

# 5<sup>TH</sup> DZL INTERNATIONAL SYMPOSIUM

„NETWORKS IN LUNG RESEARCH“

June 16-17, 2016  
Curio-Haus, Hamburg, Germany



ABSTRACT BOOK & PROGRAM



# Welcome to Hamburg

The 5th DZL International Symposium on „Networks in Lung Research“ is the first scientific meeting co-organized by three German Centres for Health Research. We are pleased that we have our partners from the German Centre for Infection Research (DZIF), the German Centre for Cardiovascular Disease (DZHK) and the Cluster of Excellence “Inflammation at interfaces” on board for the 5th edition of the International Symposium of the German Center for Lung Research (DZL).

We assembled a fascinating program that focuses on overarching aspects of different diseases. A central topic will be research on co-morbidities, because these represent a linking pin between respiratory medicine, cardiology and infectious diseases.

The Symposium “Networks in Lung Research” features oral presentations of renowned researchers from their respective fields. For each session the review committee has chosen 1-2 of the submitted abstracts for short talks. Moreover, there will be three poster sessions on the Symposium’s topics.

We hope that you’ll enjoy stimulating talks and interesting discussions on our venue – and also in the relaxing atmosphere of the nearby Alster lake in central Hamburg.



Klaus F. Rabe



Heinz Fehrenbach



Matthias Kopp



Peter Zabel

Directors of the Airway Research Center North (ARCN)  
German Center for Lung Research



## Organizers



Deutsches Zentrum für  
Lungenforschung



## Supported by



Inflammation at Interfaces



DZHK

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# **Review and Chair Committee**

## Review and Chair Committee

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# German Center for Lung Research (DZL)

German Center for Lung Research e.V. (DZL)

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- DZL Scientific Officer: Christian Kalberlah

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- DZL Manager TLRC: Birgit Teucher

Universities of Giessen and Marburg Lung Center (UGMLC)

- Director: Werner Seeger
- DZL Manager UGMLC: Sylvia Weissmann



# Program

# THURSDAY, JUNE 16<sup>TH</sup>, 2016

8:00 - 9:00	<b>Registration and coffee</b>
9:00 - 9:15	<b>Welcome</b> // Klaus F. Rabe, Werner Seeger
9:15 - 12:00	<b>DZL Opening Session: Trends in Chronic Disease</b> // Chair: Klaus F. Rabe, Werner Seeger
9:15 - 10:15	Public health, Health equity in Europe
10:15 - 11:00	Scott Budinger (Chicago) Toward a Molecular Understanding of the Age-Related Susceptibility to Chronic Lung Disease
11:00 - 11:30	Sabine Bartel (Borstel) Secretion of miRNA-containing extracellular vesicles by bronchial epithelial cells in allergic airway inflammation
11:30 - 12:00	Mario Pieper (Lübeck) Intravital microscopy of mucus transport in mice provides mechanistic insight into hypertonic saline treatment of cystic fibrosis
12:00 - 13:30	<b>Lunch and Poster Session I</b>
13:30 - 16:15	<b>Excellence Cluster I@I Session: Pulmonary immune pathogenesis</b> // Chair: Marcus A. Mall, Oliver Eickelberg
13:30 - 14:15	Benjamin A. Rybicki (Detroit) Genetic Determinants of Inflammation in Sarcoidosis
14:15 - 15:00	Annegret Fischer (Kiel) Sarcoidosis: Novel aspects in genetics and pathogenesis
15:00 - 15:45	Martin Krause (Kiel) Neonatal ARDS: inflammatory pathways and interventions
15:45 - 16:15	Sebastien Boutin (Heidelberg) Longitudinal analysis of cystic fibrosis of airways' microbiota
16:15 - 17:15	<b>Coffee break and Poster Session II</b>

17:15 - 18:30	<b>DZHK Session: Heart &amp; Lung Session</b> // Chair: Henrik Watz, Thomas Eschenhagen
17:15 - 18:00	Stephan von Haehling (Göttingen) Cachexia and muscle wasting in heart failure and COPD
18:00 - 18:30	Peter Alter (Marburg) Lung function impairment is associated with increased left ventricular cardiac wall stress in COPD: The German multicenter COSYCONET study
19:00	<b>Reception at Mozartsäle</b>
20:00	<b>Dinner</b>

# FRIDAY, JUNE 17<sup>TH</sup>, 2016

9:00 - 12:00	<b>DZL Session II: Epidemiology</b> // Chair: Holger Schulz, Klaus F. Rabe
9:00 - 10:00	Jørgen Vestbo (Manchester) COPD epidemiology – the Copenhagen experience
10:00 - 10:45	Peter Burney (London) COPD, inflammation and co-morbidities
10:45 - 11:15	Larissa Schwarzkopf (Munich) Effect of Chronic Ischemic Heart Disease on costs of care in individuals with COPD – a claims-data based analysis
11:15-12:00	Stefan Blankenberg (Hamburg) The cardio-pulmonary continuum – interaction of heart and lung at the population level
12:00 - 13:30	<b>Lunch and Poster Session III</b>
13:30 - 15:30	<b>DZIF Session: Tuberculosis &amp; Infection</b> // Chair: Sabina Janciauskiene, Stefan Niemann
13:30 - 14:15	Martin Grobusch (Amsterdam) Magic bullets, or magic guns – what is needed to control TB in Africa
14:15 - 15:00	Stefan Niemann (Borstel) Transmission of multidrug resistant Mycobacterium tuberculosis – Superbugs on the horizon?
15:00 - 15:30	Roland Diel (Kiel) Modelling cost-benefit of NGS for personalized tuberculosis control in German healthcare services
15:30 - 16:00	<b>Coffee break</b>

16:00 - 17:15

### **DZL Session III: Chronic Obstructive Lung Diseases**

// Chair: Heinz Fehrenbach, Susanne Krauss-Etschmann

16:00 - 16:45

■ Sebastian Johnston (London)

Mechanisms of virus induced asthma exacerbations and their therapeutic implications

16:45 - 17:15

■ Robin Siebers (Gießen)

A soluble factor mediates alpha-1 antitrypsin-induced inhibition of ATP-induced IL-1 $\beta$  release by monocytic cells

17:15 - 17:30

### **Closing remarks and Poster Prizes**

// Klaus F. Rabe, Werner Seeger



# **General Information**

# Conference Office

Airway Research Center North (ARCN)  
German Center for Lung Research (DZL)

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Wöhrendamm 80  
22927 Grosshansdorf  
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## Symposium Venue

Curio-Haus  
Rothenbaumchaussee II  
20148 Hamburg  
Germany

## Poster Session

We would greatly appreciate your presence at your poster during the respective poster sessions (see below). Posters will remain on display for the duration of the meeting. All posters should be available during the entire time of the Symposium.

Please, setup posters on June 16 (8:00 – 12:00) and dismantle on June 17 (after 15:30).

**Poster Sessions I and II:** Thursday, June 16<sup>th</sup>, 12:00 - 13:30 and 16:15 - 17:15

- Trends in Chronic Disease
- Pulmonary Immune Pathogenesis
- Heart & Lung
- Other Topics of Lung Research

**Poster Session III:** Friday, June 17<sup>th</sup>, 12:00 - 13:30

- Epidemiology
- Tuberculosis & Infection
- Chronic Obstructive Lung Diseases

## Poster Prizes

Three poster prizes of 500 € each will be awarded. Each participant of the Symposium will receive three ballots in order to vote for the best posters. The respective poster numbers should be written on the ballot. A ballot-box is located at the registration desk. Voting ends on June 17 at 15:00.

## Abstract Publication

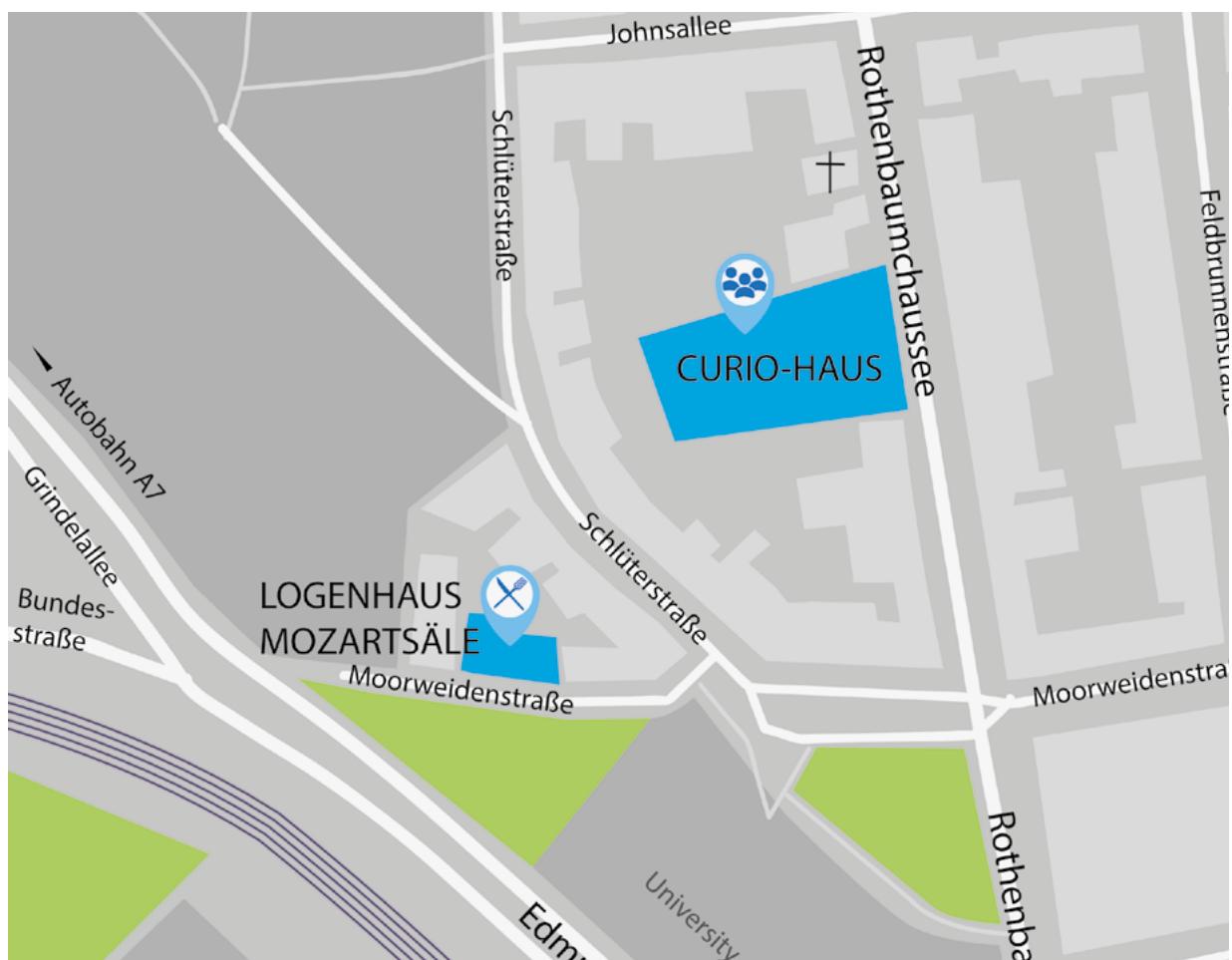
Abstracts will be published in the July edition of the "Pneumologie", the official organ of the DZL.

## Symposium's Dinner at Mozartsäle

The Symposium's Dinner will be held at Mozartsäle in Logenhaus which is in walking distance from the venue (see map). The Dinner is open for pre-registered guests. However, we have tickets for 25 additional guests. Please consult the Symposium's registration desk for tickets (€25,- each).

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## **Abstracts of Oral Presentations**

### **Invited Speakers**

## Genetic Determinants of Inflammation in Sarcoidosis

Ben Rybicki <sup>1</sup>

<sup>1</sup> Henry Ford Hospital, Department of Public Health Sciences Detroit, Michigan, US

Sarcoidosis is a multi-systemic granulomatous disorder of unknown aetiology. Most of the known or suspected genes involved in sarcoidosis play a role in an inflammatory response to an antigenic challenge, with predisposing genes mainly involved in T cell and macrophage function and antigen presentation and recognition. New data will be presented using common variant polygenic risk scores that demonstrates the underlying genetic relationship of sarcoidosis to other complex inflammatory disorders. The potential role of new candidate genes discovered from whole genome association and admixture mapping approaches, such as X-linked Inhibitor of Apoptosis Associated Factor 1 (XAF1), will be explored. Recent research based on gene-pathway analysis, gene-gene and gene-environment interactions has revealed other clues to the genes involved in the inflammatory cascade characteristic of sarcoidosis. The HLA class II DRB1 gene is known to play a major role in sarcoidosis – data on how this gene may be involved in bacterial-induced sarcoidosis, its important role in determining disease course and how DRB1 associations vary with ethnicity – will be presented. Finally, how gene sequencing studies may lead to an even deeper understanding of the genetic architecture of sarcoidosis will be discussed.

## Sarcoidosis: Novel aspects in genetics and pathogenesis

Annegret Fischer <sup>1</sup>

<sup>1</sup> Institute of Clinical Molecular Biology, Christian-Albrechts-University Kiel, Kiel, Germany

Sarcoidosis shares parts of its genetic background with distinct clinical phenotypes. Most prominently genes encoding components of the Th1/Th17 signaling pathways are associated with sarcoidosis and other diseases, like psoriasis and Crohn's disease (Fischer et al., 2015). This talk will give an updated overview of shared genetic factors between sarcoidosis and other genetically connected disorders. The potential functional consequence of the associated variants will be related to the current knowledge on disease pathogenesis and possible clinical implications will be discussed.

Fischer A, Ellinghaus D, Nutsua M, Hofmann S, Montgomery CG, Iannuzzi MC, Rybicki BA, Petrek M, Mrazek F, Pabst S, Grohe C, Grunewald J, Ronninger M, Eklund A, Padyukov L, Mihailovic-Vucinic V, Jovanovic D, Sterclova M, Homolka J, Nothen MM, Herms S, Gieger C, Strauch K, Winkelmann J, Boehm BO, Brand S, Buning C, Schurmann M, Ellinghaus E, Baurecht H, Lieb W, Nebel A, Müller-Quernheim J, Franke A & Schreiber S (2015) Identification of Immune-Relevant Factors Conferring Sarcoidosis Genetic Risk. *Am J Respir Crit Care Med* 192, 727-736.

## **Neonatal acute respiratory distress syndrome (ARDS): inflammatory pathways and interventions**

Martin F. Krause <sup>1</sup>

<sup>1</sup> Kinderklinik, Universitätsklinik Schleswig-Holstein, Campus Kiel

ARDS in adults was first described in 1967, but it took some more years until neonatal and pediatric ARDS were also recognized as similar entities in young patients. ARDS is defined by respiratory failure needing mechanical ventilation, impaired oxygenation, and diffuse infiltrations on chest X-rays reflecting pulmonary edema secondary to inflammation.

Alveolo-capillary barrier dysfunction with disruption of epithelial and endothelial tight/adherence junctions allow inflammatory cells, water, and serum proteins to flood the alveoli followed by inactivation of surfactant and alveolar collapse.

Establishing anti-inflammatory interventions, a myriad of multicenter clinical trials has been performed mainly in adults, however, besides exogenous surfactant therapy in neonatal and pediatric patients, no intervention (corticosteroids included) was identified to excel as a standard therapy for ARDS.

In the search of powerful interventions for affected neonates, we used a single-hit/triple-hit neonatal piglet model of respiratory failure (repeated airway lavage, injurious ventilation, intratracheal lipopolysaccharide). A commercially available surfactant preparation (poractant alfa) served as a carrier substance and for the mitigation of inflammation. Admixed anti-inflammatory substances ("fortified" surfactant) tackling NF-κB, phospholipase A2, lipoxygenase, and interleukin-8 were tested and compared with topical dexamethasone treatment. The inhibition of the acid sphingomyelinase (aSMase)-ceramide pathway proved to be a most powerful intervention and was achieved by admixed imipramine or fortified surfactant components, i.e. inositol-trisphosphate and phosphatidylinositol-3,5-bisphosphate. Phosphatidylglycerol subfractions reduced edema by the reduction of epithelial apoptosis and alveolo-capillary barrier dysfunction.

