Deutsche Gesellschaft für Pneumologie und Beatmungsmedizin e.V.

Gesellschaft für Pädiatrische Pneumologie e.V.

DZL Deutsches Zentrum für Lungenforschung ACADEMY

Abstract Book

Lung Science Meeting

of the DZL Academy,

the German Respiratory Society (DGP) - Section Basic and Translational Lung Research,

And the Society of Paediatric Pneumology (GPP)

in Hannover

17. – 18. November 2025

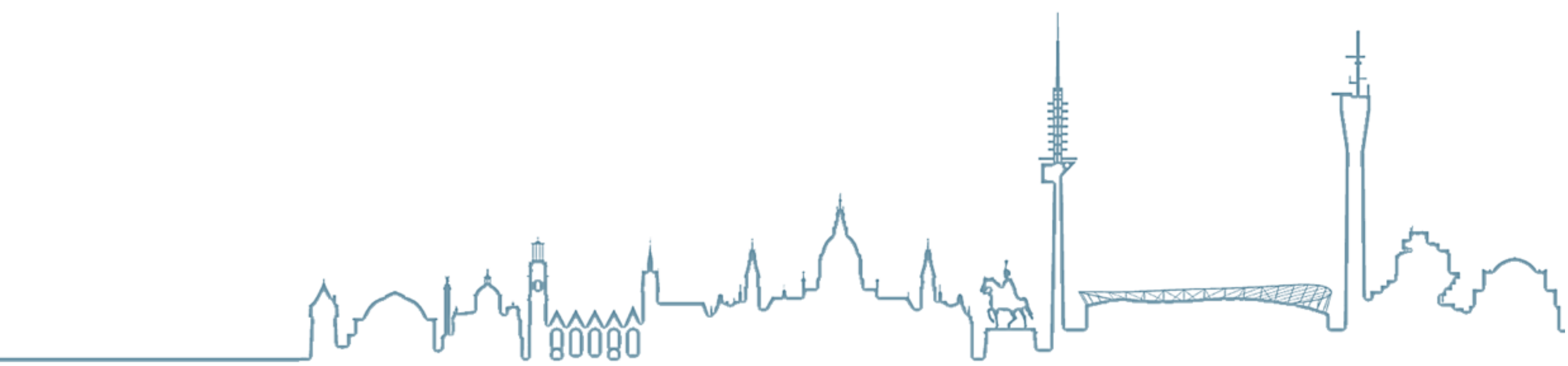


Table of Contents

Asthma and Allergies: Abstract 001 - 012

001

Comparative Genomics and Metabolomics Reveal Isolation Site-specific Metabolic Signatures in Non-typeable Haemophilus influenzae

K. Bhattarai[1], B. Baral[2], A. Sarnowicz[1], M. Diricks[3], S. Niemann[3], J. Rupp[4], K. Duda[1]

[1]Research Group of Biofunctional Metabolites and Structures, Research Center Borstel Leibniz Lung Center, Member of German Center for Lung Research (DZL), Airway Rersearch Center North, Borstel, Germany, [2]Organismal and Evolutionary Biology Research Programme, University of Helsinki, Helsinki, Finland, Finland, [3]Research Group of Molecular and Experimental Mycobacteriology, Research Center Borstel Leibniz Lung Center, Borstel, Germany, [4]Department of Infectious Disease and Microbiology, Lübeck Campus, University Hospital Schleswig Holstein, German Center for Infection Research (DZIF), Lübeck, Germany

Background: Non-typeable Haemophilus influenzae (NTHi) is a prominent respiratory pathogen implicated in otitis media and pneumonia that inhabit multiple host niches. While relevant gene content appears broadly conserved across isolation-site specific groups, extracellular metabolite profiles can differ.

Method: Culture supernatants of 9 NTHi isolates from three isolation-sites: ear smear (ES), pharyngeal smear (PS), “tracheal secretion”, “bronchial secretion” and “bronchoalveolar lavage” combined as “Lung” grown in chemically defined liquid medium were analyzed by LC–MS. Metabolites were statistically compared using MetaboAnalyst. Draft genome sequences were annotated with RAST, PROKKA, KAAS, and antiSMASH. We computed per-isolate pathway module coverage and summarized per-group coverage for significant annotated metabolites.

Results: Our results showed that despite conserved gene content across isolation-site specific groups, their extracellular metabolite profiles differed. Metabolomics data revealed inosine, hypoxanthine, uracil, phenylalanine, isoleucine, pyridoxine, niacinamide and valine as significantly varied metabolites within at least one pair of ES, PS, and Lung. Principal component analysis revealed ES group to be significantly separated from both PS and Lung groups. Inosine and hypoxanthine are reduced in ES as compared to PS and Lung groups while uracil and niacinamide are increased. Pyridoxine and valine are increased in ES as compared to PS group while isoleucine and phenylalanine increased in ES as compared to Lung group. Phenylalanine is increased in PS as compared to Lung group. Genes annotation for metabolite-related pathways shows that all 9 strains have a comparable set of relevant genes for functional proteins, indicating similar capacities for metabolism.

Conclusion: This study supports the hypothesis that NTHi isolates adapt to specific body sites through the metabolome, despite similar genomic capacities. NTHi may modulate their metabolism in a site-specific manner, affected by environmental factors. These findings show the possible application of significant metabolites as diagnostic biomarkers for site-specific NTHi infection.

ORAL PRESENTATION

002

Plasma cytokines distinguish clinical phenotypes of atopic dermatitis

M. S. Bickes[1,2,3], L. Riemann[1,4], R. Grychtol[1,2,3], A.-M. Dittrich[1,2], M. Price[1], A. Meyer-Bahlburg[5], H. Ott[6,7], T. Werfel[8,9], L. M. Roesner[8,9], G. Hansen[1,2,3,9]

[1]Hannover Medical School, Department of Pediatric Pneumology, Allergology and Neonatology, Hannover, Germany, [2]Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH), Member of the German Center for Lung Research (DZL), Hannover, Germany, [3]Else Kröner-Fresenius-Stiftung (EKFS), Bad Homburg v.d.H., Germany, [4]Hannover Medical School, Institute of Immunology, Hannover, Germany, [5]University Medicine Greifswald, Pediatric Rheumatology, Department of Pediatric and Adolescent Medicine, Greifswald, Germany, [6]Formerly at: Children's Hospital Auf der Bult, Department of Pediatric Dermatology, Hannover, Germany, [7]Currently at: Ludwig Maximilians University Hospital, Munich University Center for Children with Medical and Developmental Complexity - MUC iSPZ Hauner, Dr. v. Hauner Children's Hospital, Munich, Germany, [8]Hannover Medical School, Departement of Dermatology and Allergy, Hannover, Germany, [9]Cluster of Excellence RESIST (EXC 2155), Hannover Medical School, Hannover, Germany

Background:

Atopic dermatitis (AD) is a heterogeneous disease with several pheno- and endotypes. Distinct cytokine and lymphocyte patterns have already been described in pediatric AD [1], [2] but data integrating immunological and detailed clinical features remain scarce.

Objective:

To explore cytokine clusters in pediatric AD and examine their relation to clinical features, allergen sensitization and immune cell subsets, aiming to identify immunological signatures linked to disease phenotypes.

Methods:

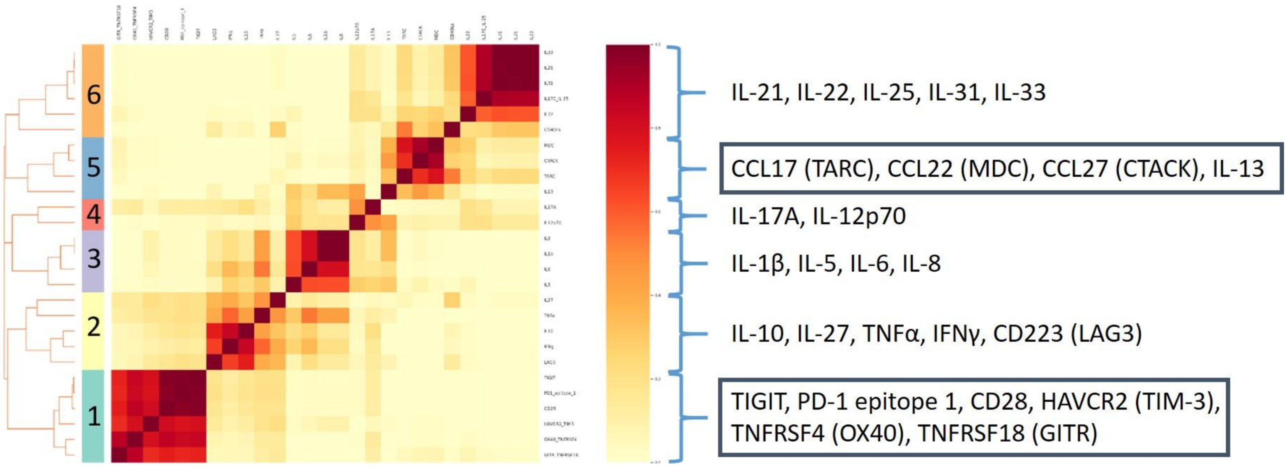
42 children with AD (median age 3.6 years, 64% male, mean SCORAD 45) were included. Plasma cytokines were measured using a multiplex panel and clustered unsupervised by CytoMod. Associations with age, disease severity, eosinophils, allergen sensitizations and peripheral B/T cell subsets were assessed.

Results:

Six cytokine clusters were identified, two showed distinct clinical associations:

Cluster 1 (T cell co-signaling molecules): Associated with younger age, lower total IgE and milder AD. Expression correlated with B cell maturation, but this was age-dependent.

Cluster 5 (Th2 cytokines): Expression increased with age and was associated with higher blood eosinophils, sensitization to inhalant allergens and more severe AD. Within this cluster, CCL17 correlated most strongly with SCORAD ($\rho = 0.67$, $p < 0.001$). A CCL17-driven upregulation of $CD4^+$ $CD25^+$ $ICOS^+$ T cells remained significant after age adjustment.



Conclusion:

Two phenotypes of pediatric AD emerged, linked to distinct cytokine clusters. CCL17 was identified as a central marker connecting cytokine expression, disease severity and T cell activation. While the role of CCL17 in AD is well known, to our knowledge this is the first pediatric study combining detailed clinical features, allergen sensitization, cytokine profiling and B/T cell subsets in peripheral blood. Future work will extend this approach to other atopic diseases such as allergic asthma to investigate shared and differential cytokine pathways between skin and lung, aiming to better understand the mechanisms underlying atopic multimorbidity.

References:

[1] Bakker DS, de Graaf M, Nierkens S, Delemarre EM, Knol E, van Wijk F, de Bruin-Weller MS, Drylewicz J, Thijs JL, (2022), Unraveling heterogeneity in pediatric atopic dermatitis: Identification of serum biomarker based patient clusters, Elsevier, Journal of Allergy and Clinical Immunology, 125-134, Issue 1, https://doi.org/10.1016/j.jaci.2021.06.029

[2] Czarnowicki T, He H, Canter T, Han J, Lefferdink R, Erickson T, Rangel S, Kameyama N, Kim HJ, Pavel AB, Estrada Y, Krueger JG, Paller AS, Guttman-Yassky E, (2020), Evolution of pathologic T-cell subsets in patients with atopic dermatitis from infancy to adulthood, Elsevier, Journal of Allergy and Clinical Immunology, 215-228, Issue 1, https://doi.org/10.1016/j.jaci.2019.09.031

003

Single-cell RNAseq analysis of peripheral blood lymphocytes in children with persistent asthma

R. Grychtol[1,2,3], S. Gaedcke[2], A. Habener[2,4], O. Dittrich-Breiholz[5], M. Bickes[2,4], M. Kopp[6], F. Brinkmann[7,8], K. Rabe[9,10], E. von Mutius[11,12], S. Bianca[11,12,13,14], A.-M. Dittrich[2,4], G. Hansen[2,4]

[1]Department of Paediatric Pneumology, Allergology and Neonatology, Hannover Medical School, Pädiatrische Pneumologie, Allergologie und Neonatologie, Hannover, Germany, [2]Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH), Member of the German Center for Lung Research (DZL), Hannover, Germany, [3]Else-Kröner Fresenius Stiftung, Hannover, Germany, [4]Department of Paediatric Pneumology, Allergology and Neonatology, Hannover Medical School, Hannover, Germany, Hannover, Germany, [5]Research Core Unit Genomics, Hannover Medical School, 30625 Hannover, Germany, Hannover, Germany, [6]University of Bern, Inselspital, Department of Pediatrics, Bern, Bern, Switzerland, Bern, Switzerland, [7]Division of Pediatric Pneumology and Allergology, University Children's Hospital Lübeck, University Medical Center Schleswig-Holstein, Lübeck, Schleswig-Holstein, Germany, Lübeck, Germany, [8]Airway Research Center North, Member of the German Center for Lung Research (DZL), Lübeck, Germany, [9]LungenClinic Grosshansdorf GmbH, Grosshansdorf, Schleswig-Holstein, Germany, Grosshansdorf, Germany, [10]Airway Research Center North, Member of the German Center for Lung Research (DZL), Grosshansdorf, Germany, [11]Institute of Asthma- und Allergy Prevention, Helmholtz Zentrum Munich, Munich, Germany, [12]Comprehensive Pneumology Center (CPC-M), LMU Munich, Member of the German Center for Lung Research (DZL), LMU Munich, Germany, Munich, Germany, [13]Department of Pulmonary and Allergy, Dr von Hauner Children's Hospital, LMU University Children´s Hospital, Ludwig-Maximilians-University, Munich, Germany;, Munich, Germany, [14]German Center for Child and Adolescent Health (DZKJ), Dr von Hauner Children's Hospital, LMU Munich, Germany, Munich, Germany

Background: Children with asthma often show first symptoms of wheeze in infancy, but it is still difficult to distinguish early-onset asthma from transient viral wheeze. Here, we performed single cell sequencing (scRNAseq) of peripheral blood lymphocytes (PBMCs) in patients with at least 8-10 years follow-up to identify cell populations and markers associated with asthma persistence or remission.

Methods: Frozen PBMCs of patients with preschool wheeze (PW, n=18, mean age 3,2 years, SD 1,5) and asthma (A, n=16, mean age 9,5years, SD 2,1) were used. Patients were selected according to outcome after a median follow-up of 9 years (IQR 8.25 - 10), with n=9/18 PW patients having asthma at school age and n= 11/16 asthma patients with disease persistence. PBMCs were sorted for live CD3+ and CD3- cells. CD3+T cells were reduced to 30% of total cells per patient and scRNAseq was performed in pooled triplets using the 10xGenomics platform after staining with a cite-seq cocktail.

Results:

Cell identification (azimuth app) resulted in 28 identified cell populations which was replicated by clustering of surface cell marker identified by cite-seq. Cell frequencies did not differ between asthma remission and persistence but differentially expressed gene analysis (DEG, FDR < 0.05 and FC 0.5) resulted in 5 cell populations with increased number of DEGs (> 100): Mucosal-associated invariant T cells (MAIT), CD14+ monocytes, double-negative T cells, CD56 bright NK cells and intermediate B cells.

Conclusions: ScRNAseq analysis of PBMCs revealed new and less recognized cell populations involved in disease persistence of children with early childhood wheeze. Further analysis will identify regulatory pathways and biomarkers for early identification of children at risk.

