation with TGF-β1.
Decreased Has1 and increased Hyal2 in CSE-stimulated fibroblasts suggests reduced synthesis and enhanced breakdown of HMW HA. This may contribute to the accumulation of LMW HA fragments, observed in CS-exposed mice.

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Cigarette smoke down-regulates the expression of β-catenin in primary human lung fibroblasts
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Rationale: Cigarette smoke is the major cause of COPD/emphysema but the etiology of these diseases is still unknown. β-catenin is a signaling molecule which is regulated through degradation/stabilization mechanism, and which promotes cell proliferation via the Wnt signaling pathway. Decreased β-catenin signaling may be involved in the parenchymal tissue destruction leading to emphysema.
Objectives: Investigate the effect of cigarette smoke on the expression of β-catenin and cell proliferation in primary human lung fibroblasts (n=6).
Methods: Fibroblasts were exposed to cigarette smoke conditioned medium (20%,

24 hours). Expression of β-catenin was determined by immuno-blotting. Proliferation was determined by [3H]-thymidine incorporation.
Results: Cigarette smoke significantly down-regulated β-catenin expression and reduced proliferation of primary lung fibroblasts.
Conclusions: Cigarette smoke down-regulates β -catenin and reduces cell proliferation in lung fibroblasts which may underlie the impaired tissue repair leading to parenchymal destruction in the lung of COPD/emphysema patients.

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Anti-inflammatory effect of beclomethasone dipropionate and formoterol on TNF-α-induced human endothelial cell activation
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Background: Among the structural cells, endothelial cells (EC) represent critical elements: they control leukocyte traffic through the adhesion molecule and chemokine expression.
Aim: To investigate the effect of beclomethasone dipropionate (BDP) and formoterol (F), either alone or in combination, on TNF-α- induced ICAM-1 expression and IL-8 release in human umbilical vein endothelial cells.
Methods: EC were incubated with BDP (10^{-11}-10^{-5}M), F (6/100 with respect to BDP concentration) or drug diluent (control cells: CC) and then exposed to TNF-α (200 U/ml; 4 hrs). For BDP/F combination (w/w: 100/6) EC were treated with low doses of BDP ($10^{-11}$and 10^{-9}M) and/or FOR (6×10^{-13} and 6×10^{-11}M). Surface ICAM-1 expression and IL-8 release were measured by ELISA.
Results: BDP reduced TNF-α-induced IL-8 release (mean±SEM% decrement) with maximal inhibition 23.7±4.2 at 10^{-7} M and 22.6±3.7 at 10^{-5} M ($p<0.05$ *vs* CC); F did not significantly affect IL-8 release (9.2±2.8% at the maximal dose tested). In a different experimental set, BDP/F inhibited IL-8 release with respect to BDP alone (10^{-9}M/6×10^{-11}M: 27.6.2±4.7 *vs* 10^{-9}M: 14.6±3.5%, $p<0.05$, n=8), achieving an effect comparable to that observed with BDP 10^{-5}M alone; BDP/F, although to a less extent, tended to decrease ICAM-1 expression (10^{-9}M/6×10^{-11}M: 17.2±0.8 *vs* 10^{-9}M: 9.3±2.1% and F:4.2±2.6, p=0.05, n=5).
Conclusions: BDP in combination with F is more effective in inhibiting EC activation as compared with BDP alone, thus allowing to use lower BDP doses to reach the maximum inhibitory effect. These results may explain some clinical anti-inflammatory activities of BDP/F combination.