Inhibition of aSMase in lung tissues reduced ceramide generation, inflammasome NLRP3 oligomerization, caspase-1/interleukin-1 $\beta$  activation, and inhibited the production of matrix substances. Therefore, aSMase is an upstream key player of pulmonary inflammation, and its inhibition mitigates both the acute-exudative and the late-proliferative phase of neonatal ARDS.

"Fortified" surfactant with naturally occurring surfactant components have the potential to be introduced into clinical medicine for the treatment of neonatal ARDS.

## Cachexia and Muscle Wasting in Heart Failure and COPD

Stephan von Haehling <sup>1</sup>

<sup>1</sup> University of Göttingen Medical Centre, Göttingen, Germany

Wasting can present in different forms in patients with chronic illness. Whilst cachexia can be easily diagnosed using weighing scales, the diagnosis of sarcopenia, i.e. loss of skeletal muscle mass and strength, requires more sophisticated technologies. Indeed, sarcopenia and cachexia are different clinical entities. In patients with heart failure or chronic obstructive pulmonary disease (COPD), both have been well recognized as common and partly reversible features that have tremendous effects on patients exercise capacity, quality of life and on survival. Therapeutic approaches may require a combined approach embracing exercise training, nutritional counselling, and drug therapy.

### References:

- Sanders KJ, Kneppers AE, van de Bool C, Langen RC, Schols AM. Cachexia in chronic obstructive pulmonary disease: new insights and therapeutic perspective. *J Cachexia Sarcopenia Muscle* 2016;7:5-22.
- von Haehling S, Anker SD. Prevalence, incidence and clinical impact of cachexia: facts and numbers-update 2014. *J Cachexia Sarcopenia Muscle* 2014;5:261-263.
- von Haehling S. The wasting continuum in heart failure: from sarcopenia to cachexia. *Proc Nutr Soc* 2015;74:367-377.
- von Haehling S, Steinbeck L, Doehner W, Springer J, Anker SD. Muscle wasting in heart failure: An overview. *Int J Biochem Cell Biol* 2013;45:2257-2265.

## COPD epidemiology – the Copenhagen experience

Jørgen Vestbo <sup>1</sup>

<sup>1</sup> University of Manchester, Manchester, UK

The Copenhagen City Heart Study (CCHS) was set up in the 1970's, modelled on the Framingham Study and initiated by Drs Gorm Jensen and Peter Schnohr, both cardiologists. By chance, spirometry was included and assessed in the 14,223 subjects surveyed. Subjects have subsequently been followed up after 5, 15, 25 and 30 years, and the CCHS has formed the basis of the Copenhagen General Population Study (CGPS), led by Prof Børge Nordestgaard and including 110,000 subjects from Copenhagen suburbs, all with questionnaires, blood biobank, spirometry, ECG, and register linkage for follow-up.

Initial COPD epidemiology in Copenhagen was focused on Peter Lange's work on tobacco and FEV1 decline, gradually spreading to look at other risk factors such as mucus hypersecretion and asthma. The strength of the surveys lies in the combination of cardiovascular and pulmonary tests and the availability of nationwide registers for mortality, hospitalisation, cancer, medicine prescription, as well as social registers. With COPD in focus, we have examined body mass and body composition, biomarkers of systemic inflammation, candidate genes, and disease incidence and/or exacerbations using hospital register and prescription data. Lately, we have been able to expand this to disease trajectories and the role of eosinophils in COPD. We have also in the 1990's used the CCHS as basis for a randomised controlled trial of inhaled corticosteroids.

The CCHS and CGPS illustrate the benefit of having well controlled and maintained populations. Cohorts like these can be used to address so-called "boring" - but much needed - questions of prevalence and incidence of symptoms and disease. However, large epidemiological studies can also be used to test hypotheses that are not suited for true experiments or controlled trials. Studies like these represent their underlying populations and replication cohorts are needed.

## COPD, inflammation and co-morbidities

Peter Burney <sup>1</sup>

<sup>1</sup> National Heart and Lung Institute, Imperial College, London, UK

Chronic Obstructive Pulmonary Disease (COPD) is a poorly defined construct that is hard to study. Nevertheless there is a commonly held view, based largely on clinical experience, that there is a strong association between COPD and both cardio-metabolic co-morbidities and common markers of inflammation. This has been further supported by the observation that medication for cardio-metabolic conditions markedly improves the outcome of patients with diagnosed COPD even where they have no history of serious cardiovascular co-morbidity.

Interpretation of many of the studies on which these conclusions are founded are complicated by a problem sometimes referred to as "Berkson's Bias" which arises when an attempt is made to generalise from association studies undertaken among patients. A second problem arises from not making a clear enough distinction between obstruction (FEV1/FVC) and lung size (FVC). This can happen directly from using the FEV1 to define obstruction or indirectly from using FEV1 to define a threshold level of obstruction (e.g. Grade II GOLD).

Much of the pathology and co-morbidity that is associated with poor lung function is associated with a low FVC. These associations are unexplained but a "low" FVC is more common than is often acknowledged. Associations with airflow obstruction have not been consistently demonstrated. How much of any such association is due to a causal association, and how much due to confounding with other factors has again yet to be established.

## The cardio-pulmonary continuum – interaction of heart and lung at the population level

Stefan Blankenberg <sup>1</sup>

<sup>1</sup> University Heart Center Hamburg, Hamburg, Germany

Heart and lung diseases are common comorbidities. They share the same risk factors and in both, dyspnea is the main clinical symptom. Coronary artery disease is the main cause of death in patients with COPD. Cardiac biomarkers predict rehospitalization and mortality in patients with acute exacerbated COPD. Even at the population level, subclinical lung function alteration is associated with measurable changes in biomarkers of cardiac stress and necrosis.

Impairment of lung function may cause symptoms of heart failure (HF), although no cardiovascular or structural heart disease is present. Patients with COPD suffer more frequently from diastolic dysfunction. This has been explained by shortening of diastolic filling due to medication-induced tachycardia and hypoxemia. Because of the mechanical compression of the heart, an increase in emphysema was associated with a reduction in cardiac chamber size. Even at the population level, mild pulmonary impairment affects the left ventricular performance. A decrease of FEV1/FVC ratio was shown to be associated with a reduction in stroke volume even after exclusion of individuals with COPD. Additionally, moderately reduced FEV1 was shown to be related to an increased incidence of HF.

The impact of COPD on the heart has repeatedly been shown in the past. Recently published studies could extend these findings to the population level and thus underline the close interaction of heart and lung.

## **Magic bullets, or magic guns – what is needed to control TB in Africa?**

Martin P. Grobusch <sup>1,2,3,4</sup>

<sup>1</sup> Center of Tropical Medicine and Travel Medicine, Department of Infectious Diseases, Division of Internal Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

<sup>2</sup> Institut für Tropenmedizin, Universität Tübingen, Tübingen, Germany

<sup>3</sup> Centre de Recherches Médicales en Lambaréne (CERMEL), Lambaréne, Gabon

<sup>4</sup> Institute of Infectious Diseases and Molecular Medicine, University of Cape Town, Cape Town, South Africa

Significant progress has been made over the past decade in the development of novel diagnostic and therapeutic tools for tuberculosis, and drug-resistant tuberculosis in particular, and for the first time, a serious debate on feasibility and obstacles of tuberculosis control towards elimination evolves.

In sub-Saharan Africa, the HIV co-pandemic, massive gaps in our understanding of the precise epidemiology of drug-sensitive and drug-resistant tuberculosis, failing health systems and financial constraints are major obstacles for successful tuberculosis control.

Two distinct country examples, namely South Africa and Gabon (Central Africa) will serve to highlight the diversity of the tuberculosis problem and in the quest for optimizing tuberculosis control; and which major factors influence the outcome of tuberculosis control efforts beyond the availability of state-of-the-art diagnostics and medication.

## Mechanisms of virus induced asthma exacerbations and their therapeutic implications

Sebastian L Johnston <sup>1, 2, 3, 4, 5, 6, 7</sup>

<sup>1</sup> Professor of Respiratory Medicine

<sup>2</sup> National Heart and Lung Institute

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<sup>6</sup> Director, MRC & Asthma UK Centre in Allergic Mechanisms of Asthma

<sup>7</sup> Asthma UK Clinical Professor

Asthma exacerbations are a major unmet medical need. Current therapies fail to prevent the majority of exacerbations, and new preventive/treatment approaches are needed.

Respiratory virus infections are the dominant cause of asthma exacerbations with human rhinoviruses the commonest precipitant. People with asthma have increased severity of upper and lower respiratory illness with virus infections. Experimental studies and models of rhinovirus induced asthma exacerbations have identified impaired innate (type I & III IFNS) and Th1-mediated (IL-12, IL-15, IL-18 and IFN- $\gamma$ ) antiviral immune responses as important in asthma exacerbation pathogenesis. The mechanisms behind this impaired immunity are poorly understood, but involve SOCS1 protein mediated as well as cross-linking of IgE bound to Fc $\epsilon$ RI on DC-mediated suppression of antiviral immunity.

Virus infections are associated with Th1, not Th2 immune responses. Asthma is a Th2 mediated disease in the stable state, but Th1 mediated inflammation is induced following viral infections. It is not known whether respiratory virus infection in asthma leads to additional amplification of Th2 inflammation in vivo, but studies reporting powerful synergistic interactions between allergen exposure and virus infection in increasing risk of asthma exacerbations, suggest that Th2 cytokines may be important. IL-25 and IL-33 are epithelial-derived mediators identified as inducers of type-2 inflammation in models of Th2 disease. The role of innate lymphoid cells in human asthma is unknown. The roles of IL-25 and IL-33 in human and mouse models of virus induced asthma exacerbations will be discussed.

The therapeutic implications of these findings in asthma exacerbations will then be discussed.





## **Abstracts of Oral Presentations**

### **Short Oral Presentations**

## **Secretion of miRNA-containing extracellular vesicles (EV) by bronchial epithelial cells in allergic airway inflammation**

Sabine Bartel <sup>1,\*</sup>, Andrea C. Schamberger <sup>2</sup>, Oliver Eickelberg <sup>2</sup>, and Susanne Krauss-Etschmann <sup>1</sup>

<sup>1</sup> Research Center Borstel

<sup>2</sup> Comprehensive Pneumology Center Munich

\* Presenting author

**Background:** miRNAs (miR) are critical regulators of the immune system. Recently they have been shown to be secreted into EVs for inter-cell communication (Valadi et al., Nat Cell Biol, 2007). We previously identified an up-regulation of miR-17 and -21 in murine lung homogenate in Ovalbumin (OVA) induced allergic airway inflammation (AAI).

**Objective:** We now asked if mir-17 and miR-21 are produced by the human bronchial epithelium and released into EVs in AAI.

**Methods:** Primary normal human bronchial epithelial (NHBE) cells were cultured at the air-liquid interface and treated with Interleukin (IL)13 to model a T-helper 2 environment. EVs were isolated by Exoquick-TC or qEV columns and quantified by bead-based flow cytometry for CD63. miRNA levels were assessed by qRT-PCR.

**Results:** CD63+ EVs were present in both the apical as well as the basal cell compartment with different secretion patterns upon IL13 treatment. Upon IL13 treatment EV miR-17 and -21 levels increased first in the basal cell compartment and later in the apical. In two murine models for AAI, OVA and house-dust mite, miR-17 and -21 were significantly up-regulated in EVs from broncho-alveolar lavage fluid (BALF) while CD63+ EV amounts were similar.

**Conclusion:** EV are secreted in vitro upon IL13 treatment of primary NHBE cells and are present in BALF of murine AAI and we have first hints for altered miRNA levels in "asthmatic EVs". Currently, we are investigating if a distribution of miRNA via EVs can perpetuate an asthmatic response and expand it to other cell types such as immune cells, or vice versa have a regenerative effect on the airway epithelium.

# Intravital microscopy of mucus transport in mice provides mechanistic insight into hypertonic saline treatment of Cystic Fibrosis

Mario Pieper <sup>1,\*</sup>, Hinnerk Schulz-Hildebrandt <sup>2</sup>, Markus Mall <sup>3</sup>, Gereon Hüttmann <sup>2</sup>, and Peter König <sup>1</sup>

<sup>1</sup> Institute of Anatomy, University of Lübeck, ARCN, DZL

<sup>2</sup> Institute of Biomedical Optics, University of Lübeck, ARCN, DZL

<sup>3</sup> Department of Translational Pulmonology, TLRC Heidelberg, DZL and Division of Pediatric Pulmonology & Allergy and Cystic Fibrosis Center, Department of Pediatrics, University of Heidelberg

\* Presenting author

In clinical studies of cystic fibrosis (CF) patients, inhalation of 7% NaCl was found to be superior compared to 0.9%, which is attributed to increased hydration of mucus. However, mechanistic insight has been hampered by the inability to visualize mucus transport *in vivo* with microscopic resolution.

To overcome this problem we developed a custom-built optical coherence microscope (OCM) providing a microscopic resolution. Images of mucus transport through the intact trachea were recorded in anesthetized spontaneously breathing wild type (WT) and  $\beta$ ENaC-overexpressing mice as a model for CF. NaCl solution (30  $\mu$ l, 0.9% and 7%, respectively) was applied intranasally.

The airway epithelium of WT and transgenic mice is covered by few  $\mu$ m thick airway surface liquid (ASL). After intranasal application of saline an early fast cough-like fluid transport was observed in both strains. This mechanism cleared a noticeable amount of liquid. After stimulation of WT mice the ASL thickness increased transiently and returned to baseline within minutes. In contrast, in  $\beta$ ENaC-overexpressing mice, application of saline induced mucus mobilization and transport over a period of more than 2 h. Imaging revealed that treatment of CF-mice with 0.9% saline resulted in an inhomogeneous transport with a high variance of ASL thickness over the tracheal epithelium over time. This also resulted in frequently clogging of the trachea and inability to efficiently remove mucus via the larynx. In contrast, treatment of  $\beta$ ENaC-overexpressing mice with 7% NaCl resulted in a more homogeneous mucus transport with lower variance of ASL thickness over the tracheal epithelium over time compared to 0.9% treatment.

Imaging of intravital mucus transport revealed substantial differences in the effects of 7% NaCl vs. 0.9% on mucus properties and transport. These results also demonstrate that OCM imaging is a promising tool for the preclinical evaluation of compounds that influence mucus transport.

## Longitudinal analysis of cystic fibrosis airways' microbiota

Sebastien Boutin <sup>1,\*</sup>, Mirjam Stahl <sup>1</sup>, Simon Y. Graeber <sup>1</sup>, Susanne Dittrich <sup>1</sup>, Marcus Mall <sup>1</sup>, and Alexander H. Dalpke <sup>1</sup>

<sup>1</sup> University of Heidelberg

\* Presenting author

In the last decades, complexity of cystic fibrosis airways microbiota just started to get explored. However, most previous studies used cross-sectional designs thus obtaining snapshots of the lung microbiota at one time point. A longitudinal approach is needed to explore the stability of the CF microbiota over time. In this study, we collected and analyzed 389 samples from the lower airways microbiota (sputum and/or throat swabs) of 91 CF patients (0 to 70 yo) over a long timeframe (4-34 samples over 2 years). The microbiota was then explored by 16S amplicons sequencing. We observe a high diversity in the microbiota of the lower airways with more than 10 types of microbial communities. One of the most represented and more stable is a *Pseudomonas aeruginosa* dominating microbiome and the second characterized by a dominance of three OTUs belonging to *Neisseria*, *Veillonella* and *Prevotella* genera. We also observed that antibiotic treatment is also a trigger of changes in the microbiota. Our results lead us to build the following model: during the early stage of CF lung disease, lower airways are colonized by members of the throat microbiota. The relatively high variability of the lower airways can be explained by a cycle of colonization-clearance. During this stage, the presence in the lower airway of three OTUs, belonging to *Prevotella*, *Neisseria* and *Veillonella* which are highly abundant in the throat, is linked to a better lung function and a low abundance of *P. aeruginosa*. In the late stage of CF lung disease, characterized by a chronic infection by *P. aeruginosa*, it seems that the colonization-clearance cycle is stopped due to a lack of clearance or a default in the re-colonization process. This break probably leads to the creation of an independent niche in the lower airways which triggers a stable colonization by *P. aeruginosa*.