ORAL PRESENTATION

004

IκBζ Overexpression in Regulatory T Cells Disrupts Immune Tolerance and Drives Type 2 Inflammation via regulation of BATF

T. Kübelbeck[1], A.-M. Kulis-Mandic[1], A. Kolb[1], E. Stylianakis[2], Z. Ergün[2], K. Singh[3], M. Klein[4,5], N. Beumer[4], B. Fischer[1], S. Hailfinger[6], K. Schulze-Osthoff[7], A. Waisman[2,5], M. Delacher[4,5], V. Heissmeyer[8,9], N. Hoevelmeyer[2,5], **S. R. Reuter**[5,10], D. Kramer[1,5]
[1]University Medical Center of the Johannes Gutenberg University of Mainz, Department of Dermatology, Mainz, Germany, [2]University Medical Center of the Johannes Gutenberg University of Mainz, Institute for Molecular Medicine, Mainz, Germany, [3]Helmholtz Munich, Institute of Computational Biology, Munich, Germany, [4]University Medical Center of the Johannes Gutenberg University of Mainz, Institute of Immunology, Mainz, Germany, [5]University Medical Center of the Johannes Gutenberg University of Mainz, Research Center for Immunotherapy, Mainz, Germany, [6]University Hospital Münster, Department of Medicine A, Muenster, Germany, [7]University of Tuebingen, Interfaculty Institute of Biochemistry (IFIB), Tuebingen, Germany, [8]Ludwig-Maximilians-Universität, Institute of Immunology, Munich, Germany, [9]Ludwig-Maximilians-Universität, Research Unit Molecular Immune Regulation,, Munich, Germany, [10]University Medical Center of the Johannes Gutenberg University of Mainz, Department of Pulmonology, Mainz, Germany

IκBζ, encoded by *Nfkbiz*, is a known co-factor of NF-κB, that activates or inhibits a subset of NF-κB target genes upon stimulation of immune cells. Its relevance as a key factor promoting pro-inflammatory responses deriving from T_H17 cells, NK cells, or keratinocytes has previously been defined, especially in autoinflammatory diseases, such as colitis, psoriasis, and EAE. Oppositely, its role in immunosuppressive cells, such as Treg cells, remains underinvestigated.
In this study, we utilized a murine model of Treg-specific overexpression of IκBζ to investigate its impact on immune regulation. Our data demonstrate that elevated IκBζ expression impairs Treg function, leading to a breakdown of immunological tolerance. This dysfunction promotes type 2 inflammatory responses especially in the lung of the mice, and subsequent B–cell–mediated autoimmunity. Importantly, this shift of Treg cells to acquire a T_H2-like function was independent of FoxP3 and TGF-β, which both remained unperturbed. Instead, single-cell RNA sequencing and ChIP-seq analyses revealed that this process was driven by an IκBζ-mediated enhanced function of the transcription factor BATF, which subsequently induced a tissue Treg program directly in Treg cells, including a Treg cell-derived T_H2 cytokine expression.
These findings suggest a selective shift in cytokine regulation that favors pathogenic immune activation upon constitutive IκBζ expression. Overall, our results identify IκBζ as a critical modulator of Treg-mediated immune suppression. Its dysregulation contributes to the emergence of inflammatory and autoimmune conditions, highlighting IκBζ as a promising therapeutic target. Understanding the molecular mechanisms underlying Treg dysfunction may open new avenues for the treatment of immune-mediated diseases

005

Lifestyle factors in respiratory health and disease – obesity-related effects on the lung

N. Mierswa[1,2], M. A. A. Alcázar[3,4], C. Mühlfeld[1,2], J. Schipke[1,2]

[1]Institute of Functional and Applied Anatomy, Hannover Medical School, Hannover, Germany, [2]Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH), Member of the German Center for Lung Disease (DZL), Hannover, Germany, [3]Institute for Lung Health (ILH), Cardiopulmonary Institute (CPI), Member of the German Centre for Lung Research (DZL), Giessen, Germany, [4]University of Cologne, Excellence Cluster on Stress Responses in Aging-associated Diseases (CECAD), Cologne, Germany

Obesity is a global health burden that is associated with a higher risk of various lung diseases including lung infection, fibrosis and asthma. It is unclear whether the age of onset of obesity or the gender plays an important role for pulmonary changes. Aim of this study is to investigate the gender-specific effects of obesity on the lung in different age groups.

Female and male C57BL/6N mice were fed either a control diet (CD, 11% fat, 7.5% sugar) or a Western diet (WD,45% fat, 22% sugar) for 24 weeks, beginning either at birth (early exposure) or at an age of 24 weeks (middle-age exposure). Weight was documented weekly and an oral glucose tolerance test (OGTT) was performed at week 22. After 24 weeks, in vivo measurements of lung mechanics were performed and arterial blood gases were determined

A gender-specific trend in body weight was observed in both age groups, with males having a higher weight than females. In the OGTT, a diet-dependent effect was observed in middle-aged mice fed with WD. The lung mechanics show no diet or gender effect for the H and IC. However, an effect related to age can be recognized, with H decreasing and IC increasing with age. Arterial HCO_3 and total CO_2 levels were reduced in WD-fed mice cross both age groups, suggesting the possibility of induced hyperventilation. These data are preliminary, structural and molecular analyses are currently being performed.

The preliminary data indicate the WD has an effect on body weight and certain blood gases, but that there appears to be an age effect on lung mechanics. Further cohorts as well as structural and molecular analyses are required to verify this finding.

ORAL PRESENTATION

006

Funktion von Gasdermin3 Mutationen in der allergischen Atemwegsreaktion in vivo

J. Raspe[1], M. S. Schmitz[1], A. Joetham[2], C. Taube[3], T. Sparwasser[4,5], M. Kabesch[6], E. W. Gelfand[2], M. Schedel[1,2,7]
[1]Universitätsmedizin Essen - Ruhrlandklinik, Klinik für Pneumologie, Translationale Pneumologie, Essen, Germany, [2]National Jewish Health, Pädiatrische Pneumologie, Denver, United States of America, [3]Universitätsmedizin Essen - Ruhrlandklinik, Klinik für Pneumologie, Essen, Germany, [4]Johannes-Gutenberg-Universität Mainz, Institut für Medizinische Mikrobiologie und Hygiene, Mainz, Germany, [5]Johannes-Gutenberg-Universität Mainz, Forschungszentrum für Immuntherapie, Mainz, Germany, [6]Universität Regensburg - Kinder-Uni-Klinik Ostbayern (KUNO), Pädiatrische Pneumologie und Allergologie, Regensburg, Germany, [7]Universitätsmedizin Essen, Klinik für Pneumologie, Essen, Germany

Asthma zählt zu den häufigsten chronischen Atemwegserkrankungen weltweit. Die chromosomale Region 17q21 ist nach wie vor der bedeutendste Risikolokus für Asthma. Neben *ORMDL3* haben neuere Studien weitere Gene, wie das *Gasdermin A (GSDMA),* in dieser Region identifiziert. Die funktionelle Bedeutung dieses Gens in der Asthmapathogenese ist jedoch bislang unzureichend geklärt. Weiterführende Analysen zeigten, dass drei kodierende Mutationen das Risiko für Asthma erhöhten, während für eine *GSDMA* Mutation eine protektive Wirkung beobachtet wurde. In der Maus existieren drei Homologe*, Gsdma1-3,* zum humanen *GSDMA*. Zur funktionellen Untersuchung von *Gsdma3* in allergischen Entzündungsprozessen wurde eine bacterial artificial chromosome (BAC) transgene Mauslinie (*Gsdma3* tg) mit Mutationen im *Gsdma3* Gen generiert.
Gsdma3 tg und Wildtyp (WT) Mäuse wurden entweder mit dem Ovalbumin (OVA) oder Hausstaubmilben (HDM) Allergen sensibilisiert (Tag 0 und 14), gefolgt von wiederholten Allergenprovokationen (Tag 28-30). Die allergische Atemwegsreaktion wurde charakterisiert und eine Immunphänotypisierung der Zellen aus Lunge, Milz und trachealen Lymphknoten durchgeführt. Zusätzlich wurden $CD4^+$ T-Zellen aus der Milz isoliert. WT $CD4^+$ T-Zellen wurden anschließend in *Gsdma3* tg Mäuse appliziert, während umgekehrt *Gsdma3* tg $CD4^+$ T-Zellen in WT Mäuse transferiert wurden, um deren Einfluss auf die allergische Immunantwort zu untersuchen.
Nach Sensibilisierung und Provokation mit OVA oder HDM zeigten *Gsdma3* tg Mäuse eine signifikant reduzierte bronchiale Hyperreagibilität (AHR) und eine verringerte Eosinophilie im Vergleich zu WT Mäusen. Dies könnte auf einen erhöhten Anteil an regulatorischen T-Zellen, insbesondere in der Milz und der Lunge, zurückzuführen sein. Der Transfer von $CD4^+$ T-Zellen aus HDM-sensibilisierten WT Mäusen in *Gsdma3* tg Mäuse führte zu einer Induktion einer AHR. Im Gegensatz dazu bewirkte der umgekehrte Transfer von *Gsdma3* tg $CD4^+$ T-Zellen in WT Mäusen eine Unterdrückung der Atemwegsreaktionen.
Diese Ergebnisse deuten darauf hin, dass Mutationen im *Gsdma3*, eines der humanen GSDMA Homologe, eine zentrale Rolle in der Asthmapathogenese einnehmen könnten.

007

Interleukin-13 sensitizes human airway epithelia to lipopolysaccharid mediated cytokine secretion

H. Schmidt[1,2], O. H. Wittekindt[2]

[1]Ulm University Hospital, Department of Pediatrics and Adolescent Medicine, Ulm, Germany, [2]Ulm University, Institute of General Physiology, Ulm, Germany

Background:

Allergic asthma is one of the most common chronic airway diseases. Its pathogenesis mainly depends on type 2 immunity airway inflammation. Toll-like receptors (TLRs) that recognize microbial antigens such as lipopolysaccharid (LPS) are expressed in the respiratory epithelium and have been shown to be involved in asthma pathophysiology [1]. However, the underlying mechanisms are not completely understood. We investigated regulation of TLRs in the airway epithelium in an in-vitro model of allergic inflammation.

Methods:

Human tracheal epithelial cells (hTEpC) were cultivated as air-liquid-interface epithelia. Differentiated epithelia were exposed to interleukin-13 10ng/ml (IL-13) for 7 days to mimic type 2 inflammatory conditions. mRNA expression of TLRs was determined by RT-PCR. Epithelial cytokine and chemokine concentrations were measured in the airway surface liquid using commercially available cytokine multiplex assays.

Results:

HTEpC expressed TRL-1, -2, -4, -5 and -6. Exposure of epithelia to IL-13 resulted in a 5.8fold upregulation of TLR-4 expression levels. The effect was preventable by inhibition of IL-13 signalling using janus kinase 1/2 inhibitors. As expected, IL-13 substantially affected cytokine and chemokine concentrations in the epithelial surface liquid with increased levels of IL-2, IL-10, IL-17 and eotaxin among other alterations. Exposure to the TLR-4 ligand LPS only had little effect on epithelial cytokine secretion. However, co-exposure of IL-13 and LPS significantly elevated levels of IL-1 beta, IL-1 alpha, IL-9, IL-12, hepatocyte growth factor (HGF) and the IL-2 receptor alpha chain compared to epithelia treated with IL-13 or LPS only.

Conclusion:

We demonstrated, that IL-13 upregulates TLR-4 in human airway epithelia and modulates epithelial immune response to the TLR-4 ligand LPS. IL-13 induced sensitization to LPS mediated epithelial cytokine secretion may contribute to aggravation of airway inflammation and asthma severity.

References:

[1] Athari SS, Athari SM, Beyzay F, Movassaghi M, Mortaz E, Taghavi M, (2017), Critical role of Toll-like receptors in pathophysiology of allergic asthma, Eur J Pharmacol, 21-27, 808, doi:10.1016/j.ejphar.2016.11.047

ORAL PRESENTATION

008

Volatile organic compounds in exhaled breath respond to specific allergen inhalation challenge and correlate to inflammation in mild allergic asthma

S. Stegemann-Koniszewski[1], L. Black[1], M. Fachet[2], S. Kahlfuss[3], J. Schreiber[1]
[1]Otto-von-Guericke University Magdeburg Medical Faculty, Department of Pneumology, Magdeburg, Germany, [2]Otto-von-Guericke University, Medical Systems Technology, Magdeburg, Germany, [3]Otto-von-Guericke University Magdeburg Medical Faculty, Molecular and Clinical Immunology, Magdeburg, Germany

Allergic asthma is a heterogeneous and variable disease involving complex pathomechanisms. Analysis of volatile organic compounds (VOC) in exhaled breath displays a non-invasive strategy for sampling the airways. Detailed, individual profiling of inflammation in patients with asthma becomes increasingly important in the light of novel, pathway-specific therapeutic options.
We aimed to characterize VOC profiles in exhaled breath as a marker for allergic asthma as well as their dynamics in the acute early and the late response to inhaled specific allergen. A cohort of 17 patients with mild allergic asthma (13 female, 4 male, 25.7 (± 8.9) years) was characterized with respect to clinical parameters as well as local and systemic inflammation before and at six time points over eight hours following specific allergen inhalation challenge. VOC in exhaled breath were analyzed before and at two time points after challenge by Proton-Transfer-Reaction Time-of-Flight Mass Spectrometry. Non-atopic controls (n = 16, 12 female, 4 male, 28.2 (± 8.9) years) without asthma were likewise analyzed following inhalation of NaCl 0.9%.
Fractionated exhaled NO (FeNO) levels significantly dropped during the acute allergic reaction. As compared to controls, patients with allergic asthma showed significantly altered VOC profiles already at baseline (8 independent components). Further, counts per second of 15 specific VOC were significantly altered during the acute early reaction and 14 during the late reaction.
Profiling of VOC in exhaled breath holds potential as a non-invasive tool for the assessment, characterization and monitoring of local disease processes in allergic asthma. Functional analysis of specifically detected VOC and correlation to clinical and inflammatory markers will allow further insights into pathophysiology on a patient-specific level.

009

Effects of nasal colonization with Staphylococcus aureus on inflammation in asthma in patients and mouse models

S. Stegemann-Koniszewski[1], S. Görges[1], A. Münchhoff[1], S. Holtfreter[2], S. Wiles[3], J. Schreiber[1]

[1]Otto-von-Guericke University Magdeburg Medical Faculty, Department of Pneumology, Magdeburg, Germany, [2]Universitätsmedizin Greifswald, Institute of Immunology, Greifswald, Germany, [3]University of Auckland, Auckland, New Zealand

The opportunistic pathogen *Staphylococcus aureus* (SA) colonizes about one third of the population, preferably in the upper respiratory tract or on the skin. There are significant associations between SA nasal carriage and asthma but the mechanisms by which SA modulates inflammation remain elusive. While there is large variability in patient samples, animal models allow controlled conditions. However, they harbor drawbacks with respect to the transferability of findings. We addressed the immunological interplay between nasal SA carriage and asthma in clinical samples and mouse models. We recruited n = 62 asthma patients and assessed SA nasal colonization, allergic sensitization and inflammation. SA-free C57BL/6J mice were intranasally inoculated with murine SA and tested for carriage. After colonization, we performed peripheral sensitization and nasal challenge with ovalbumin (OVA).
Nasal SA was detected in 38.7 % of asthma patients. These showed significantly higher serum concentrations of interleukin (IL)-4 and a significantly higher number of past nasal polyp surgeries as compared to non-carriers. IL-4 and IL-13 serum concentrations of SA-carrying non-atopic asthmatics (33.3 % of n = 36) were significantly higher as compared to non SA-carrying non-atopic asthmatics. Of the aeroallergen-sensitized asthma patients (n=26), 46.2 % were SA nasal carriers and were characterized by significantly elevated nasal TNF-α concentrations. These results suggest that the impact of SA on inflammation in asthma depends on the asthma endotype. In the mouse model, we detected successful SA colonization over a period of 35 days. While colonized mice yielded a significant increase of bronchoalveolar lavage eosinophils as compared to non-colonized control animals, the combined SA – AAI mouse model revealed only minor impact of SA nasal carriage on AAI.
While interactions between SA and inflammation in asthma potentially depend on the asthma endotype, standard mouse models are mostly limited to allergic asthma. Refinement of animal models and stringent definition of clinical cohorts will support future research into the mechanisms of SA mediated modulation of inflammation in asthma.

010

Gut and sputum microbiota significantly differ between controls, patients with asthma and patients with asthma and comorbid depression and correlate to disease parameters

S. Stegemann-Koniszewski[1], K. Lehr[2], M. Scharlach[1], A. Link[2], J. Schreiber[1]
[1]Otto-von-Guericke University Magdeburg Medical Faculty, Department of Pneumology, Magdeburg, Germany, [2]Otto-von-Guericke University Magdeburg Medical Faculty, Department of Gastrenterology, Hepatology and Infectiology, Magdeburg, Germany

Heterogeneity in asthma poses a challenge for effective treatment that can be further complicated by comorbidities. Associations between asthma and depression have been reported and there is increasing awareness for the role of microbiota composition in both diseases. Its relevance at the intersection between asthma and depression remain unclear.

We characterized inflammatory markers and microbiota in induced sputum and feces of patients with asthma and comorbid depression to detect relevant correlations. We recruited n = 20 non-asthma, non-depression controls, n = 20 asthma patients and n = 20 asthma patients with comorbid depression. Participants were interviewed for asthma control (Asthma Control Test (ACT)), depression (Beck Depression Inventory, Patient Health Questionnaire, Hamilton Depression Scale), anxiety (Beck Anxiety Inventory) and perceived stress (Perceived Stress Scale). Next to routine clinical diagnostics, inflammatory cytokines were quantified in sera and sputum. Bacterial communities in sputum and feces were analyzed by 16S rRNA sequencing.

Lung function and FeNO were not significantly altered. ACT scores were significantly lower in asthma with depression as compared to asthma alone. All psychological scores were significantly elevated in asthma with depression as compared to controls and asthma alone. Comparison of bacterial abundance in sputum and feces revealed significant changes especially between controls and asthma with comorbid depression. There were no significant changes in sputum or fecal bacterial diversity. Correlation analysis of clinical, inflammatory and psychological parameters with microbiota data yielded a number of significant correlations. These were explicitly apparent for correlations between psychological scores and bacterial abundance in the sputum.

Respiratory and fecal microbiota composition was significantly altered in comorbid asthma and depression and significantly correlated to psychological scores. Further analyses of interdependencies between microbiota and disease presentation in asthma and depression will generate valuable insight into the drivers of these comorbid conditions.

