473. Mechanistic studies in airway cell biology

Acute effect of cigarette smoke on proteasome function
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**Background:** Chronic obstructive pulmonary disease (COPD) is associated with an abnormal inflammatory response of the lungs to cigarette smoke (CS). The products of CS oxidatively modify proteins thereby inducing severe oxidative cellular damage. The ubiquitin proteasome system serves as the major disposal system for oxidatively modified proteins and is thus essential for proper cellular function.
Oral Presentation
Room A2 - 08:30 - 10:30

WEDNESDAY, SEPTEMBER 5TH 2012

4535 Decreased levels of elafin in the lungs of patients with acute lung injury as a result of proteolytic cleavage by the proteasome
Clifford Faegh1, Ansite Kerim1, Cecilia O’Kane1, Sinead Weldon1, Allen Chang2, Rodney Levine2, Thelma Craig1, Danny McAuley1, Clifford Taggart1

Objectives: Elafin is a serine protease inhibitor produced locally in the lung by epithelial and inflammatory cells with anti-inflammatory properties. In this study we assessed the temporal changes in elafin produced locally in the lung by epithelial and inflammatory cells with anti-inflammatory properties.

Methods: To determine whether elafin was susceptible to proteolytic cleavage, western blot analysis of recombinant elafin incubated with BAL fluid and protease inhibitors was carried out. Elafin was significantly increased at the onset of ALI compared to healthy volunteers (39.5 ± 5.5 ng/ml vs 5 ± 0.6 ng/ml; p < 0.0004). Elafin levels fell significantly by day 7 compared to baseline (16.4 ± 4.9 ng/ml vs 39.5 ± 5.5 ng/ml; p < 0.05). Incubation of exogenous elafin with ALI BAL fluid revealed that elafin underwent proteolytic cleavage. In contrast, proteolytic cleavage was not observed following incubation of exogenous elafin with healthy volunteer BAL fluid. Pre-incubation of ALI BALF with trypsin and chymotrypsin-like inhibitors abrogated this degradation of elafin. In addition, we demonstrated increased levels and activity of 20S proteasome in the BAL fluid of ALI patients compared to healthy volunteer BAL fluid and confirmed that 20S proteasome was responsible for cleavage of elafin in ALI BAL fluid which inactivated elafin’s anti-elastase activity.

4536 Klotho: An important protein in the formation and development of emphysema
Yan Yue1, Cheng Yuan1, Jingying Zhang1, Lin Zhou1, Mao Huang1, Tiziana Patrizia Cremona1, Christina Vock1, Ali Onder Yildirim2, Heinz Fehrenbach1

Objective: Klotho is a protein that has been associated with aging and provides a protective effect against age-related diseases. In the current study, we aimed to investigate the role of Klotho in human emphysema and its potential therapeutic implications.

Methods: Klotho expression was assessed in human lung tissues from patients with COPD and non-COPD using real-time PCR, Western blotting, and immunohistochemistry. Lung tissues from patients with COPD and non-COPD were used to determine Klotho expression levels.

Results: Klotho protein expression was significantly lower in COPD tissues compared to non-COPD tissues, indicating a possible protective role of Klotho in the pathogenesis of COPD.

Conclusions: Klotho may play an important role in the formation and development of emphysema. Further research is needed to explore the underlying mechanisms.

4537 Immune activation in α1 antitrypsin deficiency (AATD) emphysema: Beyond the protease/antiprotease hypothesis
Erika Bazan1, Simonetta Biasin1, Francesca Lunardi1, Kim Lokar Oliani1, Andrea Ballarin1, Marco Schiavon1, Grazia Turato1, Elisabetta Balestro1, Federico Rea1, Monica Loy1, Manuel Cosio1, Maria Saetta1, Fiorella Calabrese1, 2

Objective: In this study, we aimed to investigate the immune activation in α1 antitrypsin deficiency (AATD) emphysema.

Methods: Immune cell infiltration and cytokine expression were assessed in lung tissues from AATD and non-AATD patients using immunohistochemistry and qPCR.

Results: Immune cell infiltration and cytokine expression were significantly higher in AATD emphysema compared to non-AATD emphysema, suggesting a role for immune activation in the development of AATD emphysema.

Conclusions: Our data support the involvement of immune activation in the pathogenesis of AATD emphysema, highlighting the need for targeted immunomodulatory therapies.

4538 Impact of cigarette smoke exposure on Pseudomonas colonization in COPD
Triziana Patrizia Cremona1, Sharaf Benarafa, Theodor Kocher Institute, University of Bern, Switzerland

Objective: Cigarette smoke exposure is a major risk factor for the development of COPD. In this study, we aimed to investigate the impact of cigarette smoke exposure on Pseudomonas colonization in COPD.

Methods: Pseudomonas colonization was assessed in BAL samples from COPD patients with and without a history of cigarette smoking.

Results: Pseudomonas colonization was significantly higher in COPD patients with a history of cigarette smoking compared to those without.

Conclusions: Cigarette smoke exposure is a significant risk factor for Pseudomonas colonization in COPD, highlighting the need for smoking cessation interventions.

4539 Investigations on the role of region-specific IL-13 receptor alpha 1 expression along the airway tree in mucus production in asthma
Christina Vock1, Ali Onder Yildirim2, Christina Wagner1, Heinz Fehrenbach1, Michael Wegmann1, 2

Objective: In this study, we aimed to investigate the role of region-specific IL-13 receptor α1 expression in mucus production in asthma.

Methods: Mucus production was assessed in BALB/c mice sensitized with OVA and subsequently challenged with OVA for 6 weeks. Lung samples were stained with PAS to analyze mucus production.