## Lung function impairment is associated with increased left ventricular cardiac wall stress in COPD: The German multicenter COSYCONET study

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**Introduction.** Cardiovascular comorbidities are common in patients with COPD and associated with increased morbidity and mortality. Underlying mechanisms are largely unclear. We therefore studied the association between lung hyperinflation, airflow limitation, and left ventricular (LV) function, morphology and wall stress in a comprehensive manner using structural equation modeling (SEM) in a large data set from the COPD cohort COSYCONET.

**Methods.** Within COSYCONET 2,741 patients were recruited. Applying predefined criteria of completeness and quality, a subset of patients underwent baseline evaluations including forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), intrathoracic gas volume (ITGV) and echocardiography including LV mass, end-diastolic (LVEDV) and end-systolic volume (LVESV), end-diastolic and end-systolic LV wall stress. Conventional multivariate regression analysis was followed by SEM with defined latent variables (constructs) 'left heart' for cardiac and 'lung' for lung function endpoints.

**Results.** Data from 1,435 participants were analyzed (mean age 64.3 years; 54.8% male; FEV1 57.6%). In the multivariate regression analysis significant correlations were between: FEV1/FVC and LVESV; FEV1/FVC and end-systolic and end-diastolic LV wall stress; and ITGV and end-diastolic wall stress. The SEM fit the data well with a Comparative Fit Index of 0.995. There were strong correlations between LV end-diastolic and end-systolic wall stress and 'left heart', and between 'left heart' and 'lung', suggesting a direct link between impaired lung function and LV wall stress.

**Discussion.** For the first time, we have shown a direct link between lung function and LV wall stress in COPD. These associations are compatible with the view that obstruction and hyperinflation exert increased distending forces on the LV leading to increased wall stress. The findings suggest that beyond potential effects of inflammation the development of cardiovascular comorbidities could be, at least partially, influenced by mechanical factors, which supports to explain beneficial effects of COPD treatment on cardiac function.

## **Effect of Chronic Ischemic Heart Disease on costs of care in individuals with COPD – a claims-data based analysis**

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**Background:** Chronic Obstructive Pulmonary Disease (COPD) and Ischemic Heart Disease (IHD) are cost-intense and highly prevalent diseases in Germany. Owing to shared risk factors, the co-occurrence of both diseases is very likely, with consequences on morbidity, mortality, and costs of care. Our claims data-based study aimed at quantifying IHD-associated excess costs in individuals with COPD from the perspective of the German Statutory Health Insurance (SHI).

**Methods:** Based on claims data from the research database of Arvato Health Analytics GmbH which contains anonymized data of 7 million Germans insured at various SHI funds, we identified individuals with COPD with and without IHD based on multiple documentations of specific ICD-10 codes (COPD J44; IHD I21-I25) in 2011. Mean annual per capita expenditures for both groups in 2012 were analyzed via Generalized Linear Models. Age, gender, and morbidity were considered as covariates. Age-dependent developments of costs of care for both groups were analyzed with gender-stratified Generalized Additive Models.

**Results:** 10,287 of 36,605 COPD patients had comorbid IHD (28%). The prevalence of IHD in individuals with COPD increased with rising age and across the entire age range, men were more frequently concerned. Individuals with COPD and IHD incurred overall SHI expenditures of ca. €6,100 compared to ca. €4,800 in those with COPD only. Excess costs of IHD had an inverse u-shape, peaking in the early (men) respectively late seventies (women).

**Discussion:** Comorbid IHD is a cost-driving factor in patients with COPD and excess costs vary age- and to lesser extent gender-dependently. Results indicate that multi-morbidity goes along with complex disease management requirement which might not be sufficiently addressed in existing approaches that focus on a single indication (COPD or IHD). This information might provide vital input to the parameterization of real-world COPD models, since current approaches tend to disregard comorbidity.

## Modelling cost-benefit of NGS for personalized tuberculosis control in German healthcare services

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New genomic technologies such as next-generation sequencing (NGS) may yield information on multiple genetic developments of *Mycobacterium tuberculosis* (MTB). Basically, NGS allows management of contact investigation to be targeted especially at contacts likely to benefit, thus reducing secondary TB morbidity and follow-up treatment costs in the near future. However, NGS has not been implemented widely, despite of its high-throughput capacity and rapidly decreasing costs of DNA sequencing. Economic evidence could help applying NGS results to epidemiological and clinical practice, but there are several methodological challenges, including selecting the appropriate type of economic evaluation.

Currently, in Germany insufficient epidemiological methods are used to identify the origin of a MTB infection, and also traditional "fingerprinting" tools have proven to be more or less unspecific when trying to detect "clusters" including recently transmitted MTB strains.

In a retrospective analysis of the first 98 clusters assessed by NGS at the National Reference for Mycobacteria in Borstel including TB patients coming from Hamburg between 2005 and 2015 this presentation considers the challenges with respect to measuring costs of screening investigations, outcome and effectiveness prior and after implementation of NGS. This will include the resources associated with Public Health staff time, screening procedures, laboratory, appropriate drug selection and the costs of necessary epidemiological backup.

The potential cost-benefit by real-time clustering using NGS from the perspective of the Public Health Department Hamburg as well from that of the clinician will be demonstrated in terms of unnecessary TB screening and treatments avoided. Another issue is the evaluation of the predictive potential of NGS in outbreak conditions.

## A soluble factor mediates alpha-1 antitrypsin-induced inhibition of ATP-induced IL-1 $\beta$ release by monocytic cells

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**Introduction:** Alpha-1 antitrypsin (AAT), a major antiprotease of the lung, exerts anti-inflammatory functions via poorly defined pathways. At previous DZL meetings, we provided evidence that AAT is a potent inhibitor of ATP-induced release of IL-1 $\beta$  from human monocytes, and that CD36, calcium-independent phospholipase A2 (iPLA2) and nicotinic acetylcholine receptor (nAChR) subunit  $\alpha$ 9 participate in this mechanism. Here we test the hypothesis that AAT induces the release of a soluble factor X from U937 cells, which then inhibits ATP-dependent inflammasome activation via nAChR. As glycerophosphocholine (GPC) is a metabolite of membrane phospholipids cleaved by iPLA2, we tested whether GPC functionally resembles factor X.

**Methods:** A low molecular weight fraction of conditioned medium from AAT-treated U937 cells was produced by ultrafiltration (cut-off 10 kDa). LPS-primed U937 cells were stimulated with the P2X7 agonist 2'(3')-O-(4-Benzoylbenzoyl)adenosine-5'-triphosphate (BzATP) and IL-1 $\beta$  was measured in cell culture supernatants by ELISA. The low molecular weight fraction of conditioned medium or GPC was added together with BzATP in the presence or absence of nicotinic antagonists.

**Results:** Conditioned medium from AAT-treated U937 cells contained a low molecular weight factor that significantly reduced the release of IL-1 $\beta$  in response to BzATP. GPC also significantly inhibited BzATP-induced IL-1 $\beta$  release in a dose-dependent manner. General nicotinic antagonists and specific antagonists of nAChR containing subunit  $\alpha$ 9 completely reverted the inhibitory effect of conditioned medium and GPC.

**Discussion:** Our results confirm the hypothesis that AAT stimulates the release of a soluble factor X from U937 cells. This factor seems to be a potent agonist of nAChR containing subunit  $\alpha$ 9, which efficiently inhibits P2X7 receptors. Hence, we suggest a triple-membrane-passing signaling pathway triggered by AAT that inhibits inflammasome activation and release of IL-1 $\beta$  in human monocytes. Understanding this novel signaling pathway might lead to the development of efficient therapies for the treatment of systemic inflammation.





# **Poster Abstracts**

# Adverse Reactions against Biologicals – Identification and Characterization of Immunogenic Epitopes and Potential Biomarkers

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**Background:** Today there is an increase in the indication for biologicals for the treatment of various inflammatory diseases. For example, TNF-alpha blockers are used for the treatment of rheumatic disease, which also affects the lung by pulmonary hypertension and fibrosis. However, during treatment, several clinically relevant side effects occur: loss of drug efficacy, hypersensitivity and anaphylactic reactions which are mediated by IgE antibodies. These conditions lead to a discontinuation of the treatment or a switch to a different therapeutic antibody. Yet there is no existing guideline which specific therapeutic antibody should be prescribed for which patient.

**Materials and Methods:** In order to identify and analyze carbohydrate and peptide epitopes of biologicals, a blot-based test system for anti-biological antibodies was developed facilitating the parallel analysis of intact and processed therapeutic antibodies. Two patient cohorts with antibodies against the biologicals cetuximab and infliximab were used to prove the validity of the test system for anti-biological IgE. For the identification of peptide epitopes, a peptide microarray screening system was established for infliximab.

**Results:** Combined blot tests and ImmunoCAP analyses of serum from meat allergic patients reveal anti-cetuximab IgE associated with the glycosylation of cetuximab. Sera from a group of infliximab-treated patients were screened for anti-infliximab IgE. The developed test system with native and processed cetuximab/infliximab was used for the detection and characterization of anti-infliximab IgE and potential cross-reactions between the therapeutic antibodies. Furthermore, the peptide microarray screen led to the identification of six IgG epitopes on infliximab, partly located in the TNF-alpha binding region.

**Conclusion:** The development of versatile diagnostic platforms for the detection and characterization of peptide- and carbohydrate-specific IgE is a prerequisite for the prediction of clinically relevant IgE-mediated patient reactions against biologicals. These results shall lead to a more secure suggestion of a safer and more effective therapeutic antibody therapy.

## **Effects of parental preconceptional or prenatal cigarette smoke on lung development and immunity in offspring**

Barbara Hammer <sup>1,\*</sup>, Martin Wolff <sup>1</sup>, Sabine Bartel <sup>1</sup>, Sebastian Reuter <sup>1</sup>, and Susanne Krauss-Etschmann <sup>1</sup>

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**Introduction:** Epidemiological studies demonstrate that in utero smoke exposure negatively affects lung development and lung function, thereby increasing susceptibility for asthma and COPD (Svanes, Thorax 2010; Beyer, Eur J Med Res 2009; Duijts, Eur J Epidemiol 2014). More recently, it was proposed that smoking of boys at pre-teenage equally affects disease risks in offspring (Svanes, Int J Epidemiol in revision). To obtain inside into the underlying mechanisms and to develop early life intervention strategies, studies in transgenerational mouse models are needed.

**Objective:** In my PhD thesis, I will develop a murine transgenerational model of maternal and paternal cigarette smoking to analyze the effects on lung morphogenesis and disease susceptibility in offspring.

**Experimental setup:** Female mice will be exposed to mainstream smoke using a smoke exposure system (inexpose; SCIREQ) four days before mating until the end of pregnancy. In a second approach, juvenile male/female mice will be exposed to mainstream smoke. Lung and immune development will be monitored in offspring. Furthermore, we will investigate their susceptibility to lung diseases in later life in response to allergen and smoking. As a first step, we defined the optimal smoke dose for maternal smoking where we can assume smoking but not inflammation related effects on the offspring.

**Results:** Female mice were exposed to 2000 mg/m<sup>3</sup> of cigarette smoke. Comparison to higher exposure doses they show a similar decline of baseline lung compliance but only minimal signs of an airway inflammation and no changes in weight gain during the exposure. This dose therefore fulfills the criteria for further experiments.

**Purpose:** Results obtained from this study will inform how parental smoking affects lung development and susceptibility to develop lung diseases in later life. This model and the gained data can be used to develop innovative early life intervention strategies for primary prevention of lung diseases.

## Lipophilic allergens: their isolation and clinical relevance

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**Introduction:** Preliminary results on house dust mite allergens could associate lipophilic allergens with allergic asthma. The DZL-flag ship project "basic science" focuses on the effect of allergenic structure on allergenicity with particular emphasis on asthma development and molecular phenotyping. Peanut allergy in this regard serves as a model disease, since the lipid-rich fruit peanut induces severe, sometimes fatal allergic symptoms including bronchospasm. Oleosins, a class of highly lipophilic oil body proteins have been found to be triggers of severe allergic reactions to hazelnut and sesame. The aims of this study were isolation of the peanut oleosins and the assessment of their allergenicity in order to have tools for mechanistic research on sensitization and asthma development.

**Methods:** A comprehensive oleosin isolation procedure was established based on oil body extraction and subsequent step by step purification along with preparative electrophoresis. Oil body proteins were identified by N-terminal sequencing, peptide mass fingerprinting and homology search against databases. The IgE-binding potency of oleosins was evaluated by immunoblot analysis and basophil activation tests.

**Results:** Oleosins were isolated and purified from the complex lipophilic matrix of peanut. Mass spectrometry analysis identified eight peanut oleosins, ranging from 15.5 to 17.5 kDa, named Ara h 10, 11, 14 and 15. IgE binding to purified oleosins was observed in 30 of 47 sera from peanut-allergic patients by means of immunoblotting. IgE-dependent basophil activation was induced in vitro in a dose-dependent manner in peanut-allergic patients, but not in controls. Oleosin sensitization was observed exclusively in patients suffering from severe allergic reactions e. g. dyspnea, urticaria and anaphylactic shock.

**Discussion:** A novel strategy for the simultaneous isolation of the lipophilic peanut allergens, oleosins, was successfully established. Their allergenicity was demonstrated via immunoblot and basophil activation test. These early results provide some evidence that oleosins are associated with more severe allergic reactions.

## Ectopic activation of EGFR signaling in the airway epithelium of *Drosophila* induces lung cancer-like phenotypes

Judith Bossen <sup>1,\*</sup>, Karin Uliczka <sup>2</sup>, Marcus Thiedmann <sup>1</sup>, Mandy Mai <sup>2</sup>, Christine Fink <sup>1</sup>, Holger Heine <sup>2</sup>, and Thomas Roeder <sup>1</sup>

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Lung cancer is one of the most aggressive types of cancer with an extremely high overall fatality rate due to late state detection and the lack of late state treatment options. *Drosophila* is a model for studying chronic inflammatory diseases of the lung, it's tracheal system shares comprehensive structural and functional similarities with the human lung, thus making it an ideal model for studying the molecular framework underlying lung cancer development.

Targeted overexpression of human oncogenes and their *Drosophila* homologs in the airway epithelium was chosen to induce cancer-like phenotypes in *Drosophila*. The larvae were analyzed regarding hyper- and metaplasia of the epithelium. For this, we measured e.g. thickening of the epithelium or a change in number and size of the nuclei. Larvae showing a strong cancer-like phenotype were selected for treatment with potential anti-cancer drugs in a 96-well format.

A number of oncogenes were able to induce epithelial meta- and hyperplasia indicating that they can induce tumor formation in the airway system. A thickening of the epithelium and an increased number of nuclei are showing the proliferating state of the tissue. Ectopic overexpression of a constitutively active version of the EGFR gene in larval airway epithelia is lethal in early developmental stages. When treated with specific EGFR inhibitors these larvae can successfully develop into adult flies.

The results highlight the potential of *Drosophila* as a model in cancer research and its usefulness in high-throughput anti-cancer drug screenings. It is our goal to use *Drosophila* lung cancer models to understand the molecular underlying tumor formation and progression and to identify compounds that can be used as therapeutic agents.

## Dysregulation of epithelial JAK/STAT-signaling leads to malformation of the fruit fly's airways

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Worldwide, many hundred million people suffer from chronic respiratory diseases like COPD, pulmonary hypertension and asthma. Beside genetic predisposition there are many risk factors like allergens, tobacco smoke and air pollution that may promote development of asthma. However, the signaling components relevant for pathogenesis are not yet fully understood. Key members of the evolutionary highly conserved JAK/STAT pathway (STAT3/6) are known to mediate susceptibility to asthma.

We used *Drosophila melanogaster* to analyze the role of JAK/STAT signaling in the fly's airway epithelium under different stress conditions including oxidative stress, UV radiation and hypoxia. Furthermore, we investigated if and how the endogenous ligands of the JAK/STAT signaling (upd ligands) are regulated and if their enhanced expression leads to structural changes of the airways epithelia.

Our results illustrate a time dependent expression of the ligands upd2 and upd3 in oxygen undersupplied wildtype flies and therewith an autocrine activation of JAK/STAT signaling. Furthermore, we observed structural changes due to ectopic overexpression of upd ligands in the whole tracheal system. We could detect a dramatic increase in epithelial plasticity as represented by epithelial thickening of the dorsal trunks. Additionally, we were able to show that Wnt signaling is downstream of JAK/STAT signaling, which is presumably responsible for the structurally damaged epithelium.

JAK/STAT appears to be a key factor in maintaining the epithelial homeostasis of the fruit fly's respiratory system, highlighting that carriers of susceptible alleles (STAT3/6) may be prone to develop structural changes of their airways relevant for disease development.