ORAL PRESENTATION

011

miR-10 is a potential novel biomarker for predicting infant lung function prediction and targets TRPA1 in developing airways in the fruit fly

Q. Tan[1], L. Shi[1], B. Ehrhardt[1], D. Meißner[1], T. Roeder[2], T. Thum[3], J. Viereck[3], B. Liu[4], G. Hansen[5,6], C. Happle[5,6], S. Krauss-Etschmann[1,7]

[1]Research Center Borstel - Leibniz Lung Center, Early Life Origins of Chronic Lung Diseases, Borstel, Germany, [2]Christian-Albrechts University Kiel, Division of Molecular Physiology, Kiel, Germany, [3]Institute of Molecular and Translational Therapeutic Strategies Medizinische Hochschule Hannover (MHH), Hannover, Germany, [4]Hannover Medical School, Department of pediatric Pneumology, Allergology and Neonatology, Hannover, Germany, [5]Hannover Medical School, Hannover, Germany, [6]Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH), Member of the German Center for Lung Research, Hannover, Germany, [7]Christian-Albrechts University, Institute of Experimental Medicine, Kiel, Germany

Background: MiRNAs play essential roles in lung development, homeostasis, and disease pathogenesis. MiR-10 family members, have emerged as key players in cellular differentiation and immune modulation. Recent studies highlight their involvement in airway remodeling and inflammation suggesting a potential link to lung function outcomes.

Methods: Serum MiR-10a was quantified (qPCR) in children of the ALLIANCE cohort (n= 159 preschool wheeze/asthma; 0-12 yrs at baseline). Follow-up lung function (z-scores) was available from age 5 to 18 yrs and analyzed by multivariate regression analyses (FEV1/FVC). In *D. melanogaster* with airway specific loss of miR-10, tracheal morphology, alveolar structures and physical fitness were assessed. Isolated airways were subjected to mRNASeq.

Results: Children with miR-10a levels ≥median of the cohort displayed increasing FEV-1/FVC function trajectories over time, but those with miR-10a < the median of cohort declined in FEV1/FVC (n=159; p=0.02), which was most prominent in boys (n=105; p = 0.004). In the fruit fly, loss of miR-10 increased the epithelial thickness in and local airway narrowing (p = 0.0002). Male larvae had shorter “alveoli” (p = 0.005) with fewer branches (p <0.0001). Mir10 deficient larvae were shorter (p = 0.03), less successful to reach pupation (p <0.0001), and had a reduced adult lifespan versus genetic controls. TRPA1 was the top airway gene upregulated by loss of Mir-10.

Conclusion: We propose that miR-10a deficiency drives airway remodeling by upregulating TRPA1. Next, we will test if a TRPA1 inhibitor reverts this phenotype in flies. Also, we will analyze how low early miR10a-expression affects long-term outcome in pediatric asthma and evaluate it´s potential as early biomarker for the disease trajectories.

012

Gut Archaea Are Associated with Pediatric Asthma in an Age- and Sex-Specific Manner: Insights from a Multi-Platform Cohort Study

F. Xu[1,2], M. Weckmann[2,3,4], F. Brinkmann[2,3], G. Hansen[5,6], E. v. Mutius[7,8], K. F. Rabe[2,9], A.-M. Dittrich[5,6], B. Schaub[7,8], R. M. Grychtol[5,6], M. V. Kopp[10], H. Heine[1,2]

[1]Research Center Borstel Leibniz Lung Center, Priority Research Area Chronic Lung Diseases: Innate Immunity, Borstel, Germany, [2]Member of the German Center for Lung Research (DZL), Airway Research Center North, ARCN, Luebeck, Germany, [3]University Medical Center Schleswig-Holstein, Division of Pediatric Pneumology & Allergology, Luebeck, Germany, [4]Research Center Borstel Leibniz Lung Center, Priority Research Area Chronic Lung Diseases: Epigenetic of chronic respiratory diseases, Borstel, Germany, [5]Hannover Medical School, Department of Pediatric Pneumology, Allergology and Neonatology, Hannover, Germany, [6]Member of the German Center of Lung Research (DZL), Biomedical Research in End stage and Obstructive Lung Disease Hannover (BREATH), Hannover, Germany, [7]Member of the German Center of Lung Research (DZL), German Research Center for Environmental Health (CPC-M), Munich, Germany, [8]Dr. von Hauner Children's Hospital, LMU University Hospital, Department of Pediatrics, Munich, Germany, [9]LungenClinic Grosshansdorf, Department of Pneumology, Grosshansdorf, Germany, [10]University Children's Hospital of Bern, University of Bern, Department of Pediatric Respiratory Medicine, Bern, Switzerland

Background

Archaea are integral components of the human microbiome. Evidence suggests a link between gut archaea and pediatric asthma, but systematic investigations are lacking. This study aims to systematically explore the relationship between gut archaeal communities and childhood asthma.

Methods

Fecal samples from 275 children (127 healthy, 148 asthma; 171 male, 104 female; aged 6–18 years;) were analyzed using shotgun metagenomic sequencing, 16S rRNA gene sequencing and archaeal-specific qPCR assays. Archaeal diversity and abundance were compared across disease status, age, sex groups and BMI. Additionally, we employed deep learning methods to investigate the interactions between gut archaea and bacteria.

Results

Three complementary methods consistently detected archaeal presence across samples, with high concordance. Shotgun metagenomics identified 13 archaeal species, among which species *M.sp900766745* was the most abundant and *M.woesei* the least. Overall archaeal diversity did not differ significantly between asthmatic and healthy children. However, in younger children (6–11 years), asthmatic subjects exhibited significantly higher archaeal α-diversity ($p = 0.009$) than healthy controls, a difference largely confined to females. Further analysis of dominant archaeal species revealed that *M.sp900766745* was enriched in healthy children, whereas *M.woesei* was more abundant in asthmatic children - again, predominantly among younger females. BMI showed a positive association with asthma risk and correlated positively with the abundance of *M.woesei* in younger asthmatic children. Furthermore, machine learning analysis revealed a significant correlation between *Prevotella bergensis* and specific archaeal species, suggesting potential interactive roles.

Conclusion

Our findings reveal age- and sex-specific differences in gut archaeal profiles associated with pediatric asthma. These results underscore the potential role of archaea in early-life asthma and highlight the need for further studies integrating clinical data to elucidate underlying mechanisms.

Chronic Obstructive Pulmonary Disease: 101 - 116

101

Myofibroblast differentiation and monocyte activation impact granulation tissue severity in COPD patients after airway implantation

K. Barbet[1], B. Schwarz[2,3], A. L. Thiebes[4], C. Taube[1], K. Darwiche[2,3], M. Schedel[1,5]

[1]University Medical Center Essen - Ruhrlandklinik, Department of Pulmonary Medicine, Essen, Germany, [2]University Medical Center Essen - Ruhrlandklinik, Interventional Pulmonology, Department of Pulmonary Medicine, Essen, Germany, [3]DGD Lungenklinik Hemer, Department of Pulmonary Medicine, Hemer, Germany, [4]Helmholtz Institute, RWTH Aachen University, 3Department of Biohybrid and Medical Textiles (BioTex), AME - Institute of Applied Medical Engineering, Aachen, Germany, [5]University Medical Center Essen, Department of Pulmonology, Essen, Germany

Granulation tissue (GT) formation is a frequent complication after endobronchial valve (EBV) therapy in patients with chronic obstructive pulmonary disease (COPD). However, the immune–stromal interplay across different severity grades of GT remains poorly understood. We aimed to investigate if GT severity correlates with specific fibroblast phenotypes and monocyte activation by profiling stromal and myeloid cell responses across GT grades.

Using a novel scoring system, patients were classified into three groups: grade 0 (no GT), grade 1 (mild GT) or grade 2 (severe GT). Fibroblasts isolated from bronchial biopsies (n = 3 per group) were autologously co-cultured with blood-derived monocytes. Protein expression of fibroblast (FSP1) and myofibroblast (αSMA) markers were quantified by Western blot and flow cytometry (CD90, CD140a, aSMA, FSP1). Monocyte activation signatures were assessed by flow cytometry.

Fibroblast surface marker expression varied depending on the GT grade when co-cultured with autologous monocytes. Moreover, GT-derived fibroblasts demonstrated an enhanced ability to activate monocytes compared to those from EBV patients without GT. In this co-culture model, FSP1 and αSMA expression was significantly higher in grade 2 versus grade 0 fibroblasts, while CD140 tended to increase with GT severity. $CD90^{+}$ fibroblasts were more abundant in EBV patients without GT, which additionally inversely correlated with αSMA levels. In monocytes, the co-stimulatory marker CD86 was more prevalent in grade 0 compared to grade 2 EBV patients. In contrast, the percentage of $CD163^{+}$, $CD64^{+}$, $CD197^{+}$, and $CD206^{+}$ monocytes was higher in grade 1 and 2 GT. These findings indicate stronger monocyte activation in patients with more severe GT.

This study demonstrated distinct grade-dependent stromal and myeloid profiles in COPD patients following EBV implantation. Excessive GT formation was characterised by enhanced myofibroblast differentiation and altered monocyte activation. These mechanistic insights into stromal–immune interactions may inform risk-based patient selection to reduce GT-related complications after EBV treatment or the development of targeted interventions.

102

WISP1–Src–MIF–Mediated Nrf2 Dysfunction in COPD: Translational Insights from 2D and 3D Airway Models

M. E. Christopoulou, E. Papakonstantinou, D. Stolz
University of Freiburg, Freiburg, Germany

Chronic obstructive pulmonary disease (COPD) is marked by oxidative stress and inflammation driven in part by defective nuclear factor erythroid 2-related factor 2 (Nrf2) activity. We examined the role of WNT1-inducible signaling pathway protein 1 (WISP1) and macrophage migration inhibitory factor (MIF) in regulating Nrf2 expression and activity in primary airway epithelial cells, airway smooth muscle cells (ASMCs) and fibroblasts derived from COPD patients using 2D and 3D lung-on-chip cultures. WISP1 expression was significantly elevated in COPD patient biopsies and primary lung cells relative to non-diseased controls.In these cells, WISP1 modulated MIF expression in a cell type–specific manner and induced cytoplasmic sequestration of Nrf2, as demonstrated by immunofluorescence microscopy. Inhibition of MIF with ISO-1, or of Src kinase with PP2, restored nuclear localization of Nrf2, enhanced transcription of Nrf2 target genes (HO-1, NQO1, GPX1), and rescued antioxidant response element (ARE)-driven luciferase activity in all three airway cell types. Mechanistically, WISP1 activated Src-dependent NF-κB, Akt, and STAT3 signaling cascades, increased reactive oxygen species (ROS) production, and cooperated with MIF to amplify oxidative stress. Importantly, suppression of Nrf2 by WISP1 persisted even under ROS-scavenging conditions, indicating a ROS-independent mechanism of Nrf2 inhibition. Findings from 3D lung-on-chip co-culture models incorporating primary epithelial cells, ASMCs, and fibroblasts confirmed WISP1-induced cytoplasmic retention of Nrf2 and repression of its downstream antioxidant gene program, thereby validating the 2D culture observations in a physiologically relevant context.Collectively, these data identify WISP1 and MIF as critical modulators of Nrf2 dysfunction and sustained oxidative stress in COPD. Together, these findings establish WISP1 and MIF as key regulators of Nrf2 dysfunction and oxidative stress in COPD, highlighting them as potential therapeutic targets to restore antioxidant defenses.

ORAL PRESENTATION

103

Investigation of Mural and Alveolar Epithelial Cell Interactions During Emphysema Development and Alveolar Repair in COPD

D. Cojocaru, C.-Y. Wu, S. Doswada, D. Fomferra, E. Loku, A. Cilic, M. Gredic, V. Kleinhenn, O. Pak, S. Bellusci, N. Sommer, N. Weissmann, S. Hadzic
Excellence Cluster Cardio-Pulmonary Institute (CPI), Universities of Giessen and Marburg Lung Center (UGMLC), Institute for Lung Health (ILH), member of the German Center for Lung Research (DZL), Justus Liebig University Giessen, Giessen, Germany

Chronic obstructive pulmonary disease (COPD) is a progressive, incurable disease and typically results from chronic exposure to cigarette smoke (CS) or environmental stressors and is characterised by chronic bronchitis, emphysema, and pulmonary vascular alterations. However, the mechanistic interaction between pulmonary vasculature and alveolar compartments during COPD development remains unclear.

To address this, deep histological phenotyping of human COPD lungs was conducted to assess airway, vascular, and alveolar pathologies. Transgenic mice with reduced oxidative/nitrosative stress were used to investigate the underlying mechanisms of emphysema resistance. Precision-cut lung slices (PCLS) from transgenic mice were exposed to CS *ex vivo*. Viability and proliferation assays were performed to assess injury levels and evaluate repair capacity.

Two distinct COPD lung subtypes were identified: those with prominent pulmonary vascular remodelling (showing a significant increase in the thickness of the vascular smooth muscle cell layer) but milder emphysema, and those with less pronounced vascular remodelling but more severe emphysema. Lungs with pronounced vascular remodelling display characteristic extracellular matrix (ECM) alterations in distal lung parenchyma, potentially enhancing alveolar repair and providing resistance to emphysema development through enhancing endogenous repair mechanisms. *Ex vivo* analysis of transgenic mouse PCLS revealed protection from emphysema, likely due to enhanced endogenous repair mechanisms in the alveolar regions near lineage-labelled mural cells. Furthermore, using lineage-labelling in therapeutic transgenic mouse models, we investigated the possible interaction mechanisms between vascular cells and alveolar epithelial cells (AECs) in the distal lung parenchyma.

Further studies will investigate the impact of the isolated ECM on the molecular signalling of AECs, with the aim of identifying reparative ECM constituents as potential therapeutic strategies for COPD.

104

Impact of Oxidative/Nitrosative Stress Modulation on Acta2⁺ Cell-Mediated Remodeling and Regeneration in COPD

D. Fomferra[1], C.-Y. Wu[1], S. Doswada[1], E. Loku[1], M. Wujak[2], A. Cilic[1], M. Gredic[1], O. Pak[1], V. Nebel[1], S. Kraut[1], J. Koepke[1], A. Lingampally[1], J. Gote-Schniering[3], M. Bartkuhn[1], S. Bellusci[1], N. Sommer[1], N. Weissmann[1], S. Hadzic[1]
[1]Justus-Liebig-University, Excellence Cluster Cardio-Pulmonary Institute (CPI), Giessen, Germany, [2]Nicolaus Copernicus University, Department of Medicinal Chemistry, Bydgoszcz, Poland, [3]University of Bern, Lung Precision Medicine, Department of BioMedical Research (DBMR), Bern, Switzerland

Cigarette smoke (CS)-induced oxidative/nitrosative stress drives pulmonary vascular alterations and emphysema development in chronic obstructive pulmonary disease (COPD). Pulmonary vascular alterations associated with CS-exposure precede emphysema and include increased expression of oxidative/nitrosative stress mediators *Noxo1* and *Nos2* in pulmonary artery smooth muscle cells (PASMCs). Conditional deletion of *Noxo1* or *Nos2* specifically in *Acta2⁺* cells prevents CS-induced emphysema development but does not affect vascular remodeling.
We hypothesise that modulating oxidative/nitrosative stress can reprogram *Acta2⁺* cells into a pro-reparative phenotype that may influence alveolar respiratory epithelium.
We utilised laser-assisted microdissection to isolate RNA from pulmonary vessels and alveolar compartments of transgenic mice exposed to CS and histologically characterised human COPD lungs for transcriptomic analysis. In addition, we performed single-cell RNA sequencing (scRNA-seq) on lineage-labelled *Acta2⁺* cells.
Transcriptomic profiling of dissected pulmonary vessels from COPD lungs revealed significant enrichment of E2F1 transcription factor (TF) activity associated with milder emphysema. E2F1 activity coincides with a decrease of PASMC contractile marker expression. This observation was corroborated by scRNA-seq in murine *Acta2⁺* cells, which identified an "intermediate PASMC" population linked to alveolar repair. Lower oxidative/nitrosative stress and increased E2F1 activation were validated in pulmonary vessels of experimental mice and human COPD patients exhibiting milder emphysema.
Reduced oxidative/nitrosative stress in *Acta2⁺* PASMCs is associated with increased E2F1 TF activation and correlates with reduced emphysema severity in both murine models and human COPD samples.
We aim to isolate *Acta2⁺* PASMCs from transgenic animals to conduct functional *in vitro* co-culture assays with alveolar epithelial cells, assessing their effect on repair potential. We will further validate the functional role of E2F1 and screen the secretome of PASMCs for extracellular matrix components and growth factors potentially mediating the interaction with the alveolar compartment.