Results: Increased mucus production was observed in Balb/c mice sensitized with OVA, suggesting a role for region-specific IL-13 receptor α1 in mucus production.

Conclusions: Our findings support the role of region-specific IL-13 receptor α1 in mucus production in asthma, highlighting the need for targeted therapy strategies.
QPcR revealed higher expression of IL-13Rα1 in proximal versus distal airways in both acutely (1.5-fold) and chronically (1.5-fold) challenged mice. Similarly, in PBS-treated control mice expression of IL-13Rα1 was higher in proximal airways (1.3-fold) and even more prominent in epithelial cells (2-fold) isolated by LCM from proximal airways. Expression levels of transcription factors down-stream the IL-13Rα1 signalling implicated in mucus hyper-production, such as Spdef or FoxA2, were also region-specifically regulated. These results suggest, that the low expression of IL-13Rα1 and hence, the reduced sensitivity towards IL-13, might protect distal airways from mucus plugging which would impair ventilation of the alveoli of the respective acinus.

4540
Role of microRNAs in the regulation of cytokines production by human lung macrophages
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Background: In addition to its functions as a neurotransmitter, ACh may also serve as an autocrine/paracrine modulator of pulmonary inflammation. Our aims were to investigate the role of cholinergic receptors in the M1 (proinflammatory)/M2 (immunomodulatory) polarization of lung macrophages (LM).

Methods: LM were isolated from human resected lungs challenged for 24hrs with LPS to obtain M1 LM or with IL-13 to obtain M2 LM. Expression of α4/α7 nicotinic ACh receptors (nAChRs), M1-5 muscarinic receptors and cytokines was assessed with RT-qPCR. M1- (TNF-α, CCL3, CXCL12 and IL-6) and M2-cytokines (CCL18, CCL22) were quantified in supernatants.

Results: Expression of α7nAChR and M2 and M3 receptors was found in LM. The selective α7nAChR agonist and desensitizing agent GT8-21 (100 μM) inhibited (~65%) the production of M1 cytokines after LPS stimulation and of M2 cytokines after IL-13 stimulation. On the other hand, unstimulated LM in the presence of the α7nAChR antagonist α-bungarotoxin (10μM) showed an increased expression of M1 cytokines at both the transcriptional (5- to 157-fold) and protein level (2.5- to 46-fold), whereas M2 cytokines were not affected. Two agonists with mixed nicotinic/muscarinic activity that do not induce stable α7nAChR desensitization (acetylcholine and carbachol) and the muscarinic antagonists tiotropium and 4-DAMP were devoid of effect.

Conclusions: The blockade of α7nAChR in basal conditions favours LM polarization toward the M1 phenotype, whereas ligand-bound, but potentially non-constituting states of α7nAChR in proinflammatory conditions inhibit the production of M1 cytokines. α7nAChR may thus constitute a pharmacological target in lung inflammatory diseases.

4544
miRNA-17 and -144 regulate cAMP-responsive element binding protein (CREB1) signaling in murine ovalbumin-induced asthma and in human bronchial epithelial cells
Sabine Barz1, Francesca Alessandrini1, Oliver Eickelberg1. 1Comprehensive Pneumology Center, Helmholtz Zentrum München, Munich, Germany; 2Center for Allergy and Environment, Helmholtz Zentrum München, Munich, Germany

Background: MicroRNAs (miRs) are small non-coding RNAs that are essential for immune function and lung development. They are influenced by environmental exposures such as smoke or nutrition, both of which are also known to affect asthma risk development. Previously, we reported increased pulmonary expression of miR-17 and -144 in mice with OVA-induced asthma. This correlated with decreased mRNA and protein levels of CREB1, a validated target of both miRs. In addition, the cAMP-regulated transcriptional co-activators, CRTC-1 and -3, have been described to enhance CREB1-mediated gene transcription (Altarejos et al., Nat Rev Mol Cell Biol., 2011, 12) and are also predicted targets of miR-17 65%) the production of M1 cytokines after LPS stimulation and of M2 cytokines (CCL18, CCL22) were quantified in supernatants.

Methods: Human bronchial epithelial cells (16HBE) were transfected with precursor miRs or antagoniMs for miR-17 and miR-144. The expression of miR and 7nAChR antagonist α-bungarotoxin (10μM) showed an increased expression of M1 cytokines at both the transcriptional (5- to 157-fold) and protein level (2.5- to 46-fold), whereas M2 cytokines were not affected. Two agonists with mixed nicotinic/muscarinic activity that do not induce stable α7nAChR desensitization (acetylcholine and carbachol) and the muscarinic antagonists tiotropium and 4-DAMP were devoid of effect.

Conclusions: The blockade of α7nAChR in basal conditions favours LM polarization toward the M1 phenotype, whereas ligand-bound, but potentially non-constituting states of α7nAChR in proinflammatory conditions inhibit the production of M1 cytokines. α7nAChR may thus constitute a pharmacological target in lung inflammatory diseases.

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Methods: Human bronchial epithelial cells (16HBE) were transfected with precursor miRs or antagoniMs for miR-17 and miR-144. The expression of miR and endogenous CREB1, CRTC-1, -2, and -3 was assessed by RT-qPCR and Western blot analysis.

Results: The mRNA levels of CRTC-1 and -3, but not CRTC-2 in the lung were decreased in OVA-induced murine asthma. In 16HBE cells, the mRNA and protein levels of CREB1 and the mRNA levels of its co-activators CRTC-1, -2, and -3 were significantly decreased after transfection with precursor miR-17 and -144. Vice versa, their expression increased after inhibition of miRs by antagoniMs.

Conclusion: These findings suggest a role for microRNA-17 and -144 in the regulation of CREB1/CRTC signaling of potential relevance in asthma.