## **Drosophila melanogaster - an emerging model to study transgenerational effects of parental smoking**

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Health consequences of tobacco smoking are widely studied and well understood. Much less analyzed, but even more considerable are health effects which may occur in progeny, not only caused by prenatal cigarette smoke exposure, but also due to transgenerational inheritance. As prenatal exposure to (grand) maternal cigarette smoking is associated with a higher risk for childhood asthma, mechanisms of transgenerational inheritance are likewise responsible for the development of the disease. So far mouse models of tobacco smoke-related airway diseases have been used for transgenerational studies. However, they are limited to the second and third generation due to long generation times, high breeding costs and the shortage of breeding space. It is, therefore, indispensable to switch to a simple model system that allows studies on subsequent generations in a short period of time. In this context, the fruit fly *Drosophila melanogaster* is an attractive model due to its short generation time, high fertility, ease of culture and the availability of various genetic tools. In addition, *Drosophila* has been already successfully used to model innate immune dysfunctions and epithelial alterations frequently observed in asthma and COPD patients. This can be attributed to the simple epithelial architecture of the fly's airways, the lack of an adaptive immune system and the high degree of similarities between the epithelial innate immune system of flies and men.

To investigate transgenerational effects of parental smoke exposure on airway epithelial cells, we aim at establishing a *Drosophila* smoking model mimicking key features of an antioxidant response phenotype. After that, we will use this model to identify molecules and pathways mediating cigarette smoke-induced transgenerational alterations in airway epithelial cells. Ideally these results should be extrapolated to mammalian systems to identify novel regulators of asthma or COPD that also have the potential to serve as therapeutic targets in humans.

## Human macrophages differentially express long non-coding RNAs upon polarization

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**Introduction:** Macrophage polarization is a key feature of innate immunity. This inherent versatility of macrophage subtype manifestation helps the host immune system to adapt to changing requirements in the course of defence against pathogens, but it also poses a risk. Inappropriate polarization can lead to a skewed and detrimental immune response. The complex process of macrophage polarization thus needs to be tightly controlled. A newly emerging type RNAs, the long non-coding RNAs, influence cellular processes. Unlike microRNAs, lncRNAs have no defined mode of action, but can exert their function in a plethora of different ways. In this study, we aim to describe the lncRNA setup in polarized human blood derived macrophages.

**Methods:** We established a routine to obtain homogenous populations of reliably polarized macrophages (M0, M1, M2) by surface marker discrimination. We investigated their transcriptome by deep sequencing with regard to their mRNA and lncRNA configuration.

**Results:** The coding transcriptome was very well suited for the discrimination of the polarized subtypes. 500 different transcripts were used to discriminate the three macrophage subtypes. Furthermore, we identified a set of 100 lncRNAs whose expression pattern is indicative of the macrophage activation status. Similar to the coding transcriptome, changes in lncRNA expression seemed to be more pronounced upon M1 stimulation, while the majority was irresponsive or only slightly responsive to M2 stimulation.

**Discussion:** While their function remains to be elucidated, we present a comprehensive description of lncRNA expression in the context of macrophage polarization. We will correlate their expression with macrophage activation markers from the coding transcriptome to identify possible co-regulation that might be indicative of functional association.

## Systemic characterization of macrophage phenotypes in allergic airway inflammation

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**Introduction:** Macrophages are central players in lung pathology with both regulatory and effector properties. Besides activation toward the M1 subtype (classic activation), alternatively activated M2 macrophages have been implicated in pneumonia and allergy. We investigate the role of microRNAs in macrophage polarization-associated diseases. Here, we analyzed the systemic RNA phenotype of murine lung macrophages in allergic airway inflammation. We are establishing an *in vitro* model with bone-marrow-derived macrophages (BMMs) to mimick the transcriptome changes which we observed here.

**Methods:** Macrophages from the bronchoalveolar lavage fluid (BALF) and lung homogenate of mice with acute OVA-induced eosinophilic airway inflammation were sorted on the basis of the CD11 and SiglecF surface markers, and total RNA was analyzed subsequently. The transcriptome was investigated with Ingenuity Pathway Analysis to identify core regulatory units of the OVA-induced inflammation.

**Results:** Differential miRNA expression could be observed that seems to be tissue- and asthma dependent, as illustrated by principal component analysis (PCA). In the interstitial macrophage fraction, up-regulation of the M1-associated miR-155-5p and down-regulation of the equally M1-associated miR-146a-5p was observed, among others. On mRNA level, prominent markers of alternative macrophage activation were found to be up-regulated, such as Arginase (Arg1) and the IL4-induced Retnla (Fizz1), CCL17 and Mgl2. In total, IL-4 and IL-13 were identified as the crucial cytokines that cause the OVA-induced transcriptome profile. We are reproducing these findings *in vitro* in IL-4 and IL-13 treated BMMs.

**Discussion:** In accordance with the TH2-skewed environment in eosinophilic airway inflammation, we could identify IL-4 and IL-13 as decisive for the activation of macrophages upon OVA-stimulation. We could partly reproduce this pattern by administration of IL-4 and IL-13 to BMMS. We aim to interfere with this polarization trajectory by manipulating key miRNAs that target genes which are central for OVA-induced macrophage activation, such as mafB and IRF4.

## **Legionella pneumophila outer membrane vesicles are potent pro-inflammatory stimulators**

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The formation and release of outer membrane vesicles (OMVs) is a common phenomenon of gram-negative bacteria, including *Legionella pneumophila* (*L. pneumophila*), which is a causative agent of severe community- and hospital-acquired pneumonia. Upon its transmission into the human lung, *L. pneumophila* primarily replicates in macrophages.

In this study, we analyzed the influence of *L. pneumophila* OMVs on macrophages. For this, THP-1 cells were PMA-differentiated and incubated with increasing doses of OMVs, which led to a classical activation of macrophages with the release of pro-inflammatory cytokines. By use of murine bone marrow derived macrophages of different toll like receptor (TLR) knockouts, we determined the impact of TLR-signaling on the recognition of *Legionella* OMVs. To prove the effect of *L. pneumophila* OMVs on bacterial replication, THP-1 cells were pre-incubated with different doses of OMVs and were subsequently infected with *L. pneumophila*. Bacterial replication was determined by colony forming unit (CFU) assay. Treatment of THP-1 cells with OMVs prior to infection reduced replication of *L. pneumophila* in THP-1 cells. While blocking of TLR2 activation restored bacterial replication in the first 24 h of infection, NF- $\kappa$ B inhibition further reduced the replicative potential of *L. pneumophila*. With prolonged infection, OMV pre-treated macrophages became more permissive for bacterial replication than untreated cells, and showed reduced pro-inflammatory cytokine induction. That was neither dependent on OMV protein integrity nor TLR2, but NF- $\kappa$ B. Accordingly, pre-treatment of macrophages with OMVs prior to *L. pneumophila* infection enhanced host cell survival and expression of anti-apoptotic NF- $\kappa$ B target genes and resulted in an increased number of *Legionella*-containing vacuoles.

In conclusion, OMVs from *L. pneumophila* are initially potent pro-inflammatory stimulators of macrophages, acting via TLRs, while at later time points, OMVs facilitate *L. pneumophila* replication. OMVs might thereby lead to subsequent bacterial replication and promote spreading of *L. pneumophila* in the host.

## Macrophages render alveolar epithelial cells hypo-responsive to Legionella pneumophila

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Pneumonia is a leading cause of mortality worldwide. To secure organ function, pulmonary innate immune response has to be tightly regulated. Here, we analyze whether macrophages influence immune reactivity of type II alveolar epithelial cells during infection with an intracellular bacterial pathogen.

For this purpose, human macrophage-like differentiated THP-1 cells were co-cultured with human alveolar epithelial A549 cells in a transwell-setting. Infection of THP-1 cells with the important respiratory pathogen *Legionella pneumophila* (*L. pneumophila*) resulted in the release of pro-inflammatory cytokines, e.g. IL-8, by co-cultured, non-infected epithelial cells. This effect was synergistically blocked by an IL-1 receptor antagonist (IL-1ra) and a TNF- $\alpha$  neutralizing antibody (anti-TNF- $\alpha$ ). Furthermore, co-culture with infected THP-1 cells reduced cytokine expression by epithelial cells following direct encounter with *L. pneumophila*. This epithelial hypo-responsiveness could be mimicked by stimulation with IL-1 $\beta$ . It was accompanied by an accelerated pro-inflammatory mRNA decay, long-lasting degradation of interleukin-1 receptor-associated kinase 1 (IRAK-1), and reduced nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha ( $I\kappa B\alpha$ )-degradation and less nuclear factor kappa-light-chain-enhancer of activated B-cells (NF- $\kappa$ B) translocation into the nucleus. miR-146a was induced, but did not mimic epithelial hypo-responsiveness in physiological concentrations. Likewise, histone modifying enzymes did not reproduce the effect.

Corroborated by in silico simulations, our results demonstrate that macrophages can negatively regulate the responsiveness of lung epithelial cells to bacterial infection by the release of IL-1 $\beta$  resulting in a downregulation of IRAK-1. This intercellular communication may be critical for avoiding overwhelming inflammatory response in the lung.

## Differentially expressed miRNAs after *Legionella pneumophila* infection of human macrophages

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**Introduction:** *Legionella pneumophila* (L. p.) is a common cause of severe community-acquired pneumonia. This pathogen replicates primarily within alveolar macrophages and manipulates the host reaction by interfering with intracellular signaling pathways and gene transcription to support its own replication. MicroRNAs (miRNAs) have emerged as critical regulators of mRNAs and are also directly involved in the innate immunity. Therefore, they could have an important function in the regulation of the immune response to *Legionella*.

**Objectives:** The aim of this work was to identify deregulated miRNAs following infection by means of small RNA sequencing experiments and advanced bioinformatics analysis to elucidate miRNA-associated pathomechanisms.

**Methods:** Primary blood-derived human macrophages of healthy donors were infected in vitro using the wild type strain L. p. Corby for 24 and 48 h, with a multiplicity of infection (MOI) of 0.25. Total RNA was isolated and miRNA libraries were prepared for Illumina small RNA sequencing.

**Results:** Our analysis revealed infection-specific and statistically significant changes of miRNA expression in human macrophages, such as up-regulation of miR-146a and miR-155, as well as down-regulation of miR-221 and miR-125b. miRNA deregulation seems to be due to transcriptional regulation of miRNA promoters. Overexpression or knock down experiments of miRNAs were performed for functional characterization and showed an influence of selected miRNAs on bacterial replication.

**Conclusion:** In summary, the results have deepened our insight in the molecular interaction of L. pneumophila and its host cells and might help to establish potential new gene candidates for diagnosis and therapy.

## Transferrin-Polyethylenimine Nanoparticles for T Cell Targeted siRNA Delivery as Novel Anti-inflammatory Asthma Therapy

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Since asthma is a major public health problem affecting 235 million people worldwide and in a considerable section of patients the disease is still not controlled in a satisfactory way, novel efficient anti-inflammatory therapies with minimum side-effects are urgently needed. The disorder is characterized by chronic inflammation of the airways caused by infiltration of immune cells including T helper 2 (Th2) cells, a type of activated T cells (ATC), in the lung. Downregulation of genes associated with Th2 cytokines, and especially respective transcription factors such as GATA3, is a promising approach to early-on undermine pathologic pathways in asthma. RNA interference offers an auspicious therapeutic base for this strategy, as it was shown to induce transient and reversible knockdown. [1] However, the lack of efficient biocompatible siRNA carriers to overcome extra- and intracellular barriers still impedes the clinical translation. Therefore, we designed a novel ATC targeted delivery system composed of Transferrin-N-Succinimidyl-3-(2-Pyridyldithio)-Propionate-Polyethylenimine (Tf-SPDP-PEI). To achieve the challenging transfection of T lymphocytes, we utilized the iron transport molecule transferrin, whose endocytosis-mediating receptor is overexpressed by ATCs and hence displays a useful target for our purpose. As the carrier system is also equipped with a disulfide bond, this can easily be cleaved in the cells' endosome, releasing the siRNA intracellularly. Following the successful synthesis of the Tf-SPDP-PEI conjugate, size and zeta-potential of conjugate-siRNA polyplexes were characterized by dynamic light scattering (DLS) and the condensation efficiency was determined by SYBR Gold assay. Furthermore, efficient siRNA delivery was demonstrated in both human primary ATCs and a murine asthma model using flow cytometry. Finally, significant in vitro knock-down of GAPDH, a universal housekeeping gene, was quantified by real-time PCR. [2] In conclusion, our biocompatible targeted delivery system holds great promise to be an innovative therapeutic option to improve the control of asthma in the future.

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## A Bacterial Signal Peptide Increases Mucociliary Clearance in Explanted Mouse Trachea

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**Objective:** Bacterial signal peptides are known to trigger innate immunity responses by activation of formyl peptide receptors (FPRs) present in immune cells, e.g. leukocytes. Members of the FPR-family are also found in the murine vomeronasal organ where they are candidates for chemosensory recognition of bacterial pathogens. Here, we investigated the effects of bacterial signal peptides on mucociliary clearance in the murine trachea.

**Methods:** The trachea of C57Bl6, TRPM5-deficient (transient receptor potential cation channel subfamily M member 5; a crucial component of the canonical bitter and umami taste transduction) and FVB/NCrI mice was explanted and particle transport speed (PTS) was visualized by tracking directed transport of dynabeads at the surface. The transcriptome of single tracheal ciliated and brush cells, a chemosensory epithelial cell type, was analyzed by single cell deep sequencing.

**Results:** Deep sequencing showed FPR expression in both ciliated and brush cells. The N-formylated bacterial signal peptide FL185 increased PTS from  $43.48 \pm 5.05$  to  $75.96 \pm 3.56$   $\mu\text{m/s}$  ( $N=8$ ;  $p<0.0001$ ) at  $10 \mu\text{M}$  which addresses FPR1-3. Specific FPR1 and FPR2 inhibitors [cyclosporine H ( $1 \mu\text{M}$ ) and t-BOC2 ( $10 \mu\text{M}$ )] did not reduce the effect. The effect was conserved in FVB/NCrI mice which are lacking a functional FPR3. In contrast, FL185 was ineffective in increasing PTS in TRPM5-deficient mice. Four other tested bacterial signal peptides did not increase PTS.

**Conclusion:** A bacterial signal peptide stimulates cilia-driven mucociliary clearance, that could represent a novel defense mechanism against invasive bacteria in the trachea. This effect involves elements of the classical taste transduction cascade.

## Glycolipids of house dust mites – studying the impact of allergen-lipid association.

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**Introduction:** House dust mites (HDM) are important inductors of allergic asthma. HDM and its fecal pellets consist of glycoproteins and a wide range of chemically distinct substances, including lipids, which potentially initiate and/or modulate allergic reactions. Lipids can protect allergens from degradation and enhance their cellular uptake. Several major allergens from different sources bear hydrophobic domains, thus enabling them to interact with lipids.

**Methods:** In order to examine the role of HDM-derived lipids on the initiation of immune responses, lipid-containing molecules were obtained from *Dermatophagoides pteronyssinus* (D p) bodies (Greer®) and from D p culture medium (Allergopharma) utilizing chloroform/methanol/water extraction. The organic compounds were further fractionated on silica gel and by HPLC; and chemically characterized by HPTLC, GC and GC/MS. The total organic fraction and the subsequent lipid-containing fractions were tested for biological activity on human PBMC cultures based on the analysis of surface activation markers by Flow cytometry.

**Results:** With regard to polarity and chemical composition, the analysis of HDM bodies and their feces revealed the presence of a broad spectrum of lipid-containing molecules. Additionally, we developed a multiparametric panel for flow cytometry that allows the detection and analysis of innate-like lipid-reactive lymphocytes (NKT cells, gamma/delta T cells and delta/alpha/beta T cells) present on human PBMC. Ex vivo, HDM-derived lipids demonstrated their capacity to activate innate lipid-reactive lymphocytes, evidenced by their surface up-regulation of the activation markers CD25 and CD69.

**Discussion:** The full chemical characterization of HDM-derived lipids, and the further analysis of the effectors responses (cytokine release, co-stimulatory signals) induced on innate-like lipid-reactive lymphocytes, together with the co-administration of known HDM allergens with isolated lipids, may provide the rational base to understand the structure-function relationship behind the influence of lipids (as adjuvants) on the modulation of type 2 (allergic) responses towards allergens.