105

Eliminating Senescent Cells, A New Approach to Promote Repair of the Emphysematous Lung

M. Y. Gökyildirim[1], E. Born[2], E. Marcos[2], A. Vadakan Cherian[1], L. Lipskaia[2], A. Houssaini[2], J. Jacquet[2], D. Beaulie[2], R. Souktani[2], N. Vienney[2], A. Petrovic[1], E. Avci[1], D. Bernard[3], S. Savai Pullamsetti[1], S. Adnot[2]

[1]ILH, UGMLC, Justus-Liebig-Universität Gießen, Giessen, Germany, [2]INSERM U955, Département de Physiologie-Explorations Fonctionnelles and FHU SENEC Hôpital Henri Mondor, AP-HP, Créteil, France, Créteil, France, [3]Institut Leon Berard, Lyon, France

Introduction: Loss of tissue elasticity and senescent-cell accumulation are two hallmarks of ageing. Both structural damage to lung elastin (Eln) fibres and exaggerated lung-cell senescence are key pathogenic factors in pulmonary emphysema (PE) and COPD. However, whether lung elasticity, Eln expression, and senescence are linked remains unknown. We investigated whether decreased Eln levels resulted in lung cell senescence and whether this senescence promoted lung alterations.

Methods: We combined studies of human lung tissues and cells from patients with PE and studies of mice with elastase-induced PE or deletion of one elastin allele (ELN+/-). We monitored p16 activation by luminescence imaging in p16LUC/+ knock-in mice. Clearance of senescent cells was achieved by elimination of p16 positive cells in p16-ATTAC and p16LUC/LUC mice, as well as after treatment with ABT263 and Foxo4 DR1.

Results: Treatment of human cells with Eln siRNA induced senescence. This effect was attenuated when cells were seeded on an Eln-rich matrix before siRNA transfection. Similarly, cultured lung fibroblasts from patienst with COPD and from Eln+/− mice exhibited a senescence phenotype which was attenuated when cells were seeded on an Eln-rich matrix or after tropelastin transduction. Elastase-treated mice and Eln+/- mice showed increased markers of pulmonary senescence as well as accumulation of p16-expressing cells and development of PE. In p16-ATTAC mice activation of the ATTAC transgene prevented elastase-induced pulmonary functional and structural alterations. In Eln+/- mice, treatment with ABT263 or Foxo4 DR1 partially reversed PE and increased lung elastin levels and pulmonary repair processes.

Conclusion: PE is characterized by destruction of elastic fibres and increased cell senescence. Our data indicate close links between Eln dysregulation, cell senescence, and PE. Targeting senescent cells can be a new therapeutic strategy to induce pulmonary repair and stimulate elastogenesis in PE.

106

Analysis of inflammatory biomarkers and evaluation of regulatory molecular mechanisms of obstructive lung diseases with different targeted therapies: A multi-center, prospective, observational study (AERO)

K. Koethke[1], F. Tran[2,3], J. Lunow[1], M. Abdo[1], K. Gaede[4], J. Baines[5,6], J. Heyckendorf[1]

[1]University and University Medical Center Schleswig-Holstein, Campus Kiel, Department of Internal Medicine I, Pneumology, Kiel, Germany, [2]University and University Medical Center Schleswig-Holstein, Campus Kiel, Department of Internal Medicine I, Gastroenterology, Kiel, Germany, [3]Kiel University and University Medical Center Schleswig-Holstein, Campus Kiel, Institute of Clinical Molecular Biology, Kiel, Germany, [4]Research Center Borstel, Leibniz Lung Center, BioMaterialBank Nord, Borstel, Germany, [5]Institute for Experimental Medicine, Kiel University, Section of Evolutionary Medicine, Kiel, Germany, [6]Max Planck Institute for Evolutionary Biology, Plön, Germany

Chronic inflammatory airway diseases such as chronic obstructive pulmonary disease (COPD) and asthma are driven by inadequate immune response with dysregulation of the cytokine milieu. These diseases are highly heterogeneous in their clinical manifestation, and currently lacking curative therapies. Individualized treatment strategies, guided by predictive biomarkers, are therefore essential to improve outcomes.
The goal of treatment is to quickly reach inflammation control to improve life quality and, if possible, to reach a life-long remission. Although target therapies have already shown for COPD (Bhatt et al., 2023), but especially for asthma (Brusselle and Koppelman, 2022), clinical improvement, the mentioned goals have not been reached yet. One reason for this is the insufficient knowledge on the underlying immunopathological mechanisms, as well as the lack of predictive biomarkers to generate an explicit therapy stratification.
We established a multicenter, prospective study protocol with the goal of identifying prognostic biomarkers during treatment with targeted therapy. With extensive characterization of the patients including clinical phenotypes, inflammatory biomarkers and immunoregulatory mechanisms, we will identify potential predictors for therapy response. Central inclusion criteria is the prescription of a targeted therapy for patients with COPD B/E or severe, uncontrolled asthma. The choice of medications is by the treating physicians. All follow-up procedures will include clinical data, blood samples, nose curettages or brushes, sputum, urine and stool samples.
Within this study, a role of identification techniques will be used to identify signatures of biomarkers as well as mechanistic principles yielding insights on disease severity and future disease progression. With this analyses, different treatment strategies will be analyzed with a multi-omics approach by using selected technologies like transcriptome (single cell and bulk transcriptomics), proteome, methylation, microbiome and metabolome analysis. Validation experiments will be performed in a patient-derived airway organoid model.

107

Immature Neutrophil Expansion in Alpha-1 Antitrypsin Deficiency (PiZZ) COPD Revealed by Integration of Single-cell and Bulk RNA Data

B. Liu[1,2], D. S. DeLuca[1,2], B. Martinez-Delgado[3], J. Chorostowska-Wynimko[4], S.-M. Janciauskiene[1,2]
[1]Hannover Medical School, Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH), German Center for Lung Research, Hannover, Germany, [2]Department of Respiratory Medicine and Infectious Diseases, Hannover Medical School, Hannover, Germany, [3]Molecular Genetics Unit, Institute of Rare Diseases Research (IIER), Spanish National Institute of Health Carlos III (ISCIII), CIBER of Rare Diseases, CIBERER, Madrid, Spain, [4]Department of Genetics and Clinical Immunology, National Institute of Tuberculosis and Lung Diseases, Warsaw, Poland

Immature neutrophils have long been implicated in COPD pathogenesis, but their role in individuals with genetic variants such as Alpha-1 Antitrypsin Deficiency (PiZZ) remains unclear. Genetic heterogeneity and inter-individual variability pose major challenges for transcriptomic studies—particularly when using bulk RNA-seq, which has limited resolution—while single-cell RNA-seq, although more granular, remains costly and technically noisy.
To address these limitations, we applied an integrative approach combining deconvolution, weighted gene co-expression network analysis (WGCNA), pathway enrichment, and standard statistical methods to infer neutrophil states. We integrated public single-cell RNA-seq references with internal bulk RNA-seq data from PiMM and PiZZ individuals, including COPD patients (with and without alpha-1 antitrypsin [AAT] therapy) and healthy controls.
Deconvolution revealed significantly elevated proportions of immature neutrophils in both PiMM and PiZZ COPD compared to PiMM healthy controls (FDR-adjusted p = 0.0020 and 0.0081, respectively). Although variation by AAT therapy was observed within PiZZ COPD, the difference was not statistically significant, likely due to heterogeneous treatment response and a small cohort size. WGCNA identified a gene module associated with immature neutrophil abundance, enriched for ribosomal protein genes (e.g., *RPL*, *RPS*), cytoplasmic translation, and rRNA processing—indicating high biosynthetic activity typical of immature granulocytes. Additional enrichment in lamellipodium assembly genes (e.g., *FSCN1*, *CARMIL2*) suggests migratory potential.
Our results highlight the expansion of immature neutrophils in COPD, including in PiZZ cases, and demonstrate the utility of integrative transcriptomics for characterizing immune dysregulation in rare genetic subtypes with limited sample sizes.

108

Therapeutic Advances for Lung Repair Deriving from Preclinical Emphysema Models

E. Loku, C.-Y. Wu, N. Sommer, E. El-Agha, S. Bellusci, N. Weissmann, **S. Hadzic**
Justus Liebig University, Cardio-Pulmonary Institute (CPI), Universities of Giessen and Marburg Lung Center (UGMLC), Member of the German Center for Lung Research (DZL), Institute for Lung Health (ILH), Giessen, Germany

Emphysema in COPD is marked by irreversible alveolar destruction, and no approved therapy promotes lung regeneration, leaving transplantation as the only option for advanced patients. Pulmonary vascular alterations, including remodelling and capillary pruning, precede and are mechanistically linked to emphysema, while alveolar repair requires coordinated outgrowth of epithelium and capillaries. Preclinical COPD models, including transgenic and pharmacological interventions, are crucial for elucidating the mechanisms of lung injury and repair, as well as for evaluating regenerative therapeutic strategies. Evidence from experimental models implicates peroxynitrite stress as a central driver of vascular remodelling and alveolar destruction. Inducible nitric oxide synthase (iNOS) and NADPH oxidase organiser 1 (NOXO1) together generate peroxynitrite, which represses fibroblast growth factor 10 (FGF10), a key homeostatic signal in adult lungs. Impaired FGF10 signalling, whether due to environmental insults or genetic haploinsufficiency, drives vascular remodelling and emphysema and is linked to COPD susceptibility. Ablation of iNOS/NOXO1 prevents or reverses cigarette smoke–induced emphysema in preclinical models by restoring the reparative potential of the FGF10 axis. Boosting FGF10 signalling has therefore emerged as a leading regenerative strategy. Recent data show that the route of FGF10 administration critically affects outcome in the end-stage elastase-induced emphysema model. Intravenous delivery predominantly regresses vascular remodelling without repairing alveoli, whereas intratracheal delivery induces alveolar repair, revascularises septa and improves lung function. These findings indicate that FGF10 activates both epithelial and vascular repair programs, but restoration of the distal respiratory surface requires direct targeting of the alveolar compartment. Wild-type FGF10 protein is unstable and strongly binds extracellular matrix, prompting the development of engineered variants with improved stability and tissue penetration. Such engineered FGF10 peptides expand opportunities for translating these insights into feasible regenerative therapies for COPD.

ORAL PRESENTATION

109

Single-Cell Mapping Reveals CXCL11 as a Modulator of Mucus Production and Inflammation in the Airway Epithelium During Influenza A Infection in COPD

M. C. Melo-Narvaez[1,2], C. Eger[3], W. Bertrams[1], B. Benedikter[1,4], F. Ling[5,6,7], M. R. Jones[1], K. Pauck[8], B. Weckler[9], H. Pott[9], T. Procida[10,11], J. Wilhelm[10,11], E. Vollmeister[1,12], H. Garn[8], A. Kirschbaum[13], G. Rohde[9], M. Wygrecka[10], J. Nawroth[5,6,7], C. Talavera-Lopez[3], B. Schmeck[1,9,14,15,16], M. Lehmann[1,2,10]

[1]Phillips University Marburg, Institute for Lung Research, Marburg, Germany, [2]Comprehensive Pneumology Center (CPC), Institute of Lung Health and Immunity, Helmholtz Zentrum München, Member of the German Center for Lung Research (DZL), Munich, Germany, [3]Julius-Maximilians-Universität Würzburg, Institute of Systems Immunology, Würzburg, Germany, [4]University Eye Clinic Maastricht UMC+, Maastricht University Medical Center, Maastricht, Netherlands Antilles, [5]Helmholtz Zentrum München, Helmholtz Pioneer Campus, Neuherberg, Germany, [6]Helmholtz Zentrum München, Institute of Biological and Medical Imaging, Bioengineering Center, Neuherberg, Germany, [7]Technical University of Munich, Chair of Biological Imaging, Central Institute for Translational Cancer Research (TranslaTUM), School of Medicine and Health & School of Computation, Information and Technology, Munich, Germany, [8]Philipps University of Marburg, Translational Inflammation Research Division & Core Facility for Single Cell Multiomics, Member of the German Center for Lung Research (DZL) and the Universities of Giessen and Marburg Lung Center (UGMLC), Marburg, Germany, [9]Philipps-University Marburg, Department of Medicine, Pulmonary and Critical Care Medicine, University Medical Center Marburg, Marburg, Germany, [10]Institute for lung health (ILH), Giessen, Germany, [11]Universities of Giessen and Marburg Lung Center (UGMLC), Justus-Liebig-University Giessen, German Center for Lung Research (DZL), Giessen, Germany, [12]Justus Liebig University Giessen, Department of Insect Biotechnology in Plant Protection, Giessen, Germany, [13]University Hospital Gießen and Marburg (UKGM), Department of Visceral, Thoracic and Vascular Surgery, Marburg, Germany, [14]Phillips University Marburg, Core Facility Flow Cytometry - Bacterial Vesicles, Marburg, Germany, [15]Phillips University Marburg, Center for Synthetic Microbiology (Synmikro), Marburg, Germany, [16]Member of the German Center of Infectious Disease Research, Marburg, Germany

Influenza A virus (IAV) is one of the main causes of exacerbations in chronic obstructive pulmonary disease (COPD), contributing to high morbidity and mortality. The airway epithelium is the main target, and it´s responsible for initiating lung regeneration after acute lung injury. However, little is known about how it changes in COPD and its role during IAV-associated exacerbations. Here, we aimed to elucidate the cellular circuits of communication, metabolism, and transcriptional regulation of airway epithelial cells (AEC) in response to IAV-infection and determine whether pre-existent COPD hampers these circuits. AEC from donor and COPD patients were differentiated in air-liquid interface (ALI) and exposed to IAV. Using single-cell RNA sequencing, we profiled 97.573 cells, identifying all main compartments and rare cell types of the airway epithelium. Cell type proportions analysis revealed reduced deuterosomal and ciliated cells in COPD at baseline. Moreover, we found population shifts in basal, club, and suprabasal clusters after infection regardless of disease state. Cell-cell communication analysis identified two communication programs differentially regulated in ALI from COPD lungs after IAV infection. Among them, we found that the CXCL11/ACKR3-axis was activated in the COPD epithelium after infection. Interestingly, CXCL11 is a well-known secreted activator of both NF-κB and JAK-STAT signaling pathways for T-cell recruitment and is elevated in the sputum of COPD patients; however, its role in epithelial cell immunity remains unknown. Mechanistically, we found that stimulation with CXCL11 induced a dose-dependent modulation of MUC5AC expression as well as an increase in mucus viscosity in infected ALI. In conclusion, our results suggest that changes in cellular composition and deregulation of the CXCL11/ACKR3-axis might contribute to altered response to IAV in COPD patients by altering mucus production and inflammatory signaling.

110

Epigenetic analysis of COPD-patient derived airway organoids

F. Ritzmann*[1], **M. Brand***[1,2], G. Gasparoni[3], J. Walter[3], F. Langer[4], R. Bals[1,2], C. Beisswenger[1]

[1]Universität des Saarlandes, Innere Medizin V - Pneumologie, Allergologie, Beatmungs- und Umweltmedizin, Homburg (Saar), Germany, [2]Helmholtz-Zentrum für Infektionsforschung GmbH, MORE - AG Bals, Braunschweig, Germany, [3]Universität des Saarlandes, Institut für Genetik/Epigenetik, Saarbrücken, Germany, [4]Universität des Saarlandes, Klinik für Thoraxchirurgie, Homburg (Saar), Germany

*Contributed equally

Epigenetic mechanisms control gene expression and play a central role in regulating cellular processes involved in the development and progression of chronic lung diseases, including chronic obstructive pulmonary disease (COPD). In this study, we differentiated basal cells from individuals with and without COPD into 3D airway organoids and performed epigenetic profiling using the Infinium MethylationEPIC array. Promoters of inflammation-associated genes (e.g., TNFAIP2), which are expressed in secretory cell clusters of the airway organoids, were hypomethylated in both basal cells and organoids derived from individuals with COPD. Smoking was associated with global hypomethylation, including at the promoters of genes involved in airway secretions (e.g., BPIFA2). GSEA analysis revealed that genes with hypomethylated promoters in smoker-derived basal cells and organoids were significantly enriched among those highly expressed in secretory cells of differentiated organoids. Our findings reveal that smoking and COPD are associated with epigenetic alterations, including promoter hypomethylation of inflammation- and secretion-related genes, which are enriched in secretory cell populations of airway organoids.

ORAL PRESENTATION

111

Dissecting Pathogenic Cell Circuits in the in Bronchiolitis Obliterans Niche

J. Ruwisch*[1,2], H. Yilmaz*[1,2], L. M. Leiber[1,2], L. Neubert[2,3], L. Christian[1,2], L. Giercke[1], M. Greer[1,2], C. Werlein[2,3], L. Knudsen[2,4], U. Martin[5], F. Ius[2,6], D. Jonigk[2,7], C. Falk[8,9], N. Kaminski[10], J. Gottlieb[1,2], J. C. Kamp*[1,2], J. C. Schupp*[1,2,10]
[1]Hannover Medical School, Clinic for Respiratory Medicine and Infectious Diseases, Hannover, Germany, [2]BREATH, Hannover, Germany, [3]Hannover Medical School, Institute of Pathology, Hannover, Germany, [4]Hannover Medical School, Institute of Functional and Applied Anatomy, Hannover, Germany, [5]Lower Saxony Center for Biomedical Engineering, Implant Research and Development, Leibniz Research Laboratories for Biotechnology and Artificial Organs, Hannover, Germany, [6]Hannover Medical School, Clinic for cardiac, thoracic, transplant and vascular surgery, Hannover Medical Schoo, Hannover, Germany, [7]RWTH Aachen, Institute of Pathology, Hannover, Germany, [8]Hannover Medical School, Institute of Transplant Immunology, Hannover, Germany, [9]BRREATH, Hannover, Germany, [10]Yale School of Medicine, Yale, New Haven, United States of America
*Contributed equally

Background: Chronic lung allograft dysfunction (CLAD) remains the predominant cause of long-term graft loss after lung transplantation. Clinically, it manifests in two distinct phenotypes—bronchiolitis obliterans syndrome (BOS) and restrictive allograft syndrome (RAS)—which reflect the compartmentalized impact of alloimmune and non-alloimmune injury on the transplanted organ.
Aim: This study sought to delineate the cellular architecture underlying BOS lesions, to contrast these findings with alterations observed in RAS lungs, and to uncover molecular pathways that could represent therapeutic entry points in BOS.
Methods: We applied single-nucleus RNA sequencing (snRNA-seq) to characterize transcriptomes at single-cell resolution in archived explant material from 14 BOS, 15 RAS, and 13 non-diseased controls. Nuclei were isolated through enzymatic dissociation of fixed lung tissue and processed on the 10x Genomics Chromium platform. Data processing and integrative analyses were conducted with the Seurat v5.2 framework. Selected discoveries were independently validated using multiplex immunofluorescence staining to confirm spatial localization.
Results: Our dataset encompassed nuclei representing all major structural lineages—epithelial, endothelial, and mesenchymal—which were further resolved into 18 transcriptionally distinct cell populations based on canonical marker expression. Comparative analyses revealed a BOS-specific cellular fingerprint, including altered frequencies of certain populations when compared to RAS and control lungs. Multiplex validation confirmed that BOS-associated subsets were preferentially enriched at sites of histologically verified BOS lesions.
Conclusion: This work uncovers previously unrecognized structural cell circuits implicated in the emergence of BOS lesions. These findings enhance our understanding of CLAD pathogenesis and highlight potential molecular targets for precision therapies in BOS patients.

112

Repair-supportive mesenchymal cells (RSMCs) modulate epithelial WNT signaling during airway regeneration

M. Shahriari Felordi[1], A. Khadim[1], A. Lingampally[1], A. I. Vazquez-Armendariz[2], T. Procida-Kowalski[3], S. Hadzic[1], I. Alexopoulos[1], A. Günther[1], N. Weismann[1], M. Bartkuhn[3], W. Seeger[1], S. Herold[1], S. Bellusci[1], X. Chu[4], E. El Agha[1]
[1]Institute for Lung Health (ILH), Cardio-Pulmonary Institute (CPI), German Center for Lung Research (DZL), Justus-Liebig University Giessen, Germany, Gießen, Germany, [2]University of Bonn, Transdisciplinary Research Area Life and Health, Organoid Biology, Life & Medical Sciences Institute, Bonn, Germany, [3]Biomedical Informatics and System Medicine, Justus-Liebig-University Giessen, Gießen, Germany, [4]Oujiang Laboratory (Zhejiang Lab for Regenerative Medicine, Vision and Brain Health), School of Pharmaceutical Science, Wenzhou Medical University, Wenzhou, Zhejiang 325035, China, Wenzhou, China, People's Republic of

Stem and progenitor cells, as well as the surrounding niche function in a coordinated and dynamic manner to promote tissue regeneration. In the context of naphthalene injury, the crosstalk between epithelial cells, especially club cells, which are depleted, and mesenchymal cells is poorly understood. We have previously identified a novel cell population that we termed “repair-supportive mesenchymal cells” (RSMC), which is distinct from airway smooth muscle cells (ASMC) and is critical for the regeneration of the conducting airway epithelium by secreting fibroblast growth factor 10 (FGF10). In this study, we identify the RSMCs cluster as a distinct population coming from a highly Sfrp1+ cells subset, which has mostly adventitial fibroblast signatures. These cells, by secreting DKK2, a known inhibitor of the Wnt signaling pathway, can promote club cell regeneration and restore the airway epithelial composition.
Additionally, we showed the RSMCs-like cells in the COPD section, which shows the RSMCs signature upregulated compared to the donor. Organoid co-cultures revealed a reduced number of SCGB3A2+ bronchiolospheres in COPD, which may reflect fewer club cells and less mature organoids; however, this was improved following Wnt pathway inhibition, suggesting enhanced club cell differentiation and organoid maturation. Our data provide cellular and spatial evidence for the contribution of adventitial fibroblast cells to the RSMCs population emerging after naphthalene injury, which could facilitate club cell regeneration through Wnt signaling pathway inhibition

113

Epithelial CEACAM6 overexpression aggravates oxidative stress and inflammatory signalling upon short-term cigarette smoke exposure

Z. Shen, H. Treis, A. Cilic, E. Loku, V. Lakshmi, N. Sommer, N. Weissmann, C.-Y. Wu, S. Hadzic
Excellence Cluster Cardio-Pulmonary Institute (CPI), Universities of Giessen and Marburg Lung Center (UGMLC), Institute for Lung Health (ILH), member of the German Center for Lung Research (DZL), Justus Liebig University Giessen, Germany, Giessen, Germany

Chronic exposure to environmental stressors such as cigarette smoke (CS) or air pollution contributes to the development of chronic obstructive pulmonary disease (COPD). A hallmark of this incurable disease is increased oxidative stress and augmented inflammatory responses driving development of vascular alterations and emphysema. Previously, we showed that carcinoembryonic antigen cell adhesion molecule 6 (CEACAM6) impairs endogenous antioxidant defence in alveolar epithelial type II cells (AEC2s). CEACAM6 levels in AEC2s of COPD patients correlate with disease severity. However, the role of CEACAM6 during early COPD pathologies remains unclear.
To recapitulate molecular events during COPD development, we employed an *ex vivo* lung model with intratracheal delivery of fresh CS. Using a customised adeno-associated virus vector (AAV6.2FF), we achieved CEACAM6 overexpression predominantly in epithelial cells. We further used an *in vivo* model of short-term CS exposure to investigate the immune cell profiles in bronchoalveolar lavage (BAL) using flow cytometry and the alterations in alveolar compartment and pulmonary vasculature using lung function tests and right heart catheterisation.
CEACAM6 overexpression significantly elevated oxidative stress and cleaved caspase-3 levels after CS exposure. Immune cell profiling revealed an increase in number of $CD4^{+}$ /$CD8^{+}$ T-cell in CS-exposed mice with CEACAM6 overexpression. Interestingly, in CS-exposed mice, CEACAM6 increased right ventricular systolic pressure, suggesting pulmonary vascular alterations.
Further investigations aim to study the crosstalk between epithelial cells and pulmonary vascular cells upon CEACAM6 overexpression and to decipher the role of CEACAM6 in early COPD pathogenesis beyond the effect on AEC2.

ORAL PRESENTATION

114

Alveolar Macrophage based personalized therapy for the delivery of IL-37 in inflammatory airway disease like COPD

I. Theisohn[1], H. Heilmann[2], C. Herr[1], C. Beisswenger[1], B. Bufe[2], R. Bals[1]

[1]Saarland University, Department of Pulmonology, Allergology, Respiratory and Environmental Medicine, Homburg (Saar), Germany, [2]University of Applied Sciences Kaiserslautern, Department of Informatics and Microsystems Technology, Zweibrücken, Germany

Alveolar Macrophages (AMs) are self-renewing, tissue resident immune cells in the lung. Interleukin 37 (IL-37) has anti-inflammatory properties to prevent hyper-inflammation and tissue damage. It has been shown to counteract cigarette smoke induced inflammation by inhibiting expression and secretion of pro-inflammatory cytokines.
The aim of this work is the development of a cell-based personalized therapy with AMs that have been modified by adeno-associated virus (AAV) to express IL-37. The modified AM will be re-transplanted into the lung in the disease model of chronic obstructive pulmonary disease (COPD) to mitigate inflammation.
AMs were transduced with AAV expressing green fluorescent protein (GFP) to find the optimal AAV serotype, multiplicity of infection (MOI), and incubation time by confocal microscopy. Transducing AM with AAV2/6 at a MOI of 87.500 resulted in the highest transduction rate (10 – 30 %) and protein production after 6 days shown by enzyme-linked immunosorbent assay (ELISA) and Western Blot. Lipopolysaccharide (LPS) or Cigarette Smoke Extract (CSE) stimulation sharply increased IL-37 protein synthesis and secretion in a dose and time dependent manner. In a functional assay rhIL-37 decreased the expression of pro-inflammatory cytokines in tracheal epithelial cells after LPS stimulation. In a COPD animal model, mice were transplanted by intubation with AMs expressing IL-37 and smoked 3 days. The transplanted cells were rediscovered in the lung tissue. In bronchoalveolar lavage (BAL) the mice showed lower concentrations of pro-inflammatory cytokines compared to controls.
In summary AM can be successfully cultured *in vitro* and transduced. The therapeutic transgene IL-37 can be expressed and secreted after stimulation with LPS and CSE. In animal experiments mice transplanted with IL-37 expressing AM showed lower inflammation in BAL after 3 days of smoking which is an important and promising step towards the development of a cell based personalized therapy.

115

Comparative Analysis of Extracellular Vesicles from Plasma and Induced Sputum in COPD and Smoking Exposure

S. Wetzel[1], L. M. Kasperek[1], M. Müller[1,2]

[1]Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, Hannover, Germany, [2]German Center for Lung Research (BREATH), Hannover, Germany

Background/Objectives:

Chronic obstructive pulmonary disease (COPD) is a progressive inflammatory lung disease characterized by airway remodeling and irreversible airflow limitation. Reliable biomarkers for early diagnosis remain an unmet need. Extracellular vesicles (EVs) are nano-sized, membrane-enclosed particles reflecting the molecular signature of their parent cells. This study aimed to characterize EVs from plasma and induced sputum in COPD patients and healthy controls, with focus on smoking status.

Methods:

EVs were isolated from plasma and induced sputum of COPD patients (n=5 smokers, n=5 ex-smokers), healthy smokers, and healthy non-smokers (n=5 per group). Transmission electron microscopy (TEM) confirmed vesicle morphology, and dynamic light scattering (DLS) determined size distribution. Flow cytometry assessed canonical EV markers (CD9, CD63, CD81) and immune cell–related markers (CD14, CD28, CD31, CD45, CD11b). Cellular profiles from whole blood and sputum were analyzed, and correlations between immune cell subsets (identified by flow cytometry) and EV marker expression were examined.

Results:

TEM confirmed successful isolation of EVs from plasma and induced sputum. COPD patients and healthy smokers showed increased CD9 and CD14 expression in plasma-derived EVs compared to healthy non-smokers, with $CD14^+$ EVs particularly enriched in smoke-exposed groups. Notably, we demonstrate for the first time that induced sputum can serve as a reliable EV source: sputum-derived EVs also showed higher proportions of $CD14^+$ vesicles, while CD63 and CD81 expression was elevated in smokers and COPD patients. Both plasma- and sputum-derived EVs tended to display larger particle sizes in smokers and COPD patients, most consistently in plasma-derived EVs.

Conclusion:

EVs from plasma and sputum display disease- and smoking-related variations in marker expression and size, underscoring their potential as accessible biomarkers for COPD pathogenesis. The consistent increase of $CD14^+$ EVs across biofluids highlights them as candidates for less invasive COPD biomarkers. Plasma-derived EVs can further mirror lung-associated changes and serve as a minimally invasive biomarker source.

ORAL PRESENTATION

116

DUOX1 deficiency accelerates the progression of emphysema and pulmonary vascular remodeling upon cigarette smoke exposure

C.-Y. Wu, E. Loku, V. Sharma, C. Nuguri, V. Lakshmi, A. Cilic, D. Cojocaru, H. Treis, O. Pak, M. Gredic, S. Kraut, B. Kojonazarov, N. Sommer, N. Weissmann, S. Hadzic
Excellent Cluster Cardio-Pulmonary Institute (CPI), Institute for Lung Health (ILH), Universities of Giessen and Marburg Lung Center (UGMLC), Member of the German Center for Lung Research (DZL), Justus Liebig University (JLU), Giessen, Germany

Chronic obstructive pulmonary disease (COPD) is an incurable disease with a significant socioeconomic burden worldwide. A hallmark of COPD pathogenesis is augmented oxidative/nitrosative stress, which drives lung structural changes including airway remodeling, alveolar destruction, and pulmonary vascular alterations. Dual oxidase 1 (DUOX1) is a non-phagocytic NADPH oxidase and a source of hydrogen peroxide (H_2O_2). While H_2O_2 can contribute to oxidative stress, it also plays essential roles in cell signaling and host defense. DUOX1 is among the top downregulated targets in human COPD lungs. However, the role of DUOX1 and signaling mechanisms in COPD and COPD-associated pulmonary vascular remodeling remain elusive.
Here, we employed an *in vivo* model of chronic cigarette smoke (CS) exposure to recapitulate COPD-associated lung structural alterations. After 3 and 8 months of exposure, wildtype (Wt) and Duox1 knockout (*Duox1*$^{-/-}$) mice underwent clinically relevant assessments, such as micro-computed tomography (µCT), lung function tests, non-invasive transthoracic echocardiography, and right heart catheterizations. Mouse lung tissues were further analyzed by histological and molecular approaches.
Duox1 ablation accelerated early development of CS-induced emphysema, as demonstrated by increased lung compliance at 3-month time-point. After 8 months of CS exposure, *Duox1*$^{-/-}$ mice showed significantly increased mean linear intercept compared to Wt controls. Strikingly, *Duox1*$^{-/-}$ mice spontaneously developed pulmonary hypertension (PH), characterized by an increase in right ventricular systolic pressure (RVSP). CS exposure further aggravated the PH phenotype and led to pronounced impairment of right ventricular function, as revealed by echocardiography. Histological analyses in pulmonary vasculature uncovered muscularization of arterioles and pruning of capillary bed in Duox1$^{-/-}$ mice, with CS exposure further worsening capillary loss.
In summary, loss of DUOX1 accelerates COPD-associated alveolar and pulmonary vascular alterations upon CS exposure. Further investigations will focus on mechanisms underlying the distinct phenotype observed in DUOX1 deficiency.

Cross-Cutting Technologies: 201 - 208

ORAL PRESENTATION

201

Proteomic Analysis of Paediatric Bronchoalveolar Lavage Fluid: Towards a Simplified and Clinically Accessible Workflow

N. Freitag[1], D. Schramm[1], A. Stefanski[2], C. B. Schroeter[3,4], G. Poschmann[2], M. Driessen[5]
[1]Klinik für Allgemeine Pädiatrie, Neonatologie und Kinderkardiologie, Kinderpneumologie, Düsseldorf, Germany, [2]Biomedical Research Centre (BMFZ), Heinrich-Heine University, Molecular Proteomics Laboratory, Düsseldorf, Germany, [3]Harvard Medical School, Department of Cell Biology, Boston, United States of America, [4]Medical Faculty and University Hospital Düsseldorf, Department of Neurology, Düsseldorf, Germany, [5]University of Cologne, Centre of Dental Medicine, Köln, Germany

Background: Bronchoalveolar lavage fluid (BALF) provides direct access to the lower airways and is an important tool in paediatric respiratory medicine. Mass spectrometry-based proteomics can advance understanding of lung disease, but workflows tailored to children are lacking. Existing protocols are derived from adult studies, where larger sample volumes and higher protein yields are available.

Objective: To adapt an adult BALF proteomics workflow for paediatric use and establish a simplified, reproducible, and clinically accessible protocol accommodating smaller volumes and lower protein amounts.

Methods: BALF (1–10 mL) was collected from five children (ages 1–6 years) undergoing bronchoscopy for different indications. Four workflows were compared: ultracentrifugation (UC) with depletion, UC only, depletion only, and a simplified protocol omitting both steps. After 3 kDa ultrafiltration and S-Trap digestion, proteins were analysed on an Orbitrap Fusion Lumos mass spectrometer. Proteome coverage, reproducibility, and efficiency were assessed through technical replicates, coefficient of variation (CV) analysis, and processing time estimation.

Results: Across workflows, 643 proteins were identified, with the simplified protocol detecting the most (632 proteins from 1 mL BALF). A conserved proteome of 425 proteins (72%) was shared among all patients despite differing diagnoses. Technical reproducibility was high across workflows. Protein recovery was lowest in the combined UC+depletion workflow (<1%) and highest in the simplified workflow (21.6%). Estimated processing time for 30 samples decreased from three working days (UC+depletion) to two with the simplified workflow. The simplified workflow was successfully applied on 59 paediatric BALF samples.