## The Contribution of the Airway Epithelial Cell to Host Defense in the Context of Cystic Fibrosis

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The monogenic disease cystic fibrosis is characterized by dysfunctional CFTR, encoding a chloride- and bicarbonate channel expressed in epithelial cells. We have characterized modifying genes in cystic fibrosis by studying informative markers at 52 candidate genes that were derived from differential transcriptome analysis in patient's biosamples or heuristic reasoning in 101 CF families with a total of 171 F508del-CFTR homozygous subjects enrolled in the European Cystic Fibrosis twin and sibling study. Genotype-phenotype-association was tested for by comparing patient subsets with contrasting endophenotypes of cystic fibrosis, including two assessments of the cystic fibrosis basic defect in nasal and intestinal epithelia of F508del-CFTR homozygous CF patients by nasal potential difference measurement and intestinal current measurement of biopsies. For many genes prominently known for their role in host defense and inflammation, we could describe an association with the cystic fibrosis basic defect [1]. The apical membrane of epithelial cells is equipped with a variety of receptors that sense the presence of pathogens or the inflammatory state such as TLRs and cytokine receptors. In other words, the airway epithelial cell is in a unique position to recognize the need for host defense as well as contributing to it via an activation of the chloride and bicarbonate channel CFTR. In conclusion, the observed association of genes such as TNFR1, IL1B and IFNGR1 with the basic defect in cystic fibrosis likely reflects the properties of the epithelial cell to activate chloride, bicarbonate and fluid secretion by airway epithelial cells in order to promote clearance and host defence against pathogens [2].

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## Immunomodulatory role of IL-37 in asthma pathogenesis

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**Introduction:** The recently identified cytokine IL-37 has been described as a negative regulator of innate immunity. Since the production of IL-37 is impaired in allergic asthmatics, it is likely that IL-37 is involved in asthma pathogenesis. We have previously shown that IL-37 ameliorates experimental asthma via a mechanism that requires the IL-18 receptor (R)  $\alpha$  and the single Ig IL 1 related receptor (SIGIRR). We aim to further clarify, how the anti-inflammatory effect of IL-37 on asthma pathogenesis is mediated.

**Methods:** Experimental asthma was induced in IL-18R $\beta$ - and IL-18 binding protein (BP)-deficient mice. IL-37 was applied intra-trachealy during allergen challenge. Airway inflammation and airway hyperresponsiveness (AHR) were compared to an untreated and a healthy control group. Expression of IL-18R $\alpha$  and SIGIRR was analyzed in different cells and tissues. Production of T helper 2 (Th2) cytokines in mononuclear cells (MNC) was assessed by cytometric bead array.

**Results:** Application of IL-37 ameliorated experimental asthma in IL-18R $\beta$ - as well as in IL-18BP-deficient mice by points of airway inflammation and AHR, which was comparable to the effects in wildtype animals. IL-18R $\alpha$  and SIGIRR were expressed by airway epithelial cells (AEC), smooth muscle cells (SM cells), dendritic cells (DC) and T cells. In vitro IL-37 reduced the production of Th2 cytokines within MNC.

**Discussion:** IL-37 ameliorated experimental asthma via a mechanism that did neither require IL 18R $\beta$  nor IL 18BP. This demonstrates that IL-37 does not simply function via a SIGIRR-mediated blockade of the pro-inflammatory IL-18 signaling or a IL-18BP-mediated neutralization of IL 18. AEC, DC and T cells expressed IL-18R $\alpha$  and SIGIRR and therefore represent potential target cells for IL-37. In vitro IL-37 impaired Th2 cell functions.

## The influence of the probiotic compound D-Tryptophan on the differentiation of murine naïve CD4+ spleen cells

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**Introduction:** Current therapies for asthma improve symptoms but cannot cure the disease or its prevalence prompting demands for alternatives. The use of probiotic bacteria to prevent chronic diseases could represent a cost-effective approach. However, clinical studies have shown contradictory findings, possibly explained by the individual and highly complex interaction between probiotics and host. To reduce this complexity, our group investigates the use of isolated probiotic compounds, instead of living bacteria. Recently we identified D-Tryptophan (D-Trp) showing immune modulatory properties. As a next step we plan to investigate D-Trp's influence on particular immune cells.

**Methods:** In a first approach we analyzed the effect of D-Trp on T-cell differentiation using murine, naïve spleen CD4+ T-cells which were differentiated in vitro (IL-2, anti-CD3/CD28) into Th1 (IL-12, anti-IL-4), Th2 (IL-4, anti-IFN $\gamma$ ) and iTreg (TGF- $\beta$ ) in the presence or absence of (A) 10 or (B) 50  $\mu$ M D-Trp. Differentiation was assessed on cytokine and mRNA levels by qRT-PCR, flow cytometry and cytometric bead array. Results were obtained from three independent experiments and evaluated using mean fold change (FC) to untreated control (no D-Trp).

**Results:** In the presence of D-Trp naïve Th0-cells showed a decreased differentiation into Th2-cells, as indicated by down-regulated mRNA levels of IL-4 (FC A: 0.85, B: 0.75) and IL-5 (FC A: 0.82, B: 0.89) and by lowered cytokine production of IL-5 (FC A: 0.97, B: 0.78) and IL-13 (FC A: 0.92, B: 0.75). D-Trp treatment also resulted in slightly increased differentiation towards Treg. In these cells IL-10 mRNA levels were elevated (FC A: 1.01, B: 1.17). A consistent effect of D-Trp on Th1 development was not observed.

**Discussion:** Our results indicate that D-Trp affects T-cell differentiation by suppressing Th2 and promoting Treg differentiation. Prospectively, we aim to confirm this effect in a more physiological co-culture system with dendritic cells.

## **$\alpha$ -Linoleic acid enhances the capacity of $\alpha$ 1-antitrypsin to inhibit lipopolysaccharide-induced IL-1 $\beta$ in human blood neutrophils**

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Alpha1-antitrypsin (A1AT, SERPINA1), a major circulating inhibitor of neutrophil elastase (NE) and proteinase-3 (PRN3), has been proposed to reduce the processing and release of IL-1 $\beta$ . Since the anti inflammatory properties of A1AT are influenced by the presence of polyunsaturated fatty acids, we compared effects of fatty acid free (A1AT-0) and  $\alpha$ -linoleic acid bound (A1AT-LA) forms of A1AT on lipopolysaccharide (LPS) induced synthesis of IL-1 $\beta$  precursor and the release of IL-1 $\beta$  from human blood neutrophils. The presence of A1AT-LA or A1AT-0 significantly reduced LPS-induced release of mature IL-1 $\beta$ . However, only A1AT-LA reduced both steady state mRNA levels of IL-1 $\beta$  precursor and IL-1 $\beta$  released. In LPS-stimulated neutrophils, mRNA levels of TLR2/4, NFKBIA, P2RX7, NLRP3, and CASP1 decreased significantly in the presence of A1AT-LA but not A1AT-0. A1AT-0 and A1AT-LA did not inhibit the direct enzymatic activity of caspase-1, but we observed complexes of either form of A1AT with NE and PRN3. In common with the effect on TLR and IL-1 $\beta$ , only A1AT-LA inhibited LPS-induced gene expression of NE and PRN3. Increased gene expression of PPAR- $\gamma$  was observed in A1AT-LA treated neutrophils independent of LPS stimulation, and the selective PPAR- $\gamma$  antagonist (GW9662) prevented the reduction in IL-1 $\beta$  by A1AT-LA. We conclude from our data, that the ability of A1AT to reduce TLR and IL-1 $\beta$  gene expression depends on its association with LA. Moreover, the anti-inflammatory properties of A1AT-LA are likely to be mediated by the activation of PPAR- $\gamma$ .

## Plasma drug-concentrations in patients with pulmonary arterial hypertension on combination treatment

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**Introduction:** Combination therapy of the phosphodiesterase-type 5 inhibitors (PDE-5i) sildenafil or tadalafil and endothelin receptor antagonists bosentan, ambrisentan, or macitentan may cause mutual pharmacokinetic interactions in patients with pulmonary arterial hypertension (PAH). The objective of this study was to analyse plasma drug concentrations in PAH-patients during different combination treatments.

**Methods:** PAH patients receiving a stable combination treatment with ERA and PDE-5i with targeted dosage for at least one month were routinely assessed, including clinical parameters and plasma drug concentrations. Plasma concentrations were measured at trough before the second daily dose was taken. Time, type and dosage of last medication intake were documented. Plasma concentrations were normalised considering dose and time from last medication intake and presented as multiples of the expected mean (MOM) of the respective monotherapies. Differences in plasma concentrations between each treatment group were analysed by Wilcoxon rank sum test.

**Results:** 125 patients (84 female, 57% idiopathic/heritable, mean pulmonary arterial pressure  $47 \pm 15$  mmHg) were included. Mean PDE-5i plasma concentrations were significantly lower when co-administered with bosentan than with ambrisentan or macitentan (both  $p < 0.001$ ). Patients receiving sildenafil-bosentan who performed clinically indicated transition to macitentan or ambrisentan showed a significant increase in sildenafil concentrations ( $p < 0.001$ ).

**Conclusions:** Only the combination with macitentan or ambrisentan treatment led to sufficient mean PDE-5i plasma concentrations and should therefore be preferred to bosentan. The study was not powered to analyse if lower PDE-5i concentrations cause unsatisfying clinical response or clinical worsening. However, plasma concentrations within a targeted range are desirable and may become of increasing importance.

## A new fast molecular genetic diagnostic approach for pulmonary arterial hypertension

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**Background:** In this study we developed a new specific gene panel for pulmonary arterial hypertension (PAH) including major disease genes and further candidates.

**Methods:** We assessed 37 patients with invasively confirmed PAH and 5 relatives of affected patients for genetic testing. A new PAH-specific gene panel was designed using next generation sequencing including 12 known disease genes and 17 further candidates. Any potential pathogenic variants were reassessed by Sanger sequencing.

**Results:** Twenty-six of the 42 subjects (62%) had a mutation in BMPR2, ALK1, ENG or EIF2AK4 genes identified by panel and Sanger sequencing. In addition, 12 unclassified variants were identified in 7 genes (known and candidate genes). A sensitivity of 100% was met after quality parameters were adjusted. The positive predictive value for all newly investigated genes based on panel results increased to 100% when Sanger technique was additionally applied.

**Conclusions:** The new PAH-specific gene panel developed in this study allowed for the first time the assessment of all known PAH genes and further candidates at once and markedly reduced overall sequencing time and costs. Sensitivity and positive predictive value reached 100% when Sanger technique was additionally applied. Thus, this technique will potentially change the routine diagnostic genetic testing in PAH patients.

## **Erythropoietin exhibit angiogenic Potential and the role of Erythropoietin Receptor in lung cancer cells**

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**Background:** Recombinant human erythropoietins (rHuEPOs), a hormone regulating proliferation and differentiation of erythroid cells is one of the prescription drugs to treat cancer associate anemia. However, rHuEPOs administration to lung cancer patients has been reported to associate with decreased survival, and the mechanism remains controversial.

**Objectives:** 1. To investigation the angiogenic effect of EPO on human vascular endothelial cells in an in vitro 3D cell culture model. 2. The expression of EPO-Receptor in lung cancer cells including EGFR(epidermal growth factor receptor ) wild type/EGFR gene mutation cell lines and whether EPO treatment modifies lung cancer cells growth.

**Methods:** EPO-Receptor expression in lung cancer cell lines H838, H1650, H1975, HCC827 and small cell lung cancer cell H1339 were measured by ELISA. The cells proliferations were monitored by a real-time cell monitor technology icelligence system in the presence of PBS or EPO for seven days. Vascular endothelial cells HUVEC tube formation assay in EPO or PBS treatment groups were performed in a 3D collagen gel cell culture model.

**Results:** EPO-Receptor can be detected in EGFR wild type lung cancer cell line H838, and small-cell-lung-cancer cell line H1339 while in EGFR gene mutation lung cancer cell lines H1650, H1975 and HCC827 were not measurable. Although EPO-Receptor was expressed in H838 and H1339, rHuEPO $\alpha$  treatment did not alter the cells proliferation in vitro. However, rHuEPO $\alpha$  significantly promoted HUVEC tube formation in a 3D culture model in vitro.

**Conclusion and Outlook:** EPO-R is not necessary for lung cancer cells proliferation in vitro. The role of EPO is beyond erythropoiesis, it can be as a strong angiogenesis as well, an animal model will be established in the future. EPO-R may co-expression with EGFR, EPO-R mutation or chimeric receptor between EGFR and EPO-R could be further investigated in the mechanism of EGFR-TKI (tyrosine kinase inhibitors ) resistant on the possibility of cross-talk signaling pathway.

## Potential protective role of secretory leukocyte protease inhibitor (SLPI) in transplanted organs

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**Introduction:** During self-limiting acute rejection of experimental allografts, numerous leukocytes, predominantly monocytes, accumulate in the vasculature. They interact with graft endothelia and produce diverse factors able to influence graft outcome. SLPI, in addition to its well-known anti-protease function, was reported to possess potent anti-inflammatory properties, mainly associated with inhibition of nuclear factor-kappa B activity. SLPI was described to be expressed by monocytes, however, its potential role in the context of organ transplantation has not yet been assessed.

**Materials and Methods:** Kidney transplantation was performed in the Fischer to Lewis rat strain combination to study reversible acute allograft rejection. Isografts were performed in Lewis rats. On day 9 after transplantation during the peak of reversible acute rejection, intravascular leukocytes were harvested by intensive perfusion of the grafts and SLPI expression was analysed by real-time RT-PCR. Furthermore, monocytic U937 cells transiently overexpressing SLPI were primed with LPS. Thereafter, ATP-induced IL-1 $\beta$  release was monitored by ELISA. In addition, the effect of conditioned medium from cells overexpressing SLPI on ATP-induced IL-1 $\beta$  release was investigated.

**Results:** We demonstrated that intravascular graft leukocytes, obtained during reversible rejection, expressed elevated mRNA levels of SLPI in comparison to the cells harvested from renal isografts. Overexpression of SLPI in U937 cells resulted in significant inhibition of ATP-mediated IL-1 $\beta$  release in comparison to the cells transfected with control empty vector. Unexpectedly, mRNA level of pro-IL-1 $\beta$  and the inflammasome component NLRP3 remained unchanged. In line with this observation, U937 cells treated with conditioned medium from cells overexpressing SLPI secreted significantly less IL-1 $\beta$  in response to ATP.

**Conclusions:** SLPI efficiently inhibits ATP-mediated IL-1 $\beta$  release. Elevated expression of SLPI in graft monocytes during reversible acute rejection might serve as negative feedback loop to protect grafts and graft recipients from detrimental effects caused by uncontrolled IL-1 $\beta$  secretion.

## TGF- $\beta$ on the loose: How downregulation of BAMBI contributes to perturbed signaling in NSCLC

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**Introduction:** Despite recent developments in targeted therapies, non-small cell lung cancer still remains among the most fatal types of cancer. Most patients are diagnosed with an advanced state of the disease and exhibit poor prognosis independent of therapeutic efforts. The TGF- $\beta$  signaling pathway is known to be involved in several different diseases as well as crucial cellular or immunological processes. Especially in malignant diseases such as lung cancer, the pathway is known to exert a double-edged role. In early stages of carcinogenesis, the tumor suppressive effects prevail, while at later stages invasiveness and metastasis are favored.

**Methods:** A comprehensive analysis of different pathway members was performed via IHC on >130 patient samples and corresponding tumor-free lung tissues as well as NSCLC cell lines. Furthermore, epigenetic as well as transcriptome-based studies were performed to explore the differential regulation and methylation of the TGF- $\beta$  pathway. In vitro studies using the TetOn system to overexpress the negative regulator BAMBI were applied to study the effect on downstream signaling, invasion and migration. The invasion and tumor-forming capacity of re-expressed BAMBI in human A549 cells were investigated in a mouse xenograft model.

**Results:** TGF- $\beta$  pathway activation as well as differential expression of its core mediators was found to be a feature of human lung cancer. Furthermore, epigenetic and transcriptome analyses revealed alterations in DNA methylation and gene expression patterns. The TGF- $\beta$  pseudo-receptor BAMBI was discovered to be significantly down regulated in tumor tissues as well as cell lines and might be epigenetically silenced. Re-expression by retroviral TetOn system resulted in reduced migration and invasion capacity as well as tumor growth and size in the in vivo model.