Conclusion: This first systematic evaluation of BALF proteomics workflows in children shows that a simplified, adapted protocol achieves high proteome coverage, reproducibility, and efficiency while minimizing protein loss. It is particularly suited for paediatric samples due to smaller volumes, lower protein content, and its clinical accessibility, supporting future use in translational respiratory research.

ORAL PRESENTATION

202

Photon-Counting CT for Dynamic Lung Perfusion: Validation of a Low-Dose Protocol in a Porcine Lung Transplantation Model

A. M. Hunkemöller[1], T. Werncke[1], J. Dittrich[1], C. Schaefer-Prokop[2,3], F. Söbbeler[4], C. Figueiredo[4], A. Enzig-Strohm[4], M. Avsar[5], A. Ruhparwar[5], F. K. Wacker[1], H.-o. Shin[1]

[1]Hannover Medical School, Institute of Diagnostic and Interventional Radiology, Hannover, Germany, [2]Department of Radiology, Radboud University, Nijmegen, The Netherlands, [3]Department of Radiology, Meander Medical Center, Amersfoort, The Netherlands, [4]Institute of Transfusion Medicine and Transplantat Engineering, Hannover Medical School, Hannover, Germany, [5]Department of Cardiothoracic, Transplant and Vascular Surgery, Hannover Medical School, Hannover, Germany

Background:

Quantitative lung perfusion imaging is important for evaluating diseases such as pulmonary embolism, COPD, and CTEPH. Energy-integrating CT (EICT) perfusion is limited by high radiation dose, leading to routine use of perfused blood volume (PBV) as a surrogate. Photon-counting CT (PCCT) improves dose efficiency and spectral resolution, enabling dynamic functional imaging at standard chest CT dose levels.

Purpose:

To evaluate the quantitative performance of dynamic low-dose PCCT for lung perfusion imaging compared to reference-dose PCCT and static perfused blood volume (PBV) imaging.

Materials and Methods:

Six Göttingen minipigs with allogenic left lung transplants underwent dynamic perfusion imaging using PCCT at both, reference and low-dose settings, along with a static PBV scan. Lungs were divided into six regions. Perfusion metrics – Blood Flow Deconvolution (BFD), Mean Transit Time Deconvolution (MTTD), Flow Extraction Product (FEP), and Time to Start Deconvolution (TTSD) – were normalized and analyzed using Kruskal-Wallis tests and Bland-Altman plots.

Results:

Dynamic low-dose and reference-dose PCCT showed strong agreement across all perfusion parameters (BVP bias: 0.03; BVD bias: 0.04), with no significant differences in BVP ($p = 0.995$) or BVD ($p = 0.374$). Kinetic metrics were stable across dose levels (all $p > 0.2$). While low-dose imaging showed slightly increased perfusion heterogeneity, especially for BVD, BVP remained robust. Static PBV differed significantly from dynamic BVP ($p < 0.001$; $p = 0.04$), indicating consistency. Left-right lung perfusion differences were detected in two animals by all methods.

Extrapolated to a dynamic acquisition of 20 time points over a 35 cm range, cumulative DLP was 151.2 mGy·cm (2.37 mSv) for reference-dose and 86.8 mGy·cm (1.36 mSv) for low-dose PCCT – comparable to a single chest CT including a PBV map (95.2 mGy·cm, 1.49 mSv), and substantially below perfusion EICT (3 – 10 mSv).

Conclusion:

Photon-counting CT enables dynamic, low-dose lung perfusion imaging at radiation doses comparable to those of a single CT acquisition used for PBV imaging.

203

Providing context for clinical lung CT – A multi resolution imaging approach for bronchopulmonary dysplasia

J. Labode[1,2], Y. Regin[3], J. Toelen[3], M. Greyling[3], C. Mühlfeld[1,2,4]

[1]Hannover Medical School, Institute of Functional and Applied Anatomy, Hannover, Germany, [2]Biomedical Research in Endstage and Obstructive Lung Disease (BREATH), Member of the German Center for Lung Research (DZL), Hannover, Germany, [3]KU Leuven, Department of Development and Regeneration, Leuven, Belgium, [4]Hannover Medical School, Research Core Unit Electron Microscopy, Hannover, Germany

The diagnosis of bronchopulmonary dysplasia (BPD) is currently based on the need for supplemental oxygen, without the integration of a medical imaging protocol. Thus, the site and the extent of pathological changes in the patient’s lung are usually unknown. This impairs individualized therapy approaches.

Computed tomography (CT) examinations of the lung show high correlations with the clinical outcomes in patients with BPD. This method offers an overview of both the site and the extent of disease activity. In its clinical application however, it is restricted by its limited resolution. As CT scans come with a high radiation dose, exposure times and in consequence image quality are limited to a minimum. This is especially the case in pediatric CTs. Movements due to e.g. heartbeat and breathing activity further limit the achievable image quality. While the superficial pattern of pathological changes in the lung can be gathered from the scans, the histological properties of these changes stay beyond reach.

It would therefore be advantageous to know, what histological changes cause a specific signal pattern visible in CT scans. To address this question, an imaging workflow was developed to follow up an initial clinic-like in-vivo CT scan of the chest with a consecutive ex-vivo µCT scan and light microscopy of the lung. This workflow was employed on n=6 animals from a rabbit model of BPD at either 3- or 7-days post-partum. Through a series of image registrations between the resulting datasets, it proved to be possible to trace points of interest from the initial CT scan through the higher resolution ex-vivo imaging steps and uncover their cellular structure.

This knowledge can aid the interpretation of clinical CT scans, and may serve as a basis for the formulation of targeted treatment strategies.

204

Modeling environmentally triggered injury with human iPSC-derived lung organoids to evaluate cellular responses

U. Navarro[1], C. le Barro[2,3,4,5], L. Han[2], S. Gaedcke[6], T. Stoeger[2], C. Voss[1,2]

[1]Hannover Medical School, Clinic for Cardiac, Thoracic, Transplantation and Vascular Surgery, Leibniz Research Laboratories for Biotechnology and Artificial Organs, Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH), Member of the German Center for Lung Research (DZL), Hannover, Germany, [2]Institute of Lung Health and Immunity (LHI), Helmholtz Munich, Comprehensive Pneumology Center (CPC-M), Member of the German Center for Lung Research (DZL), Munich, Germany, [3]Translational Immunology in Environmental Medicine, School of Medicine and Health, Technical University of Munich, Munich, Germany, [4]Translational Immunology, Faculty of Medicine, University of Augsburg, Augsburg, Germany, [5]Department of Pediatrics, Klinikum rechts der Isar, School of Medicine and Health, Technical University of Munich, Munich, Germany, [6]Department of Respiratory Medicine and Infectious Diseases, Hannover Medical School, Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH), German Center of Lung Research (DZL), Hannover, Germany

Although nanomaterials (NM) have revolutionized many applications from chemistry to medicine, their interaction with biological compartments pose a threat to human health. One exposure pathway is inhalation, where NM can deposit deep in the lungs and lead to the development of respiratory diseases. Interestingly, lung diseases affect different men and women; however, the contribution of sex-specific differences in lung diseases has received little attention in air pollution studies. Carbon nanoparticles (CNPs), double-walled carbon nanotubes (DWCNT), and multi-walled carbon nanotubes (MWCNT) are representative of NM in environmental and industrial settings. Previous research into the effect of these particles on the lungs has been widely used in animal and in vitro systems. Due to the interspecies differences and the need for a more realistic representation of human lung biology, lung organoids might be a better option for understanding cell-specific responses and pose a new approach methodology. The study examines activated cellular pathways and reveal the mechanisms behind sex-specific responses. We use biological male and female human alveolar organoids generated from iPSC according to established protocols and expose them to CNPs, DWCNT, and MWCNT for 12h, 6d, or up to several weeks. Cell characteristics, viability, cytotoxicity, and RNA sequencing will be employed as endpoints to explore the time dependency of cell circuits and evaluate the model relevancy compared to pre-existing in vivo and in vitro data. Epigenetic analysis will elucidate differences in susceptibility to respiratory disease between men and women. Due to the maintained cellular plasticity in lung organoids, we expect to understand the modes of action of these particles in a human model system.

205

Volume and distribution of mitochondria in alveolar epithelial type I cells

A. Schierz, G. Roeßler, J. P. Schneider, J. Schipke, C. Mühlfeld
Medizinische Hochschule Hannover, Institute of Functional and Applied Anatomy, Hanover, Germany

The epithelial surface area of the air-blood-barrier of the lung is formed to 95 % by alveolar epithelial type I (AE1) cells. Serial block face scanning electron microscopy (SBF-SEM) investigations led to the hypothesis that mitochondria of AE1 cells are preferentially distributed as aggregates along connective tissue pillars between capillaries but far from the nucleus [Schneider2019]. Such a localization would not increase the diffusion distance for oxygen and carbon dioxide but provide energy substrates to the whole cytoplasm. Moreover, due to morphological changes during lung development like the flattening of the air-blood-barrier [Zeltner1987], it was hypothesized that distribution of mitochondria in AE1 cells differs between infants and adults. Lung tissue from three infants (26/30 d., 6 mo.) and three adults (20/39/40 yr.) was examined by microscopic stereology. Furthermore, adult lung tissue was used for immunohistochemistry and confocal laser scanning microscopy (CLSM) to analyze the distribution of mitochondria. The amount of AE1 cell mitochondria (V_V(Mito/AE1)) and their distribution were similar in infants and adults. Mitochondria were mostly found as single objects dispersed in the cytoplasm of AE1 cells with only a minor fraction located as aggregates in cellular niches not directly contributing to the air-blood barrier. In summary, the relative volume and distribution of AE1 cell mitochondria does not differ between infant and adult human lungs. No preferential localization of mitochondrial aggregates was observed.

References:

[Schneider2019] Schneider, J. P., Wrede, C., Hegermann, J., Weibel, E. R., Mühlfeld, C., & Ochs, M, (2019), On the topological complexity of human alveolar epithelial type 1 cells, American Journal of Respiratory and Critical Care Medicine, 1153-1156, 199(9), https://doi.org/10.1164/rccm.201810-1866LE

[Zeltner1987] Zeltner, T. B., Caduff, J. H., Gehr, P., Pfenninger, J., & Burri, P. H, (1987), The postnatal development and growth of the human lung. I. Morphometry, Respiration Physiology, 247-267, 67(3), https://doi.org/10.1016/0034-5687(87)90057-0

206

Advancing the BETA/CIVIC mini-lung model for nanotoxicity studies

M. Stremlau, C. Gabriel, A. Chakraborty, A. Schröppel, O. Schmid
Helmholtz Munich, Institute of Lung health and Immunity, Oberschleißheim, Germany

Lung diseases remain among the leading causes of morbidity and mortality worldwide, yet the translation of *in vitro* findings into effective *in vivo* research continues to be a major challenge. Conventional *in vitro* lung models are often overly simplistic and fail to mimic the complex architecture and environment of the alveolar space. One key aspect that is frequently overlooked is the mechanical stretch the lung undergoes during the breathing process.
The previously published BETA/CIVIC mini-lung model addresses this gap by combining epithelial, endothelial, and primary human fibroblast cells on a unique, stretchable membrane, enabling the study of mechanobiology in a human-relevant context.
Recently, we have advanced this model by implementing THP-1–derived macrophage-like cells, thereby establishing a complex quadruple co-culture that more closely recapitulates the alveolar niche. These macrophage-like cells were extensively characterized by qPCR, confirming their identity and robust pro-inflammatory responses to LPS as seen in other studies. Current efforts are focused on investigating the response to pro-inflammatory stimuli in the quadruple co-culture model.
In parallel, the effect of cyclic stretch on the mini-lung model is being investigated by comparing cultures with and without stretch to characterize stretch-dependent differences in morphology and biological response. Building on this characterization, future work will explore mechanosensitive pathways and nanomaterial-induced lung injury, which remains a pressing concern given the potential burden of nanomaterials on the alveolar region.
Together, these advances contribute to the development of a more predictive *in vitro* lung model that integrates key biological and mechanical cues.

207

Boost Efficiency: Use of artificial intelligence in a clinical setting

C. Valtin, S. Gaedcke, B. Liu, M. Zurawski, J. Fuge
BREATH, Hannover, Germany

Clinical workflows in pneumology involve a constant influx of external medical documents alongside the time-intensive creation of doctor's letters. Each month, about 1,500 patient-related documents are received, often as scanned faxes. They vary in quality and structure, requiring manual review, classification, assignment to the correct patient record, and manual transfer into the electronic health record (EHR). At the same time, doctor's letters remain central to clinical communication but are usually written manually, adding to administrative workload and variability in quality. Together, these processes consume valuable resources and can delay access to critical information.

This project addresses both challenges through a two-part AI-based solution. First, optical character recognition (OCR) converts scanned documents into machine-readable text. Large language models trained on medical terminology and document structures then identify document types, ensure correct patient assignment, and extract key clinical data directly into the EHR.

The second component transforms structured data into usable clinical correspondence. Based on the patient's EHR, the system generates draft doctor's letters summarizing key findings such as laboratory results, lung function metrics, and medical history. Retrieval-augmented generation integrates prior records and context, supporting comprehensive, coherent drafts. Physicians retain full control, reviewing and editing drafts before approval, reducing documentation time while improving consistency across letters.

Both components will be systematically evaluated through benchmarking of OCR methods, comparison of language model configurations, and user-centered testing. By linking document intake with automated correspondence, the project establishes a continuous workflow that reduces administrative burden, improves data quality, and enables structured information to be used directly for clinical decision-making, communication, and research. In the long term, this integrated approach is expected to support more efficient documentation workflows and promote the adoption of AI-based tools in routine clinical practice.

208

Enhancing Pulmonary Particle Clearance by Redirecting Alveolar Macrophages Using a SiglecF-cre Lineage Tracing System

Z. Yin
Helmholtz Zentrum München, Munich, Germany

Background:
Alveolar macrophages (AMs) are long-lived, self-renewing immune cells that reside in the alveoli and play a central role in particle clearance and immune homeostasis. Traditionally, AMs are considered sessile. However, recent evidence suggests that under certain stimuli, they can migrate toward the airways, potentially joining the mucociliary escalator for particle removal. Whether this migration can be intentionally induced to enhance clearance remains unknown.
Objective:
To determine whether chemokine-based reprogramming can induce AM migration toward the mucociliary escalator, thereby improving pulmonary particle clearance and reducing inflammation.
Methods:
SiglecF-cre × Ai14 reporter mice will be used for lineage tracing of SiglecF^{+} AMs. Specificity and mosaicism of labeling will be evaluated by flow cytometry, immunofluorescence, and intravital microscopy. Candidate chemokines (CCL2, CCL4, CCL7) will be screened in vitro using Boyden chamber assays with MHS cells and primary AMs from BAL. The most selective chemokine will be aerosol-delivered in vivo to central lung regions. AM migration will be monitored via intravital imaging and endpoint histology. Functional assessment will include fluorescent nanoparticle retention, BAL cytokine analysis, and inflammatory cell profiling.
Results (Expected):
We anticipate identifying at least one chemokine capable of selectively inducing AM migration without significant recruitment of neutrophils or monocytes. Chemokine-treated mice are expected to show increased AM accumulation in bronchial regions, improved clearance of fluorescent particles, and reduced levels of pro-inflammatory cytokines.
Conclusion:
This study will establish whether spatial reprogramming of AMs is a viable therapeutic strategy for enhancing pulmonary clearance. The SiglecF-cre × Ai14 system provides a robust platform for visualizing AM dynamics in vivo, offering translational potential for lung diseases involving particulate burden.

Cystic Fibrosis and Bronchiectasis: 301 - 307

ORAL PRESENTATION

301

Effects of elexacaftor/tezacaftor/ivacaftor on CFTR function in children with CF aged 2-11 years

J. Berger[1,2,3], J. Berges[4,5], Y. Yu[4,5], A. González[1], S. Hämmerling[4,5], R. Morty[4,5], S. Thee[1,2,3,6], M. Stahl[1,2,3,6], O. Sommerburg[4,5], M. A. Mall[1,2,3], S. Y. Graeber[1,2,3]

[1]Department of Pediatric Respiratory Medicine, Immunology and Critical Care Medicine and Cystic Fibrosis Center, Charité Universitätsmedizin, Berlin, Germany, [2]German Centre for Lung Research (DZL), Associated Partner, Berlin, Germany, [3]German Center for Child and Adolescent Health (DZKJ), partner site Berlin, Berlin, Germany, [4]Division of Pediatric Pulmonology and Allergology and Cystic Fibrosis Center, Department of Pediatrics, University of Heidelberg, Heidelberg, Germany, [5]Department of Translational Pulmonology, Translational Lung Research Center Heidelberg (TLRC), German Center for Lung Research (DZL), University of Heidelberg, Heidelberg, Germany, [6]Berlin Institute of Health at Charité Universitätsmedizin Berlin, Berlin, Germany

Objective: Elexacaftor/tezacaftor/ivacaftor (ETI) improves CFTR function in the intestinal epithelium to 40–50% of normal levels in patients with cystic fibrosis (CF) ≥12years with at least one *F508del CFTR* mutation. In children with CF aged 2-11years, ETI therapy reduced sweat chloride concentration (SCC) in clinical trials. However, its effect on CFTR function in the intestine in this age group remains unknown. Therefore, the aim of this study was to investigate the effect of ETI on CFTR function in intestinal epithelium in children with CF aged 2-11years with one or two *F508del* alleles.