**Discussion:** Our data suggest that deregulated TGF- $\beta$  signaling, enhanced by silencing of BAMBI, is a common feature of human lung cancer and might be of therapeutic relevance.

## Modeling of TGF $\beta$ pathway dynamics in lung cancer cells

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**Rationale:** Transforming growth factor- $\beta$  (TGF $\beta$ )-induced effects are different, even opposite, depending on the cellular type and conditions. TGF $\beta$  signaling has been widely acknowledged to contribute to cancerogenesis via enhancing tumor cell metastasis. Numerous attempts to target TGF $\beta$  pathway to reduce cancer spread proved to be ineffective.

**Aim:** In the current work we aim to use a systems biology approach to obtain better understanding of information processing in the TGF $\beta$  signaling pathway. Identifying emerging systems-level properties that arise from network components could help discovering novel therapeutic strategies in targeting the TGF $\beta$  pathway.

**Methods:** Quantitative immunoblotting and mass-spectrometry were used to acquire time- and dose-resolved measurements of TGF $\beta$ /Smad signaling dynamics in NSCLC cell lines. A mathematical model was developed to explain Smad signaling data.

**Results:** We show that in all tested NSCLC cell lines Smad2/3 phosphorylation peaks at about 1 hour after stimulation, then gradually declines and remains steadily elevated until the ligand is consumed. Pathway perturbations can alter such dynamics. Actinomycin D treatment resulted in a switch from transient to sustained Smad2/3 activation, while application of the proteosomal inhibitor MG-132 inhibitor caused an increased amplitude of Smads phosphorylation. On the basis of dynamic measurements of the pathway we have established mathematical model, which is able to describe the experimental data.

**Conclusion:** TGF $\beta$  pathway demonstrates complex regulation on the receptor level: transcriptional negative feedback loop as well as ligand-independent constant receptor turnover are essential in determining pathway dynamics.

# The human lung lipidome mirrors histopathological phenotypes of lung tissues

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Lipid metabolism is closely connected to respiratory functions. Especially, pulmonary surfactant is constantly synthesized, stored, and secreted by type II pneumocytes located in the alveoli. Disruption of this metabolism affects lung functions in a not yet well characterized manner. To gain insight into these fundamental physiological processes, we present a systematic lipidomics study of 43 human lung tissue samples from 26 lung-cancer patients.

Each biopsy was split equally to perform histopathology and shotgun lipidomics. Lung samples were characterized by a tissue-specific scoring system that covered, among others, stroma, necrosis and vital tumor for 22 cancer tissues, and emphysema as well as fibrosis for 21 alveolar tissues. Quantitative lipid profiles comprising 311 lipids and 11 classes were determined using shotgun lipidomics. Employing multivariate methods like hierarchical clustering, PCA and PLS-regression, we studied the association between lipidome alteration and histological phenotypes as well as clinical data.

The lipidome discriminates alveolar and tumor-containing tissues. Tumor-free tissues are characterized by high amounts of phosphatidylglycerol and saturated phosphatidylcholine species originating from the pulmonary surfactant. In tumor-containing tissues we always found elevated amounts of cholesterol esters and triacylglycerols. From the lipidome data we developed a sensitive and specific score that discriminates the major two entities of Non-Small-Cell-Lung-Cancer, adenocarcinomas and squamous-cell carcinomas (AUC 0.91, ROC analysis).

The lipidome composition showed correlations to histologic scores. Specific lipidome features could be set into relation to presence of necrosis, content of stroma, and percentage of metabolic active tumor cells. In tumor-free tissue we found a correlation between lipidome and the emphysema grade (Thurlbeck) indicating an altered lipid metabolic state. We started to collect tissue samples for a cohort of approximately 200 individuals in order to substantiate our findings concerning lipid metabolic perturbations in lung-cancer and COPD.

## Genome-wide chromatin profiling of *Legionella pneumophila*-infected human macrophages reveals activation of the pro-bacterial host factor TNFAIP2

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**Introduction:** *Legionella pneumophila* (*L. pneumophila*) is a causative agent of severe pneumonia. Infection leads to a broad host cell response, as evident e.g. on the transcriptional level. Chromatin modifications, which control gene expression, play a central role in the transcriptional response to *L. pneumophila*.

**Methods:** We infected human blood-derived macrophages with *L. pneumophila* and used chromatin immunoprecipitation followed by sequencing (ChIP-Seq) to screen for gene promoters with the activating histone H4 acetylation mark (pan-acH4). We transferred these findings to investigate the effect of *L. pneumophila* on blood-derived macrophages.

**Results:** We found the promoter of tumor necrosis factor, alpha-induced protein 2 (TNFAIP2) to be acetylated at histone H4. This factor has not been characterized in the pathology of *L. pneumophila*. TNFAIP2 mRNA and protein were upregulated in response to *L. pneumophila* infection of human blood-derived macrophages and human alveolar epithelial cells (A549). We show that *L. pneumophila*-induced TNFAIP2 expression is dependent on NF-κB. Importantly, a knockdown of TNFAIP2 led to reduced intracellular replication of *L. pneumophila* Corby in A549 cells. The TNFAIP2 promoter furthermore controls the expression of the long non-coding RNA linc00677 which might be accessory to TNFAIP2 expression.

**Discussion:** Taken together, genome-wide chromatin analysis of *L. pneumophila*-infected macrophages demonstrated induction of TNFAIP2, a NF-κB-dependent factor relevant for bacterial replication. Besides linc00677, we found numerous long non-coding RNAs that were promoter-acetylated upon *L. pneumophila* infection. These RNAs might also contribute to *Legionella* pathogenesis.

## The effect of the antiinflammatory IL-1R antagonist anakinra in mice with CF-like lung disease and *Pseudomonas aeruginosa* infection

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**Introduction:** Spontaneous neutrophilic inflammation can be triggered by hypoxic epithelial necrosis in mucus-obstructed airways via the release of IL-1 $\alpha$  in Scnn1b-Tg mice with CF-like lung disease, as recently reported. In this model, the inhibition of the IL-1R-MyD88 pathway with the IL1-R antagonist anakinra reduced airway neutrophilia and structural lung damage (Fritzsching B. et al., AJRCCM 2015). However, effects of anakinra on airway inflammation and antibacterial host defense in the context of a *Pseudomonas* infection remain unknown. Thus, the aim of this study was to evaluate the effects of anakinra on neutrophilic inflammation and *Pseudomonas* infection *in vivo* in wild-type and Scnn1b-Tg mice with CF-like lung disease.

**Methods:** Scnn1b-Tg and wild-type mice were treated with anakinra (5 mg/10 g bodyweight) or vehicle (NaCl) subcutaneously b.i.d. and subsequently challenged with the *P. aeruginosa* strain PAO1 ( $\sim 2.5 \times 10^7$  cfu/mouse) or vehicle (PBS) by intratracheal instillation. Broncho-alveolar lavage (BAL) was performed 24 h after *Pseudomonas* infection and analyzed using differential cell counts, cytometric bead assay to measure proinflammatory cytokines and quantitative microbiology.

**Results:** A robust neutrophilic inflammation in both wild-type and Scnn1b-Tg mice was induced by the acute infection with PAO1. However, treatment with anakinra reduced neutrophils in infected wild-type and Scnn1b-Tg mice ( $n=13-15$ ,  $P<0.01$ ). Despite this reduction, anakinra treatment did neither aggravate the acute PAO1 infection in wild-type nor in Scnn1b-Tg mice ( $1.9 \times 10^3 \pm 1.5 \times 10^3$  cfu/ml (untreated) vs.  $5.6 \times 10^3 \pm 3.8 \times 10^3$  cfu/ml (treated),  $n=13-14$ ,  $P>0.4$ ).

**Discussion:** Our results support that treatment with the IL-1R antagonist anakinra reduces neutrophilic inflammation without exacerbating bacterial infection in CF-like lung disease in mice. Thus, anakinra may be used as a novel anti-inflammatory approach to control overwhelming neutrophilic airway inflammation without aggravating bacterial infection in CF. However, clinical studies are warranted to test the safety and efficacy of this anti-inflammatory strategy in patients with CF.

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## **Stem cell transplantation in a cystic fibrosis mouse model induced Cftr genotype conversion and improved outcome of *P. aeruginosa* lung infection**

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Infections with *Pseudomonas aeruginosa* still cause severe morbidity in people with Cystic fibrosis (CF). The Gram-negative, opportunistic pathogen is nearly perfectly adapted to the microaerophilic conditions within the CF lung characterized by impaired ciliary function, mucus plugging and progressive remodeling. This scenario of chronic inflammation and loss of lung function is not only caused by the loss of function of the CFTR channel in the respiratory epithelium, but also by an impaired function of professional phagocytes in CF lung disease as it was demonstrated by several studies during the last years.

Here, we tested in a CF mouse model (B6.129P2(CF/3)-CftrTgH(neoim)Hgu) whether the immune response against *P. aeruginosa* airway infection can be improved by transplantation of wild type hematopoietic stem and precursor cells (HSPCs). Infection experiments revealed reduced lung bacterial numbers as well as increased survival in CF mice which were transplanted with wild type HSPCs (CFB6) compared to CF mice which received isogenic cells (CFCF). To check whether the improved outcome of infection was based on HSPC transplantation, we analyzed the Cftr locus in blood samples of chimeric mice. First, we used wild type mice (C57BL/6JHanZtm) congenic to our CF mice and established genotyping with several genetic markers localized in the Cftr locus. Later on, we also applied wild type mice, with the trackable leucocyte allele CD45.1 (B6.SJL-Ptprrca-Pep3b/BoyJ). In recipients transplanted with HSPC from these mice (CFCD45), chimerism can be easily characterized by FACS analysis.

Results showed an almost complete donor chimerism in CFB6 and in CFCD45 chimeras. We therefore conclude that HSPC transplantation is an effective tool of improving the immune response towards infection in a CF mouse model and might even be a therapeutic approach worth thinking of in treating lung infections in CF patients.

## miRNAs as biomarkers in pneumonia and COPD-exacerbation

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MicroRNAs (miRNAs) are small regulatory RNA molecules. Their dysregulation has been associated with many inflammation-mediated diseases. In recent years, miRNAs have been recognized as important post-transcriptional regulators of the innate immune response. Their property to be stable and detectable in almost all body fluids makes them attractive new biomarker candidates. Community-acquired pneumonia (CAP) is one of the leading causes of death worldwide. An abrupt onset of severe illness, the lack of identification of the relevant pathogenic agent in the majority of the episodes and the difficulty to distinguish CAP from other acute airway infections demonstrate the urgent need to find new diagnostic (and prognostic) tools.

In the present work, the miRNA profile of peripheral blood mononuclear cells (PBMCs) of CAP patients was assessed in a clinical pilot study and compared to patients suffering from acutely exacerbated chronic obstructive pulmonary disease (AE-COPD), a chronic pulmonary disease aggravated by an acute viral or bacterial infection. Receiver operation characteristic (ROC) analysis revealed single miRNAs to be sufficient to distinguish healthy controls from critically ill patients. Furthermore, by discriminant function analysis a panel of five miRNAs was found to discriminate between CAP, AE-COPD patients and healthy controls. In comparison with healthy controls, analysis of PBMCs of a first cohort of CAP and AE-COPD patients showed a significantly reduced expression of anti-inflammatory miR-146a. In the analyzed healthy controls relative expression of miR-146a correlates with age and inversely correlates with the absolute number of monocytes.

Taken together, PBMCs of CAP and AE-COPD patients show a very distinct miRNA expression pattern compared to healthy controls. ROC and discriminant function analysis revealed miRNAs as good candidates to discriminate between the two groups or between ill and healthy state, respectively.

## **Smoking fruit flies - Impact of cigarette smoke exposure on the airway epithelium of *Drosophila melanogaster***

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Chronic obstructive pulmonary disease (COPD) and coronary artery disease (CAD) are global epidemics with overlapping mechanisms and pathophysiologic processes. Cigarette smoking is their common major risk factor and the possibility that they are associated in the same patient is very high. Recent studies strongly suggest that CAD is common in COPD patients. However, our knowledge about the underlying molecular framework linking COPD and CAD is still fragmentary. Herein, we introduce *Drosophila melanogaster*, as a highly attractive model for basic COPD/ CAD research. The fruit fly consists of a primitive vascular system in comparison to other invertebrate models. For example, the molecular mechanisms that regulate the formation of the tracheal tube seem to be similar to those that are involved in shaping the vascular tube in mammals.

In this study, we address the *in vivo* impact of cigarette smoke exposure (CSE), using 3rd instar larvae of *D. melanogaster* and focused on the epithelial defense of the trachea. To identify a complete repository of genes involved in the response to cigarette smoke, we have sequenced transcriptomes of trachea after CSE by Illumina HiSeq. Our data show that the tracheal epithelium is highly responsive to smoking and that CSE triggers an immune response indicated by the expression of antimicrobial peptide genes and activation of JAK-STAT signaling along with its cytokine-like ligands upd, upd2 and upd3. Moreover, genome-wide association studies (GWASs) indicated that gene-smoking interactions in CAD are in part mediated by ADAMTS-7. ADAMTS-7 plays a crucial role in the build-up of cells in the coronary arteries, making them narrower, which finally lead to coronary heart disease. Interestingly, our RNA-seq analysis revealed that the ADAMTS-7 homologue got highly expressed in the trachea of smoke exposed animals making it an interesting candidate to further investigate its role and function.

## Endoplasmic Reticulum Stress Is a Danger Signal Boosting Inflammatory Responses in Bronchial Epithelial Cells

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**Introduction:** Endoplasmic reticulum (ER) stress has been associated with proinflammatory signaling and is implicated in several pulmonary pathologies, including cystic fibrosis and other chronic obstructive pulmonary diseases. ER stress activates the unfolded protein response (UPR) in order to restore cell homeostasis. As UPR cascades seem to intersect with immune functions, we hypothesized that UPR signalling might increase inflammatory reactions of Toll-like receptors (TLR) upon microbial stimulation within airway epithelial cells.

**Methods:** Airway epithelial cells were treated with thapsigargin to induce UPR and subsequently stimulated with TLR agonists. Secreted cytokines and their relative mRNA expression levels were determined by ELISA and qRT-PCR, respectively. Silencing of the UPR branches was done using small interfering RNAs. MAPKs activation was investigated by Western blotting.

**Results:** ER stress increased reactivity of BEAS-2B and human primary bronchial epithelial cells to microbial stimulation with respect to IL6 and IL8 production. UPR induction resulted in an increased p38 and ERK activity by LPS and polyI:C, yet NF $\kappa$ B activation was not affected. Notably, pharmacological inhibition of p38 and ERK MAPKs could inhibit the observed boosting effect. Knockdown of UPR branches revealed that mainly PERK, and to some extent ATF6, mediated p38 and ERK MAPKs activation leading to synergistic proinflammatory activity with TLR stimulation in airway epithelial cells.

**Discussion:** We demonstrate that combined activation of ER stress and TLR signaling leads to synergistic proinflammatory activity of bronchial epithelial cells. We suggest that ER stress, present in various chronic pulmonary diseases, acts as a costimulatory danger signal activating MAPKs in airway epithelial cells. MAPKs activation is the crucial trigger for full-blown epithelial cell activation upon TLR stimulation. ER stress might contribute to microbial sensitization and infectious exacerbation of chronic airway diseases.

## The effect of comorbidities and respiratory symptoms on healthcare costs in patients with COPD. Results from the COSYCONET cohort.

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**Background:** Healthcare costs in patients with COPD are rising with increasing disease severity. This study investigates the role of comorbidities and symptoms as additional drivers of costs based on data from the COSYCONET COPD cohort.

**Methods:** Healthcare costs for the year 2012 were calculated from self-reported data on healthcare utilization from the baseline examination of patients with physician-diagnosed COPD in GOLD grade 1-4.

From a list of 33 possible self-reported comorbidities, 26 with a prevalence >3% and information on cough, dyspnea (mMRC grade), and sputum were used as predictors of costs in generalized linear regression models. The final model comprised the top-10 comorbidities and symptoms with significant effects. Additional models also included interaction terms. Age, sex, education, smoking status, and BMI were considered as confounders.

**Results:** Unadjusted mean annual healthcare costs of 2,139 COPD patients (mean age 65 years, 61% males) were €6,725 [SD 9,424]. Higher COPD grade was significantly associated with increased costs (+23%, +63%, +85% for grades 2,3,4 compared to grade 1, all p<0.003). Higher levels of dyspnea significantly increased costs (+24%, +65%, +206% for mMRC grades 2,3,4). Cough and sputum did not have significant effects.

A history of stroke showed the strongest association with costs (+57%), but osteoporosis(+41%), psychiatric disorders(+35%), peripheral polyneuropathy(+30%), heart disease(+29%), sleep apnea(+25%), cancer(+23%), migraine(+18%), cholelithiasis/cholecystitis(+16%), and peptic ulcer(+14%) were also significantly associated with higher costs.

When studying interactions, higher COPD grade, a high number of comorbidities, and a high degree of dyspnea were significantly associated with an increase in costs, but interactions between these predictors showed sub-additive effects and reduced their independent cost driving effects.

**Discussion:** Besides higher COPD grade, dyspnea and several comorbidities are important drivers of healthcare cost in patients with COPD. Knowledge about predictors of costs may support the targeting of appropriate interventions to reduce the economic burden of COPD.

## Investigating the role of cathepsin S in the pathogenesis of cystic fibrosis-like lung disease

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**Introduction:** Elevated levels of the cysteine protease cathepsin S (cat S) are found in Cystic fibrosis (CF) lung secretions, however, the role of cat S in CF lung disease is unclear. Cat S is capable of maintaining its activity at a neutral pH allowing it to remain active outside of the cell. Consequently, cat S has the capacity to promote remodelling of the extracellular matrix via its potent elastolytic activity. In addition, cat S can cleave and inactivate key antimicrobials in the CF airways. On the basis of findings to date, we hypothesise that active cat S contributes to the pathogenesis of CF lung disease and represents a viable therapeutic target for the treatment of chronic lung disease.

**Methods:** Pharmacological knockdown of cat S activity was achieved in the  $\beta$ ENaC-Tg mouse, a mouse model recapitulating features of chronic CF lung disease such as airway mucus obstruction and inflammatory lung damage using the cat S inhibitor VBY-999 or via the genetic knockout of cat S. Mice were injected daily from birth for 14 days with VBY-999/Dextrose control. Mice were culled, BAL was performed and lungs were collected for histology or protein/RNA analysis.

**Results:** Findings to date suggest that inhibition of cat S reduces inflammatory cell infiltration into the lung as well as levels of pro-inflammatory cytokines in bronchoalveolar lavage fluid and lung tissue destruction with the genetic knockout and pharmacological inhibition of cat S in the  $\beta$ ENaC mouse model. Furthermore, concomitant reductions in mucus plugging were also observed.

**Discussion:** These results support the hypothesis that active cat S plays a role in the pathogenesis of chronic lung disease and may be a viable and promising target in the treatment of diseases such as CF. Further work will be directed toward determining a mechanism through which cat S exerts these effects.

## Identification of biomarkers predicting COPD exacerbations

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**Introduction:** COPD affects 600 million patients worldwide (WHO). The disease is mainly caused by long-term smoking of cigarettes. Symptoms include progressive airflow limitation and chronic airway inflammation. Several triggers (e.g. bacterial or viral infections) can induce acute exacerbations. These episodes of acute worsening of disease symptoms are a significant burden for both patients and health care systems. Moreover, frequent exacerbations lead to more rapid lung function decline and increased mortality.

**Early prediction of exacerbations** could help to initiate treatment before symptoms reach a critical level. Therefore, the aim of this study is to identify biomarkers which predict an imminent COPD exacerbation. In a first step, we aimed to set up an animal smoke model mimicking COPD airway inflammation to serve as a basis for future exacerbation experiments.

**Methods:** Animals were exposed for 24 days to two doses of cigarette smoke (1 or 4 puffs/min) for 1 hour daily (InExpose®Scireq, CA). Smoke exposure concentrations and animal weight course were recorded daily and compared to air exposed handling controls. 24 hours after the last exposure, lung function, BAL and lung inflammation, and pulmonary immuno-phenotypes were analyzed.

**Results:** 4 puffs/min lead to a 4-fold higher particle concentration than 1 puff/min (7631 mg/m<sup>3</sup> vs 2065 mg/m<sup>3</sup>). 4 puffs/min significantly reduced weight gain over time in comparison to the air and 1 puff/min groups. Pulmonary baseline compliance was declined both in 1 and 4 puffs/min exposed animals. They further had increased numbers of total BAL cells.

Neutrophils were increased in animals exposed to 4 puffs/min only ( $p < 0.05$ ). These animals also demonstrated increased numbers of proinflammatory and conventional lung dendritic cells.

**Discussion:** Exposure to 4 puffs/min induced an inflammatory phenotype comparable to that seen in COPD patients. Thus, we suggest that this animal model can be used in the next step for infection induced exacerbations.

## Accelerometric assessment of physical activity in COPD: Correlation between measures by two different devices

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A sedentary lifestyle is associated with the progression of COPD and its systemic consequences. Physical activity (PA) can be assessed during daily life by accelerometry but there is considerable heterogeneity regarding methodological approaches and devices. The present study examined the correlation between measures by two common accelerometers in COPD. COPD patients from the German COSYCONET study wore two different accelerometers simultaneously during daily life over 6 full days. A Bodymedia SenseWear MF armband was worn at the upper arm all the time and an ActiGraph GT3X device was worn at the hip during the day and on the wrist during the night. Additionally, patients recorded their daily routine in an activity diary. For the present analysis, only days with a weartime >22h for both devices were evaluated. Minutes spent in the common activity levels, sedentary (SPA), light (LPA), and moderate-to-vigorous physical activity (MVPA), as well as number of steps were determined per day and the correlation between both devices was examined using Spearman's rank correlation coefficient.

18 COPD patients were examined (10 females; mean age 69.4 years) and a total number of 86 valid days were included in the analysis. Patients spent a median of 237 min/day in LPA and 83 min/day in MVPA. Over subject-specific activity means, correlation coefficients were 0.34 for SPA, 0.16 for LPA, 0.38 for MVPA, and 0.95 for number of steps per day ( $p<0.01$  for number of steps). Correlations between PA measures by both devices were low to moderate for activity levels and markedly better for number of steps per day. For comparative analyses of daily activity in studies which use the examined devices a primary focus on the number of steps appears to be most appropriate.

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## Influence of Exposure Parameters and Iterative Reconstruction on MDCT-based Lung Densitometry – An ex vivo Phantom Study

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**Objectives:** To evaluate the influence of exposure parameters and raw-data-based iterative reconstruction (IR) on lung densitometry on multidetector CT (MDCT) by dedicated in-house software.

**Material and Methods:** 10 fresh porcine heart-lung-explants (4 simulated high, 6 simulated low attenuating chest wall) were prepared in an ex vivo chest phantom. MDCT scans were performed with 120 kV and 80 kV, each combined with 120, 60, 30 and 12 mAs, and reconstructed with both filtered back projection (FBP) and IR, resulting in 16 datasets per lung. Mean air density (AD), noise and lung density (LD) were measured by automated ROI analysis with a total of 80 ROIs per dataset, with 120 kV 120 mAs serving as reference.

**Results:** Overall mean noise averaged for AD was  $5.5 \pm 0.9$  to  $69.5 \pm 2.4$  HU with FBP, and  $4.2 \pm 1.1$  to  $49.9 \pm 1.8$  HU with IR, IR reduced noise by 28 % ( $p < 0.001$ ). AD increased significantly with lower exposure settings, e.g. from -995.1 (reference) to -948.5 HU (80 kV 12 mAs) with high and low attenuating chest wall and FBP ( $p < 0.05$ ). Similarly, mean LD was increased from -917.6 (reference) to -902.5 HU (80 kV 12 mAs) with high attenuating chest wall, and decreased from -920.5 (reference) to -926.6 HU (80 kV 12 mAs) with low attenuating chest wall and FBP ( $p < 0.05$ ). Noise reduction by IR induced minor changes to AD and LD compared to corresponding reconstructions FBP, for example in LD with high attenuation chest wall -926.6 in FBP and -925.4 in IR at 80 kV 120 mAs.

**Conclusions:** AD as a background signal for densitometry and LD are significantly influenced by radiation dose. Though IR effectively reduced noise at each exposure setting with high and low attenuating chest phantoms, AD and LD are not restored in a clinically meaningful magnitude. IR may thus be used in the setting of densitometry but does not correct for systematic errors in attenuation values in low-dose chest CT.

## Nasal epithelia cultures of patients with chronic rhinosinusitis show altered ion transport capacities

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**Rationale.** Chronic rhinosinusitis (CRS) is a very common chronic disorder of the upper airways and frequently observed in patients with cystic fibrosis (CF). The underlying pathophysiological mechanisms may result in sinonasal inflammation, deficient mucus clearance or altered nasal epithelial ion transport, however, it is still rather unexploited. The purpose of this study was to functionally characterise the epithelial ion transport in cultured nasal epithelia of patients with CRS.

**Methods.** Nasal tissues of patients with CRS undergoing polyps resection and turbinate tissue from healthy controls were utilized to freshly isolate nasal epithelial primary cells (hNEpC). hNEpC were cultured under air-interfaced conditions and transepithelial short-circuit currents (Isc) were measured in Ussing chambers in the absence and presence of pre-treatment with the type 2 cytokine IL-13.

**Results.** Bioelectrical studies revealed a significantly reduced basal Isc in hNEpC cell monolayers of patients with CRS compared to controls (CRS Isc =  $18.2 \pm 1.7 \mu\text{A}/\text{cm}^2$  vs. control Isc =  $38.1 \pm 6.5 \mu\text{A}/\text{cm}^2$ ,  $p < 0.05$ ). Further, the amiloride-insensitive current reflecting constitutive Cl<sup>-</sup> secretion was significantly diminished in CRS ( $15.5 \pm 1.7 \mu\text{A}/\text{cm}^2$ ) compared to control cell monolayers ( $33.8 \pm 6.6 \mu\text{A}/\text{cm}^2$ ,  $p < 0.05$ ). UTP-induced responses reflecting Ca<sup>2+</sup>-activated Cl<sup>-</sup> secretion were substantially lower in CRS ( $0.0 \pm 0.2 \mu\text{A}/\text{cm}^2$ ) vs. control ( $0.7 \pm 0.2 \mu\text{A}/\text{cm}^2$ ). Based on previous studies demonstrating activation of the Ca<sup>2+</sup>-activated Cl<sup>-</sup> channel TMEM16A by the type 2 cytokine IL-13, we next compared UTP-mediated Isc in CRS and control cultures in the presence of IL-13. UTP-mediated Cl<sup>-</sup> secretory responses in IL-13 pretreated cultures increased in CRS to  $1.9 \pm 0.5 \mu\text{A}/\text{cm}^2$  ( $p < 0.01$ ), but only ~2-fold ( $1.2 \pm 0.4 \mu\text{A}/\text{cm}^2$ ) in control cultures.

**Conclusions.** We observed substantial differences in the ion transport capacity of nasal epithelial cultures from patients with CRS compared to controls. Our results suggest an abnormal epithelial ion transport partially accountable to TMEM16A and support further investigation of this epithelial dysregulation in the pathogenesis of CRS.

## Cigarette smoke exposure induces substantial changes in the airway epithelium of a *Drosophila* COPD model

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Cigarette smoke with over 4000 different components is the primary risk factor of a disease called chronic obstructive pulmonary disease (COPD). COPD is predicted by the WHO to become the third leading cause of death in 2030 and already today over 64 million people suffer from it. The term COPD comprises all diseases resulting in an airflow limitation due to the destruction of lung tissue (emphysema), fibrotic changes in the lung tissue (fibrosis) and mucus hypersecretion. As there is no cure yet, the need to understand this disease in its full complexity is great, to counteract the postulated effects.

In order to understand the responses of the airway epithelium to prolonged cigarette smoke exposure (CSE), we used the fruit fly *Drosophila melanogaster* as a model. For this, the tracheal epithelium was subjected to CSE with smoke of three cigarettes at an interval of three hours on two consecutive days each. After manual dissection of the tracheal system, RNA sequencing and qRT-PCR was performed.

Daily low dose CSE (1 cigarette per day) reduced the lifespan of adult *Drosophila* significantly in comparison to matching controls. In addition, cigarette smoke exposed *Drosophila* larvae showed an increase in tracheal epithelial thickness. Results of RNA sequencing suggest the involvement of the Keap1/Nrf2 and JAK/STAT pathway in the reaction to CS exposure. Among the regulated genes are those encoding for the cytochrome P450 family, Glutathione S-transferases and Mucin-like proteins.

Based on these results, we will further expand the *Drosophila* COPD model to better understand the molecular framework underlying COPD development.

## **MRI and CT phenotyping of 600 subjects from the German COPD trial COSYCONET: study concept and current status**

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**Introduction:** Imaging allows for precise characterization of regional lung alterations in COPD beyond global parameters such as spirometry. Since MRI allows for radiation-free regional morphological and functional assessment of the lung, it is the aim of this multi-centre study (ClinicalTrials.gov identifier NCT02629432) to evaluate sensitivity and specificity of morpho-functional MRI to diagnose emphysema-and airway-phenotypes of COPD, with CT as standard of reference.

**Methods:** Prospective lung imaging is being performed in a subcohort of 600 participants from COSYCONET, the national COPD Cohort. CT and MRI data are acquired at 16 study centres, which include all DZL-sites. Standardized protocols were defined for 1H-MRI combining morphological and contrast-enhanced 3D dynamic perfusion acquisitions, and non-enhanced low-dose CT in inspiration and expiration (<3.5 mSv). Phenotyping is performed by visual scoring and software-based analysis, providing quantitative CT-based airway- and emphysema parameters and quantitative MRI-based perfusion data.

**Results:** The study protocol, patient information and standardized SOPs for imaging were established. Quality control beyond regular scanner calibration is done by periodical phantom scans for CT and MRI. The workflow was implemented on occasion of initial training visits. The trial started in December 2013. Today, all centers were initiated and have already examined 592 participants. The recruiting period will end in June 2016.

**Discussion:** This study will define the role of MRI in phenotyping COPD and validate innovative biomarkers for interventional COPD trials. It also represents the close collaboration of radiology experts from the DZL-Platform Imaging and the representatives of the Disease Area COPD.

## Optogenetic mouse strains for studying airway innervation

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Airway function is profoundly controlled by parasympathetic, sympathetic and peptidergic sensory nerve fibers. Traditionally, the function of airway innervation is studied by electric field stimulation or by pharmacologic activation or inhibition. These methods allow only poor discrimination between fiber subtypes. Optogenetics is a new promising tool to solve these questions of specificity and selectivity. A blue light sensitive channelrhodopsin 2 (ChR2) from Chlamydomonas has been inserted in mice to create cell-specific expression of ChR2. Light stimulation induces depolarization and release of neurotransmitters from ChR2-expressing neurons. We here generated two mice strains expressing ChR2 in the major subtypes of neurons expected to induce bronchoconstriction, i.e. cholinergic (parasympathetic, expressing choline acetyltransferase = ChAT) and peptidergic sensory neurons, expressing transient receptor potential cation channel subfamily V member 1 (TRPV1), respectively, by crossbreeding ChAT and TRPV1 cre driver lines with Ai27D mice, expressing a ChR2-tdTomato fusion protein following exposure to Cre.

In "cholinergic mice", native tomato-fluorescence was observed in known cholinergic neurons in the central nervous system, in parasympathetic postganglionic fibers in the airways, heart, urinary bladder and in the enteric nervous system. Non-neuronal cholinergic epithelial cells of the trachea, gall bladder, urethra and thymic medulla lacked native tomato-fluorescence. In organ-bath experiments, light stimulation activated cholinergic nerves of this strain leading to contraction of the urinary bladder and colon. In TRPV1-ChR2-tomato mice, native tomato-fluorescence was observed in sensory neurons of dorsal root ganglia and in nerve fibers in the trachea. Additionally, some cells in the gut wall and in very small arteries displayed tomato-fluorescence.

These optogenetic mice offer a novel tool to functionally dissect the various neuronal airway constrictor pathways and to discriminate between non-neuronal and neuronal cholinergic mechanisms in particular.