Methods: This prospective, multicenter study assessed clinical outcomes including ppFEV$_1$% and BMI, and the CFTR biomarkers SCC and intestinal current measurement (ICM) in children with CF with at least one *F508del CFTR* mutation aged 2-11years before and after ETI.

Results: 26 children with CF including 8 with one *F508del* and a minimal function mutation and 18 *F508del* homozygous patients were enrolled in this study. ETI improved ppFEV$_1$% by 10.5% (SD ±19.7, P = 0.519) and SSC by -46.0mmol/L (IQR, -54.0 to -39.5; P < 0.001). In rectal epithelium, ETI improved cAMP-dependent chloride (Cl^-) response to 104.4 µA/cm² corresponding to rescue of CFTR function to a level of 89.5% (IQR, 60.0 to 146.1, P < 0.001). Total Cl^- response increased to 317.8 µA/cm² (IQR, 212.2 to 462.2, P < 0.001) corresponding to a level of 99.2% of normal CFTR activity. In addition, a secondary ICM data analysis of our previous study on the effects of ETI on CFTR function in patients with CF ≥12years and the present study showed correlations of the change in cAMP-mediated and cholinergic Cl^- response with the age (r = -0.63, $P < 0.001$ and r = -0.65, $P < 0.001$).

Conclusion: ETI leads to near-normal CFTR function in children with CF aged 2-11years with at least one *F508del CFTR* mutation. Our study supports early initiation of ETI therapy in children to improve long-term benefits.

302

Linking highly neutralizing human antibodies targeting multidrug-resistant Pseudomonas aeruginosa to clinical and immunological phenotype in people with cystic fibrosis

R. Brock[1,2,3,4], D. Lange[5,6], D. Meyer[6,7], J. Kutschera[5,6], D. Holzmann[5,6], T. Procida-Kowalski[8,9], M. Bartkuhn[8,9], J. C. Thomassen[2,3,4], E. Rietschel[2,3,4], S. J. Theobald[5,6,10], B. Schumacher[6,7], J. Rybniker[5,6,10], A. Simonis[5,6,10], M. A. Alejandre Alcazar[1,2,3,4,8,11], S. van Koningsbruggen-Rietschel[2,3,4]

[1]Translational Experimental Pediatrics, Department of Pediatric and Adolescent Medicine, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany, [2]Cystic Fibrosis Center, Department of Pediatric and Adolescent Medicine, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany, [3]Center for Rare Diseases, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany, [4]Member of the German Centre for Lung Research (DZL), Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany, [5]Department I of Internal Medicine, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany, [6]Center for Molecular Medicine Cologne (CMMC), Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany, [7]Institute for Genome Stability in Aging and Disease, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany, [8]Institute for Lung Health (ILH), Cardiopulmonary Institute (CPI), Member of the German Centre for Lung Research, Justus-Liebig-University Giessen and Marburg Lung Center, Giessen, Germany, [9]Biomedical Informatics and Systems Medicine, Justus-Liebig-University Giessen, Giessen, Germany, [10]German Center for Infection Research (DZIF), Partner Site Bonn-Cologne, Cologne, Germany, [11]Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases (CECAD), Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany

Multidrug-resistant Pseudomonas aeruginosa (PA) poses an emerging threat to human health with urgent need for novel therapeutic approaches. In people with cystic fibrosis (pwCF), PA frequently establishes chronic infections in the lung. We previously demonstrated that repeated exposure to PA can elicit highly neutralizing antibodies directed against its type III secretion system (T3SS). However, the immunological dynamics underlying chronic infection and the clinical significance of these antibodies remain poorly understood.
To address this, we integrated clinical data and serum T3SS-neutralizing activity from 99 pwCF and 12 healthy individuals with immunophenotyping of immune cell populations and bulk RNA sequencing of peripheral blood mononuclear cells (PBMCs). As expected, prolonged PA infection was associated with reduced lung function. Although chronic infection correlated with the presence of high titers of T3SS-neutralization antibodies (PA-Ab^{high}), these antibodies did not confer measurable protection against lung function decline, consistent with previous findings that the T3SS plays a subordinate role in chronic lung infections. Importantly, comparison of pwCF with high versus low neutralization capacity revealed distinct immune signatures: Flow cytometry demonstrated altered CD8^{+} T-cell profiles in PA-Ab^{high} individuals, marked by expansion of terminal effector subsets and reduction of naïve CD8^{+} T cells. These cellular alterations were mirrored in transcriptomic differences, particularly within cytokine–cytokine receptor interaction and adaptive immunity pathways.
In conclusion, our findings provide mechanistic insight into the development of highly neutralizing anti-T3SS antibodies and identify a distinct immunological profile associated with chronic PA infection and impaired lung function. Incorporating this immune signature into clinical practice may support the early identification of patients at risk of disease progression.

303

Bronchoalveolar lavage as a diagnostic tool for assessing disease activity in bronchiectasis

L. Kunz[1,2], L. Bauer[1,2], C. Sieber[1,2], S. Plein[1,2], A. Ryba[1,2], R. Grosser[3], W. Mellin[4], U. Sommerwerck[1,2]
[1]Cellitinnen-Severinskloesterchen Krankenhaus der Augustinerinnen, Klinik für Pneumologie, Allergologie, Schlaf- und Beatmungsmedizin, Köln, Germany, [2]Universität Witten/Herdecke, Fakultät für Gesundheit, Witten, Germany, [3]Labor Dr. Wisplinghoff, Köln, Germany, [4]Institut für Pathologie & Zytologie, Köln, Germany

Background

Bronchiectasis is a condition characterized by radiologically detectable, irreversible bronchial dilatation combined with typical symptoms (cough with sputum production, dyspnoea, recurrent infections, fatigue, haemoptysis, chest pain, bronchospasm). In the prospective analysis of a cross-sectional group of patients with bronchiectasis, more than 100 patients were examined.

Results

In this prospective interim analysis of this cross-sectional group of nearly 50 patients in a stable phase of their condition (no exacerbations), the mean age was 61.1 (SD +/- 15,7) years. Female patients predominated in this group, at 71,4%. Most patients were never smokers or non-smokers (61,3%). Overall, a large proportion of patients had underlying conditions such as COPD (33%) and bronchial asthma (34,7%). In terms of lung function, we observed a mean FEV1 of 1,81 Liters which corresponds to 63,7% of the target value and FVC of 2,68 Liters (74%).

In the differential cytology of bronchoalveolar lavage (BAL), surprisingly 40% of patients showed a relevant neutrophilia, while they were clinically stable.
In 50% of subjects, pathogenic germs were detected in the BAL despite a clinical stable phase of their disease, which could not consistently be reflected in sputum. Pseudomonas aeruginosa dominated with 42,8% followed by Haemophilus influenzae (19%), Staph. aureus (14,2%) Klebsiella oxytoca (9,5%). Other pathogens included Moraxella catarrhalis, Streptococcus pneumoniae E. coli and Streptococcus anginosus.

Conclusion

Clinically stable patients with bronchiectasis need to be followed up regularly. In line with the data from Prognosis registry, there are initial indications that BAL may be superior to systemic markers for assessing disease activity.

ORAL PRESENTATION

304

Novel polymeric reducing agents as mucus modulators for cystic fibrosis

A. Loewe[1,2,3], J. Arenhoevel[4], J. Ivković[4], R. Bej[4], A. Addante[1], S. Y. Graeber[1,2,3], J. Duerr[1,2,3], M. Gradzielski[5], D. Lauster[6], R. Haag[4], M. A. Mall[1,2,3]

[1]Charité - Universitätsmedizin Berlin, Department of Pediatric Respiratory Medicine, Immunology and Critical Care Medicine, Berlin, Germany, [2]German Center for Lung Research (DZL), associated partner site Berlin, Berlin, Germany, [3]German Center for Child and Adolescent Health (DZKJ), partner site Berlin, Berlin, Germany, [4]Freie Universität Berlin, Department of Chemistry and Biochemistry, Berlin, Germany, [5]Technische Universität Berlin, Institute of Chemistry, Berlin, Germany, [6]Freie Universität Berlin, Institute of Pharmacy, Biopharmaceuticals, Berlin, Germany

BACKGROUND Airway mucus obstruction is a key features of cystic fibrosis (CF) lung disease due to increased mucin crosslinking, which results in abnormal viscoelastic properties and impaired mucociliary clearance. Therefore, targeting these excess crosslinks is a promising mucolytic strategy. The only reducing agent approved for clinical use N-acetylcysteine (NAC) lacks potency and clinical efficacy resulting in an unmet medical need for more effective mucolytic therapies. The aim of this study was therefore to determine the mucolytic activity of s monothiol-functionalized mucolytic (dPGS-SH) in βENaC-Tg mice. Additionally, a second-generation dithiol-functionalized polymeric mucolytic (dPG-DTBA) was assessed in vitro.

METHODS βENaC-Tg mice were treated with three intratracheal instillations in one day to test acute effects of dPGS-SH on airway mucus plugging and inflammation. CF sputum samples and a synthetic hydrogel were treated with dPG-DTBA for 30 minutes at 37 °C and analyzed by Western blot and rheology respectively.

RESULTS Compared to vehicle control, acute dPGS-SH treatment decreased airway mucus content and neutrophil counts in adult βENaC-Tg mice. dPG-DTBA showed dose dependent reducing activity on mucin multimers in Western blot analysis of MUC5B and MUC5AC. Further, rheological measurements demonstrated that dPG-DTBA decreased the elastic modulus G' of a synthetic hydrogel compared to samples treated with PBS.

CONCLUSION Our results show dPGS-SH has potent mucolytic activity in βENaC-Tg mice. Data on the second-generation dPG-DTBA show effective mucolytic activity in vitro and provide the rationale for advancing preclinical testing to in vivo studies.

ACKNOWLEDGEMENTS Funded by the Deutsche Forschungsgemeinschaft (DFG) - SFB 1449 - 431232613; sub-project C04.

ORAL PRESENTATION

305

Immune-regulatory effects of CFTR triple modulator therapy in cystic fibrosis

V. Meyboom[1], M. Welsner[1,2], D. Westhölter[1], S. Straßburg[1,2], C. Taube[1], S. Sutharsan[1,2], M. Schedel[1,3]
[1]University Medical Center Essen - Ruhrlandklinik, Department of Pulmonary Medicine, Essen, Germany, [2]University of Duisburg-Essen, Adult Cystic Fibrosis Center, Essen, Germany, [3]University Medical Center Essen, Department of Pulmonology, Essen, Germany

Cystic fibrosis (CF) is a life-limiting genetic disease associated with altered immune regulation. While CFTR triple modulator therapy (elexacaftor/tezacaftor/ivacaftor, ETI) has transformed clinical outcomes in people with CF (pwCF), its impact on adaptive immunity remains unclear.
We analyzed peripheral blood $CD4^+$ T cells from healthy controls (n=22) and pwCF (n=13) at baseline, 3, 6, and 9 months after ETI initiation. Cells were immunophenotyped by flow cytometry, both *ex vivo* and following *in vitro* culture with or without activation (αCD3/CD28). Plasma levels of 12 cytokines were quantified at each time-point.
At baseline, pwCF compared to controls exhibited higher $CD69^+$ and terminally differentiated effector memory (Temra) $CD4^+$ T cells, along with reduced effector regulatory T cells (eTreg), suggesting chronic activation and impaired T cell regulation. ETI progressively reduced $CD69^+$ and Temra cells towards levels of controls, while giving rise to eTregs and total Tregs, particularly after activation of cultured cells, reaching values comparable to controls. CD69 and CD103 expression, markers of tissue-resident memory T cells, showed dynamic regulation following ETI therapy, indicating shifts in tissue-homing properties. Naïve, central memory, and effector subsets remained unchanged. Plasma cytokines mirrored some of the cellular effects. IL-9 and IL-10, both linked to Treg function, were lower in pwCF at baseline than in controls but increased post-ETI. Classical Th2 cytokines (IL-5 and IL-13) showed similar trends. This may reflect partial recovery of Treg-associated immune function and reversal of a suppressed Th2 response. In contrast, IL-22 remained below control levels before and following ETI, suggesting persistent deficits in epithelial barrier-associated pathways.
Together, ETI not only improved clinical outcomes in pwCF but also promoted immune rebalancing of $CD4^+$ T cells and partial restoration of cytokine profiles. These findings support the concept that CFTR correction may recover functional properties of the adaptive immune system contributing to the therapeutic benefit in pwCF.

306

Longitudinal Effects of Elexacaftor/Tezacaftor/Ivacaftor Therapy on the Respiratory Microbial Metagenome in Children with Cystic Fibrosis Aged 6-11 Years

I. Rosenboom, T. L. Hertramph, R. Dalferth, M. E. Baud, R. Minso, K. Schütz, A.-M. Dittrich
Hannover Medical School, Hannover, Germany

Introduction:
The introduction of mutation-specific triple combination therapy with Elexacaftor/tezacaftor/ivacaftor (ETI) has significantly improved lung function and quality of life for people with cystic fibrosis (pwCF). As shown in culture- and sequencing-based studies that involve respiratory secretions of pwCF 12 years and older, ETI decreases the abundance and load of typical CF pathogens in the airways. For the past two years, ETI treatment has also been available for children with CF (cwCF) aged 6-11 years. To investigate the impact of early ETI treatment on airway microbial communities, our study employs shotgun metagenomics that provides higher resolution identification of bacteria, fungi and DNA viruses compared to amplicon sequencing commonly applied to study this habitat.
Methods:
In this prospective post-approval study, cough swabs from 15 cwCF aged 6-11 years at baseline, 3 months, and 24 months after initiation of ETI therapy were collected and analyzed for microbial metagenomics. DNA was extracted under ultra-clean wet-lab conditions, followed by shotgun metagenome sequencing. Fragment libraries were sequenced on an Illumina NovaSeq XPlus. Processing of the short sequencing reads was performed by our in-house developed sequencing alignment pipeline Wochenende prior to appropriate statistical analyses.
Results:
In this prospective post-approval study, treatment with ETI improved CFTR function and several clinical parameters in children with CF aged 6–11 years. At the global level, microbial α-diversity remained unchanged throughout ETI therapy. In contrast to therapy, age significantly influenced the abundance of several commensal taxa.
Conclusion:
Metagenomic profiles appeared to be more strongly influenced by age than by ETI treatment status, suggesting that, in this age group, inter-individual variability and developmental factors may play a more dominant role in shaping the respiratory microbiome than therapeutic intervention.

ORAL PRESENTATION

307

Synergistic Enhancement of CFTR Function by Short-Chain Fatty Acids and CFTR Modulators in Cystic Fibrosis Cell Models

M. Wang[1], A. Balázs[2], A. Tony-Odigie[3], A. Dalpke[4], M. Mall[5]

[1]Heidelberg University, Mannheim, Germany, [2]Department of Pediatric Respiratory Medicine, Immunology and Critical Care Medicine, Charité - Universitätsmedizin Berlin, Berlin, Germany, Berlin, Germany, [3]Department of Infectious Diseases, Medical Microbiology and Hygiene, Medical Faculty Heidelberg, Heidelberg University, Heidelberg, Germany, Heidelberg, Germany, [4]Department of Infectious Diseases, Medical Microbiology and Hygiene, Medical Faculty Heidelberg, Heidelberg University, Heidelberg, Germany, Heidelberg, Germany, Heidelberg, Germany, [5]Department of Pediatric Respiratory Medicine, Immunology and Critical Care Medicine, Charité - Universitätsmedizin Berlin, Berlin, Germany

The elexacaftor-tezacaftor-ivacaftor (ETI) triple combination CFTR modulator therapy has improved clinical outcomes for up to 90% of patients with cystic fibrosis (CF) who carry at least one F508del-CFTR allele. Evidence links a more diverse airway microbiome with improved clinical outcomes in patients with CF. The hypothesis suggests that some commensal bacteria may play protective roles in the airways by producing short-chain fatty acids (SCFAs) that inhibit pathogens and reduce inflammation.

To investigate the effects of SCFAs on F508del-CFTR function and their potential synergism with ETI, we performed transepithelial short-circuit current measurements using Ussing chambers in CFBE41o-cells. To investigate the effect of SCFAs on CFTR expression and maturation, we performed real-time qPCR and Western blot analysis on whole-cell lysates. Further, we tested SCFAs in primary nasal epithelial cultures from patients with CF homozygous for the F508del mutation.