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## Caveolin-1 and Caveolin-3: Structural and Functional Insights into Their Role in Murine Airway Smooth Muscle Constriction

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Serotonin and acetylcholine are bronchoconstrictors clinically relevant for airway diseases associated with airway hyperreactivity. Caveolins (cav-1, -2 and -3), which are binding partners for receptors and enzymes, are structural components of caveolae. The process of cav assembling into caveolae requires cytoplasmic adapter proteins (cavin-1 to -4).

Here, we addressed the role of cav-1, cav-3, cavin-1 and -4 in airway smooth muscle (SM) constriction utilizing immunofluorescence, RT-PCR, electron microscopy, western blotting, co-immunoprecipitation and functional analysis (organ bath and videomicroscopy) in cav-1 and cav-3 deficient (KO) and wild-type mice.

In mouse airways, cavin-1 and -4 interact with cav-1 and cav-3, respectively. Cav-1 expression was decreased in tracheal SM in cav-3-KO mice. Caveolar numbers were diminished in cav-1-KO but not in cav-3-KO tracheal SM. Muscarine-induced tracheal constriction was not altered in either KO-strain but was increased in intrapulmonary bronchi of cav-3-KO mice. However, serotonin-induced constriction was lost in the trachea of both KO-strains. Protein and mRNA for cavin-1, -4 and cav-1 and -3 were also detected in human intrapulmonary bronchi and primary SM cells.

In conclusion, cav-1 and cav-3 are essential for serotonin-induced tracheal SM constriction pointing to an involvement of different serotonin receptors in the trachea and bronchi that differ in their coupling to caveolae. Cav-3 was previously found to interact with muscarinic receptor type-2 (m<sub>2</sub>AChR) in bronchial SM cells. Thus, depletion of cav-3 might cause a disturbance of m<sub>2</sub>AChR coupling to caveolae resulting in increased muscarine-induced bronchoconstriction.

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## Cigarette Smoke and Cigarette Smoke Condensate Induce Inflammation and Cytotoxicity in Precision-Cut Lung Slices (PCLS)

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COPD is characterised by emphysema, mucus hypersecretion and airway inflammation. COPD is the fourth leading cause of death worldwide. Major reason to develop COPD is cigarette smoke (Cs). Therefore, a human relevant Cs model is needed. Currently several human cell culture models and non-human animal models are well-established. Most are lacking the human microenvironment, which is necessary to induce inflammatory, destructive and cytotoxic effects of Cs. Thus, the project's aim is to establish features of COPD in the 3D model of Precision-Cut Lung Slices (PCLS) of different species by using Cs and cigarette smoke condensate (Csc).

Rodent, rhesus and human PCLS were prepared and submersely exposed to Csc (extract of the particulate smoke phase) or to Cs in Air-Liquid Interface (ALI) and cultured up to 96 hours. Toxicity and viability of tissue was assessed. Pro-inflammatory immune responses and changes in extracellular matrix proteins were determined by ELISA. Therapeutical intervention was assessed using dexamethasone.

Toxicity in tissue after Csc exposure was determined by metabolic activity. Therefore, EC50 values were calculated. Pro-inflammatory cytokines interleukin-1 $\alpha$ , - $\beta$  and matrix metalloproteinase-9 (MMP-9) showed increased release from rodent, rhesus and human PCLS after Csc exposure. Release of pro-inflammatory cytokines was inhibited using dexamethasone. A cultivation of human lung tissue up to 96h showed no loss of vitality. PCLS were also exposed to Cs at ALI using the P.R.I.T.® ExpoCube® that allows the exposure of lung tissue to native whole smoke. Cs aerosol induced concentration-dependent tissue damage in rat, rhesus and human PCLS as well as inflammation and increased MMP-9 production.

Csc and Cs induced tissue damage and early biomarkers of inflammation in rodent, rhesus and human PCLS. Further intervention of inflammation can be studied in PCLS. The exposure of lung tissue to the whole complex mixture of Cs closely reflects the in vivo situation in PCLS.

## Airway mucins – suitable biomarkers to predict an upcoming exacerbation in COPD and asthma?

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Mucins are large glycoproteins lining and protecting the epithelial airway surface. However, their amount, composition and glycosylation pattern can be affected by the airway environment including the inflammatory status of the lung. In COPD amount and ratio of the two most important airway mucins MUC5AC and MUC5B are altered compared to the healthy situation. Moreover, variations in the glycosylation pattern of airway mucins were observed in asthma animal models as well as in patients with cystic fibrosis, making mucin composition and characteristics potential biomarkers to detect and even predict upcoming, life threatening exacerbations in chronic inflammatory airway disorders.

To analyze whether distinct alterations of the mucin composition are representative for inflammatory airway processes like COPD and asthma, we purified mucins from bronchoalveolar lavage fluid (BALF) of healthy and airway-diseased individuals and subjected them to a comprehensive lectin-binding assay to screen for disease-specific glycosylation patterns. With this assay we are currently identifying combinations of lectins, corresponding to distinct glycostructures, characteristic for mucins from healthy, airway-diseased and exacerbated individuals.

Although very informative, the lectin-binding assay depends on laborious mucin pre-purification and cannot distinguish between different airway mucins. To make use of mucin alterations as potential biomarker in a more general, versatile test system, it is therefore essential to catch and analyze specific mucins from human samples donor- and disease-status independent and with little effort. Antibodies specifically targeting the protein backbone of the different mucins could be viable tools. However, commercially available antibodies against the major airway mucin MUC5AC are not able to detect equal amounts of protein in a patient- and disease-independent manner making them unsuitable for our purpose. In an attempt to change this, we are currently identifying epitopes within the protein backbone of MUC5AC consistently accessible for antibodies and thereby potential targets for universally reactive mucin capture reagents.

## **Chemosensory cholinergic signaling network in the thymic medullary epithelium**

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**Objective:** A subset of medullary epithelial cells in the thymus (mTECs) was previously shown to be cholinergic and express components of bitter taste cascade. In this study we set out to further characterize these cells and elucidate their function.

**Methods:** Immunohistochemistry, real-time RT-PCR and intracellular calcium measurements were conducted on thymi from ChAT- (choline acetyltransferase) and Chrna3-eGFP (nicotinic receptor subunit alpha3) reporter mice, mice expressing diphtheria toxin A driven by TRPM5 promoter (TRPM5: channel in taste transduction signaling), and wild-type mice with streptococcal pneumonia. Newborn human thymi were subjected to immunohistochemistry.

**Results:** Analysis of thymi at different age stages revealed that expression of ChAT and chemosensory components in the mTECs starts at birth but not before. The ChAT-positive cells in the thymus are in proximity to terminally differentiated mTECs (Hassall-like bodies) carrying Chrna3. In human newborn thymus, these cells closely surround or are integrated in the outer layer of the Hassall's corpuscles. Hassall-like bodies were not observed in TRPM5-DTA mice lacking chemosensory cells. These cholinergic cells respond to the bitter substance denatonium with an increase in intracellular calcium concentration. Thymic mRNA expression of TRPM5 and alpha-gustducin was up-regulated in streptococcal pneumonia-infected mice.

**Conclusion:** We identified a novel chemosensory cholinergic cell type in the thymic medulla and hypothesize that there is paracrine acetylcholine signaling between these cells and Hassall's corpuscles, and that this signaling plays a role in bacterial pathogen detection and defense.

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## Innate immune network in asthma: studies on dendritic cell interaction with airway epithelium.

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**Background:** The interaction of various types of innate and adaptive immune cells during ongoing allergic asthma is pivotal for the outcome and the resolution of the disease. Dendritic cells (DC) are key regulators of this network, however, the airway epithelium is the first entry site for airborne allergens and signals derived from these cells can affect DC function decisively.

**Methods:** A 3D co-culture model involving the airway epithelial cell line Calu-3, cultured under air-liquid interface (ALI) conditions, and monocyte-derived DC was used to study the interaction of these cell types when challenged with airborne allergens. In addition, we monitored changes in cell morphology with a real-time cell analyzer (RTCA).

**Results:** Stimulation of Calu-3 cells with the allergens Der p1, Der p 2 and Bet v1 resulted in minor activation of these epithelial cells whilst allergen-treatment of DC led to differential induction of immune stimulatory cytokines such as IL-6, CXCL8 and CCL22. In contrast to the stimulation of the individual cell types, we observed further changes in cytokine induction under ALI co-culture conditions. These were already detected under conditions where direct epithelium/DC contact was prevented but it was at maximum when contact was enabled, indicating the importance of soluble factors in addition to direct cell interaction. Furthermore, using the RTCA we see a modification of Calu-3 morphology when challenged with Bet v1 only in the presence of DC.

**Conclusion:** The establishment of cell culture systems that enable allergen challenge studies on DC/airway epithelial cell co-cultures underline the importance of the direct cell contact in this context and will provide further insights into the nature of the DC-epithelium interplay.

## Effects of TRPA1 agonists on murine airways

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TRPA1 is a cation channel of the transient receptor potential channel family that is predominantly expressed in sensory C-fibers and activated by a wide variety of environmental irritants and endogenous inflammatory mediators relevant for asthma. Activation increases  $[Ca^{2+}]_i$ , induces inward currents and action potential discharge in lunginnervating C-fibers in vitro and elicits central reflexes in vivo. However, it remained unclear if TRPA1-dependent central reflex activity is associated with changes in lung function. To examine our hypothesis that TRPA1 activation in C-fibers induces bronchoconstriction, we performed head-out-bodyplethysmography in conscious mice and measured the midexpiratory tidal flow (EF50) during inhalation of increasing concentrations of cinnamaldehyde (CA), a TRPA1 agonist. Unexpectedly, CA induced a dose-dependent increase of EF50, characteristic for bronchodilation. Organ bath experiments were performed by using explanted, preconstricted tracheal rings to elucidate the nature of bronchodilation. Our results revealed that the CA effect was independent from central reflexes and could be mimicked by a variety of electrophilic and non-electrophilic TRPA1 agonists, including arcolein, AITC, 2-APB, thymol and carvacrol. Surprisingly, the bronchodilatory effect of CA was increased by pre-treatment with TRPA1-antagonists (HC-030031, AP-18, Ruthenium-Red) as well as in tracheas of TRPA1-KO mice, indicating that CA causes a TRPA1-mediated bronchoconstriction which is superimposed by a TRPA1-independent bronchodilation. Since a 5-day organotypic culture of the tracheas or pretreatment by RP-67580, a neurokinin 1 receptor (NK1R) antagonist, respectively, was associated with an increased bronchodilation, we conclude that the TRPA1-dependent bronchoconstriction involves sensory neurons and is dependent on NK1R signalling. However, the nature of the TRPA1-independent bronchodilation remains unclear since iberiotoxin, indomethacin, tetrodotoxin, propranolol, Nω-nitro-L-arginine and triphenylphosphine-oxide were ineffective. We conclude that activation of TRPA1 in C-fibers induces bronchoconstriction superimposed by a bronchodilatory effect of unknown etiology.

## Characterization of cellular sources for non-quantal ACh release in mouse airways

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Acetylcholine (ACh) is the major transmitter to induce contraction of the airway smooth muscle (ASM). In addition to the quantal vesicular ACh release after stimulation of parasympathetic neurons, non-quantal ACh (nqACh) release is observed in tracheal preparations after eserine treatment, which is capable to induce ASM contraction. In order to identify which cell population in the airways contributes to nqACh synthesis, expression of the choline acetyltransferase (ChAT), high affinity choline transporter-1 (CHT1), and the low-affinity choline transporter-like family (CTL1-5) was evaluated by RT-PCR in primary isolated vagal and dorsal root ganglia, parasympathetic neurons, tracheal epithelial cells, lymphocytes, granulocytes, and in a smooth muscle and fibroblast cell line. All evaluated cell populations expressed ChAT-, CHT1- or CTL-mRNA, respectively. ACh release was functionally investigated by using M3WT4 cells, a reporter cell line overexpressing muscarinic M3 receptors. An increase in  $[Ca^{2+}]_i$  was induced in M3WT4 cells by supernatants of eserine-treated tracheal preparations and inhibited by atropine co-application. However, supernatants of eserine-treated isolated cell populations or cells co-cultured cells with M3WT4 cells, respectively, had no effect on  $[Ca^{2+}]_i$  levels. To address the question if mast cells might contribute to nqACh release, organ bath experiments with tracheas of mast cell-deficient B6.Cg-KitW-sh/HNihraeBsmGlliJ mice (kindly provided by Frank Petersen, Leibniz Forschungszentrum Borstel, ARCN) were performed. ASM contraction in response to eserine in mast cell-deficient mice was unaltered in comparison to wild-type mice. In conclusion, we were able to show that many different cell populations located in the trachea express transporters and/or enzymes associated with nqACh release. The fact that we were not able to identify the major source of nqACh may be due to a low sensitivity of the M3WT4 cell assay. Alternatively, the possibility that the effect of nqACh-dependent ASM contraction is the sum of low-level ACh released from different cell populations has to be discussed.

## Towards automated evaluation of mucus transport measured by microscopic OCT (mOCT) during hypertonic saline treatment of Cystic Fibrosis

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Effective mucus transport in the airways is essential for infection defense. Malfunction caused by diseases like cystic fibrosis (CF) can result in severe and even life threatening complications. Time resolved imaging of mucus transport *in vivo* is essential to get mechanistic insight in factors influencing the transport and for developing and testing therapeutic interventions to increase mucus transport.

Microscopic OCT (mOCT) was used successfully to image mucus transport in trachea of spontaneous breathing mice. Image series over more than 2 hours containing 35,000 frames were captured in wild type (WT) and  $\beta$ ENaC overexpressing mice, which served as a model for CF. In order to evaluate these large amount of data an automatic quantitative evaluation is need. This has to include an efficient correction of tissue motion and an automatic identification and rejection of non-evaluable frames.

In this study we compared two algorithms for motion correction. A pairwise correlation of the different frame for calculation of the motion vector was matched to a maximization of the overlap of segmented images. Image series were evaluated with both algorithms and bench marked against a manual motion correction.

Due to the dominating speckle noise in the OCT images and tissue motion perpendicular to the plane of the cross-sectional images, the correlation algorithms was not able to correctly determine a correct tissue motion in all cases. Results of the optimization algorithms were more reliable after dedicated preprocessing of the images. In cases of strong motion both algorithms failed.

In conclusion, automatic motion correction of mOCT image series taken from mice trachea is possible. However, at the current stage manual supervision is still necessary.

## Mass spectrometric analysis of ΔF508 CFTR interactome identifies new drug targets for Cystic Fibrosis

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Cystic fibrosis (CF) is one of the most common genetic childhood diseases, affecting 1:2500 newborns in Europe. The disease is caused by loss-of-function mutations in the anion channel Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). Deletion of F508 in CFTR ( $\Delta$ F508 CFTR) is the most prevalent mutation, occurring in more than 70 % of CF patients. Children with CF suffer from unusually thick mucus in the lung, which promotes recurrent bacterial infections, causes obstruction of the airways and eventually leads to inflammation, tissue damage and respiratory failure (1).

We recently reported the complete protein-protein interaction network of wt and  $\Delta$ F508 CFTR using co-immunoprecipitation and mass spectrometry (2). We showed that deletion of F508 re-organizes the CFTR protein-protein interaction network and causes CFTR to acquire new, disease-specific interactions that drive the disease for example by preventing trafficking of  $\Delta$ F508 CFTR to the plasma membrane and promoting its premature degradation at different stages of biogenesis. Analysis of interactome changes upon treatment of human CF bronchial epithelial cells with HDAC inhibitors or shift to permissive temperature, both of which partially restore channel function, then allowed us to identify novel key interactors whose removal rescued  $\Delta$ F508 CFTR function at least partially. These key interactors can now be used for targeted drug development.

Mass spectrometric analysis further revealed that rescuing conditions also alter appearance and abundance of post-translational modifications (PTMs) of the  $\Delta$ F508 CFTR protein. Preliminary results indicate that a specific pattern of  $\Delta$ F508 CFTR PTMs might be utilized as an alternative read-out for small molecule screens aiming to rescue CFTR channel function. In summary, in-depth analysis of the CFTR interactome as well as its post-translational modifications can guide the development of new compounds to treat Cystic Fibrosis, which is urgently needed as current therapies are mostly directed towards symptoms and no cure is available yet.

- (1) F. S. Collins, Cystic fibrosis: molecular biology and therapeutic implications.  
*Science* 256, 774-779 (1992).
- (2) S. Pankow et al., F508 CFTR interactome remodelling promotes rescue of cystic fibrosis.  
*Nature* 528, 510-516 (2015).





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