Our results showed relatively small standalone effects but strong synergy between several SCFAs and ETI in restoring F508del-CFTR function. The combination therapy of butyrate and ETI enhanced CFTR-mediated currents fourfold compared to ETI alone in CFBE41o cells. Additionally, propionate and valerate demonstrated similar synergism, increasing CFTR-mediated currents approximately twofold when combined with ETI. Real-time qPCR and Western blot analysis demonstrate that butyrate upregulates F508del-CFTR transcription and enhances protein maturation. Furthermore, preliminary data from nasal epithelial cultures show an increase in CFTR-mediated ion transport when combining ETI with either butyrate.

Our findings demonstrate that SCFAs amplify the ETI-mediated pharmacological restoration of F508del-CFTR chloride channel function, thereby identifying a further putatively beneficial effect of commensals' metabolites. While the detailed molecular mechanisms remain to be identified, these findings help to explore an additional therapeutic approach to further improve F508del-CFTR function.

Infectious Lung Diseases - from vaccination to therapy: 401 - 408

401

Exploring the therapeutic potential of novel antimicrobial peptides using human bronchial organoids

A. Carvalho[1,2], J. H. Pflugmacher[1], C. Falciani[3], A. Pini[3], F. Mungenast[2], R. Ecker[2], I. Ellinger[4], H. Garn[1]
[1]Translational Inflammation Research Division & Core Facility for Single Cell Multiomics, Marburg, Germany, [2]Research and Development Department of TissueGnostics GmbH, Vienna, Austria, [3]SetLance srl, Siena, Italy, [4]Institute of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria

Antibiotics have played a crucial role in controlling infectious diseases. However, as we are now facing rising rates of antimicrobial resistance worldwide, there is an urgent need to develop novel antimicrobial strategies. Recently developed antimicrobial peptides (AMPs) from the SET-M33 family may be one such strategy, as they have been shown to exert antibacterial activity against relevant respiratory pathogens, such as *P. aeruginosa*, including resistant strains. Preliminary results from a resazurin-based cell viability assay using a human bronchial epithelial cell line (BEAS-2B), which were exposed for 72 hours, showed a therapeutic window of concentrations up to 1.5 µM for two enantiomers of these AMPs (SET-M33D/L). As these drugs are being developed for inhalation administration, this study aimed to evaluate their safety and host-specific effects on the human airway epithelium. Thus, three doses that proved effective against the aforementioned bacteria within and above the predicted therapeutic window — 0.7, 1.4 and 3.0 µM — were investigated using primary somatic cell-derived bronchial organoids. Both initial and preformed cultures were exposed to each AMP for 2 weeks. Thereafter, cell viability was measured using live/dead cell staining and transcriptome analyses were conducted to assess the effect of each AMP on epithelial differentiation and activation. At a concentration of 3.0 µM, both AMPs significantly reduced the cell viability by up to over 60%. This effect was less pronounced at lower concentrations with no significant differences observed between untreated organoids and those treated with 0.7 µM AMP. The gradual reduction in viability with increasing doses of AMP was AMP-dependent. Image analyses confirmed these findings, showing a concentration-dependent impact on organoid morphology parameters. Moreover, the enantiomers affected – mostly in opposite directions - the expression of certain signalling molecules and marker genes, particularly for secretory cells. This study provides valuable insights into the differential activity of two AMP enantiomers, providing overall support for their potential as novel antimicrobial drugs.

ORAL PRESENTATION

402

Pulmonary glycosaminoglycans and fucosylation regulate lung barrier function and defense against S. pneumoniae

C. Goekeri[1], K. Linke[1], K. Hoffmann[1], E. Lopez-Rodriguez[2], V. Gluhovic[2], A. Voß[3], S. Kunder[3], A. Zappe[4], S. Timm[2,5], A. Nettesheim[1], S. Schickinger[1], C. Zobel[6], L. Bechtella[4], G. Vos[4], K. Pagel[4], A. Gruber[3], M. Ochs[2,5], M. Witzenrath[1,7], G. Nouailles[1,7]

[1]Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Infectious Diseases, Respiratory Medicine and Critical Care, Berlin, Germany, [2]Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Institute of Functional Anatomy, Berlin, Germany, [3]Freie Universität Berlin, Institute of Veterinary Pathology, Berlin, Germany, [4]Freie Universität Berlin, Institute of Chemistry and Biochemistry, Berlin, Germany, [5]Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Core Facility Electron Microscopy, Berlin, Germany, [6]Bundeswehrkrankenhaus Berlin, Department of Internal Medicine, Berlin, Germany, [7]German Center for Lung Research (DZL), Berlin, Germany

Background: Lungs are decorated with glycosaminoglycans, *N*- and *O*-glycans, which are exposed to invading pathogens. Shedding or alteration of such structures may influence susceptibility to infection.
Aims: Investigating how heparan sulfate and hyaluronan, as well as terminally fucosylated glycans influence Streptococcus pneumoniae (S.pn.) infection and lung barrier function.
Methods: Murine models of pneumonia were employed utilizing enzymatic cleavage of pulmonary hyaluronan and heparan sulfate, as well as systemic inhibition of terminal fucosylation using 2-deoxy-D-galactose (2d-Gal). The murine glycome was evaluated using mass spectrometry. Barrier function was assessed through ELISA, impedance sensing of primary lung epithelial cells and use of a human alveolus-on-a-chip model.
Results: Heparinase treatment of mice resulted in increased lung barrier permeability during pneumonia. Exposure to heparinase in vitro accelerated loss of barrier function of human primary alveolar epithelial cells following S.pn. infection. In a human alveolus-on-a-chip model, heparinase treatment combined with S.pn. infection resulted in increased epithelial permeability [1]. Systemic inhibition of fucosylation in vivo using 2d-Gal led to reduced bacterial burden and quicker resolution of neutrophilic inflammation in BAL of infected mice. Treatment with 2d-Gal led to reduced lung permeability and lower cytokine levels in BAL fluid of mice infected with S.pn.
Conclusions: Our findings reveal a protective role of intact heparan sulfate on lung epithelial barrier function, while restricting availability of fucosylated glycans may limit S.pn. infectivity and pulmonary inflammation.

References:

[1] Goekeri C et al., (2024), Enzymatic Modulation of the Pulmonary Glycocalyx Enhances Susceptibility to Streptococcus pneumoniae, American Thoracic Society, American Journal of Respiratory Cell and Molecular Biology, 646 - 658, 6, https://doi.org/10.1165/rcmb.2024-0003OC

403

Breaking Barriers: Probing the Role of Airway Mucin Glycoproteins in Pneumococcal Infection with a Mucin Selective Protease.

N. Hardiman[1], C. Goekeri[1], U. Behrendt[1], K. Hoffmann[1], S. Kunder[2], A. D. Gruber[2], S. A. Malaker[3], M. Witzenrath[1], G. Nouailles[1]

[1]Charité, Fächerverbund für Infektiologie, Pneumologie und Intensivmedizin, Berlin, Germany, [2]Freie Universität Berlin, Institute of Veterinary Pathology, Berlin, Germany, [3]Yale University, Department of Chemistry,, New Haven, CT, United States of America

Decoding how the airways epithelial glycocalyx governs infection outcomes could unlock new strategies to protect against respiratory infections. The airway glycocalyx, dominated by mucin-type *O*-glycoproteins, is the first microbial point of contact, yet its role in shaping infection outcomes remains largely unknown. This underexplored barrier offers the potential to uncover new mechanisms that strengthen respiratory immunity. Here, we employed the mucin-selective Secreted Protease of C1 Esterase Inhibitor (StcE) as a tool to selectively cleave pulmonary mucin-type glycoproteins.

Mice were treated with active or heat-inactivated StcE, with or without pneumococcal infection, and were monitored over 48 hours. At 48 hours post-infection, lungs were harvested for histopathological assessment and bacterial burden was determined in blood and spleen samples. Infected animals receiving active StcE exhibited significant weight loss at 24-, 36-, and 48-hours post-infection, with evidence of clinical deterioration in some individuals. At 48 hours, blood and spleen bacterial burden was not significantly different between groups. A trend towards hypothermia in StcE-treated infected mice was observed. Ongoing histopathological analyses will reveal the impact of StcE treatment on murine lungs following pneumococcal infection.

These findings so far suggest that intranasal treatment with mucinase StcE is associated with more severe clinical outcomes during infection. Ongoing histopathological and immune analyses aim to clarify how mucin-type glycoproteins influence epithelial damage and host responses, while human *in vitro* models, such as lung-on-chip infection studies, will extend these insights. Overall, this work aims to provide novel mechanistic insight into host-pathogen interactions and the role of the glycocalyx in supporting respiratory defence.

ORAL PRESENTATION

404

Mechanical strain exacerbates Pseudomonas infection in an organoid-based pneumonia-on-a-chip model

K. Hoffmann*[1], U. Behrendt[1], P. Pennitz[1], H. Kirsten[2], J. Pohl[3], E. Lopez-Rodriguez[4], C. Weissfuss[5], J. Kollmeier[6], M. Tönnies[6], S. Brill[7], K. Steinestel[7], M. Witzenrath[1,8], W. Wenzel[9], C. Zobel*[9], G. Nouailles*[1]
[1]Charité Universitätsmedizin Berlin, Department of Infectious Diseases, Respiratory Medicine and Critical Care, Berlin, Germany, [2]University of Leipzig, Institute for Medical Informatics, Statistics, and Epidemiology, Leipzig, Germany, [3]Bundeswehrkrankenhaus Berlin, Department of Internal Medicine, Berlin, Germany, [4]Charité Universitätsmedizin Berlin, Institute of Functional Anatomy, Berlin, Germany, [5]Bundeswehrkrankenhaus Berlin, Department of Internal Medicine and Department of Microbiology and Hospital Hygiene, Berlin, Germany, [6]Helios Klinikum Emil von Behring GmbH, Berlin, Germany, [7]Bundeswehrkrankenhaus Ulm, Ulm, Germany, [8]German Center for Lung Research (DZL), Berlin, Germany, [9]Bundeswehrkrankenhaus Berlin, Berlin, Germany

*Contributed equally

Pseudomonas aeruginosa, an opportunistic and often multidrug-resistant pathogen, is a leading cause of ventilator-associated pneumonia (VAP) in mechanically ventilated ICU patients, with mortality rates ranging from 24% to 76%. Yet, the mechanisms driving VAP are still poorly understood, largely because of the lack of physiologically relevant human models.

We used a human alveolus-on-a-chip system (Emulate) that reproduces the epithelial–endothelial barrier under air exposure, vascular perfusion, and mechanical stretch. While commercially available primary epithelial cells failed to display essential alveolar functions such as surfactant production, the integration of adult stem cell–derived organoid cells enabled a more representative alveolar epithelium. On-chip, these cells acquired features of mature surfactant-producing alveolar type 2 (AT2) cells and showed upregulation of alveolar type 1 (AT1) markers.

To investigate how mechanical forces influence epithelial states, we performed single-cell RNA sequencing under 5% and 10% stretch conditions. The analysis confirmed the presence of AT1-like cells and revealed that while overall cellular composition was stable, 10% hyper-physiological stretch broadly suppressed gene expression.

We next established *P. aeruginosa* infection in the chip to study how strain impacts host–pathogen interactions. While 10% stretch alone slightly weakened barrier integrity, infection under this condition caused a pronounced increase in barrier permeability and vascular bacterial burden compared to 5% stretch. Surprisingly, inflammatory cytokine secretion (IL-6, IL-8) was reduced under higher strain, suggesting impaired epithelial defense mechanisms.

In summary, this pneumonia-on-a-chip model demonstrates how epithelial differentiation state and mechanical strain together influence infection progression. These findings provide new insight into how ventilation-associated mechanical forces may exacerbate pneumonia and offer a platform to dissect stretch-dependent mechanisms of barrier disruption and immune failure.

ORAL PRESENTATION

405

Investigating Redox-Signaling Mechanisms Underlying Trained Immunity in Alveolar Macrophages

M. Kumari[1,2], A. Ö. Yildirim[1,3], C. Lindermayr[1,2]

[1]Institute of Lung Health and Immunity (LHI), Helmholtz Munich, Comprehensive Pneumology Center (CPC-M),Germany, Member of the German Center for Lung Research (DZL), Munich, Germany, [2]TUM School of Life Sciences, Technical University of Munich, Munich, Germany, [3]Institute of Experimental Pneumology, Ludwig-Maximilians University (LMU), Munich, Germany

Trained immunity is a functional state of the innate immune response, which is characterized by long-term epigenetic and metabolic reprogramming of the innate immune system. This enables a fast and strong response to future challenges. The production of nitric oxide (NO) is part of the response to pathogen attack, but less is known, if these molecules are essential for establishing innate immune memory.

We are investigating the role of redox-signaling in trained immunity in alveolar macrophages (AM). We demonstrated that treatment with flagellin is inducing innate immune training. This results, in comparison to non-trained cells, in enhanced expression of immune marker genes, such as TNF and NOS2, after treatment with a second stimulus (LPS). Both, flagellin and LPS treatment, are inducing NO production. Interestingly, inhibition of flagellin-induced NO production with the NO synthase inhibitor significantly reduced the training effect. On the other hand, treatment with the NO synthase inhibitor as first stimulus is also inducing innate immune training. We also demonstrate that ambient flagellin exposure rewires AM metabolism, which further impacts the metabolic regulation in trained immunity. Further, RNA-seq analyses identified genes, which are regulated by NO in trained immunity and which of these NO-dependent genes are required for establishing innate immune memory. Moreover, NO target proteins provide an insight into regulatory mechanisms of NO in trained immunity.

Overall, a detailed understanding of redox-mechanisms in trained immunity might allow us to develop immunotherapies to promote trained immunity on one side and to treat excessive or defective trained immunity on the other side.

406

Influence of host fucosylation and secretor status on pneumonia pathogenesis and infection dynamics

S. Mukherjee*[1], S. Schickinger[1], L. Salmon[1], K. Hoffmann[1], U. Behrendt[1], K. Schmidt[2], F. Röpken[3], G. Vos[4], S. Hedtrich[2], A. Braun[3], K. Pagel[4], G. Nouailles[1,5], CAPNETZ Study Group[6], M. Witzenrath*[1,5,6], C. Goekeri*[1]
[1]Charité – Universitätsmedizin Berlin, Department of Infectious Diseases, Respiratory Medicine and Critical Care, Berlin, Germany, [2]Center of Biological Design, Berlin Institute of Health (BIH) at Charité – Universitätsmedizin Berlin, Berlin, Germany, [3]Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Member of the German Center for Lung Research (DZL), Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH), Hannover, Germany, [4]Freie Universität Berlin, Institute of Chemistry and Biochemistry, Berlin, Germany, [5]German Center for Lung Research (DZL), Associated Partner Site, Berlin, Germany, [6]CAPNETZ Stiftung, Hannover, Germany
*Contributed equally

Background: The alveolar epithelium harbors glycans in glycoproteins including terminal sugars, like fucose. Systemic inhibition of terminal fucosylation in mice with 2-deoxy-D-galactose (2d-Gal) lowers bacterial loads and improves physiological parameters after Streptococcus pneumoniae (S.pn.) infection. In Europe, approximately 23% of healthy people are reported to be non-secretors (sese) due to a fucosyltransferase (FUT)2 gene mutation, preventing terminal fucosylation in mucosal secretions. Clinical impact of FUT2 expression on pneumonia pathogenesis remains unestablished.
Aims: We aim to explore the role of α-(1,2)-fucose in S.pn. infection of human preclinical models including primary epithelial cell-derived air liquid interface (ALI) cultures and precision cut lung slices (PCLS).
Methods: Single nucleotide polymorphism (SNP) genotyping was performed to detect non-sense mutations in the FUT2 gene in DNA of patients with community-acquired pneumonia (CAP), in addition to primary epithelial cell donors, using TaqMan SNP Genotyping assays and real-time PCR. Primary bronchial epithelial cells were cultured in ALI before infection. PCLS were treated with 2d-Gal for 48 hours prior to S.pn. infection. Planktonic, cell-adherent, and translocated bacteria were quantified by colony-forming unit (CFU) enumeration, and microscopy was performed to visualize adherent and invasive bacteria.
Results: Significantly higher number of hospitalized patients with CAP were secretors (SeSe or Sese) compared to sese. The prevalence of homozygous SeSe genotype amongst hospitalized patients with CAP was higher than that of healthy individuals. Preliminary data suggest that following infection of lung epithelia cultured under ALI, increased numbers of planktonic and cell-adherent bacteria were quantified in SeSe compared to sese cultures at 16 hours post infection (hpi). Productive infection in PCLS was seen at 16 hpi via CFU enumeration, in addition to microscopic visualization of extracellular and intracellular S.pn.
Conclusions: Lack of terminal fucosylation may limit S.pn. adherence and infection of lung epithelia.

ORAL PRESENTATION

407

From Lungs to Heart: scRNA-seq Illuminates Post-Pneumonia Cardiovascular Risks

P. Pennitz[1], M. Brack[1], K. Mueller[2,3], R. Steinhorst[1], E. Wyler[4], C. Goekeri[1,5], J. Bagli[1], A.-E. Saliba[6,7], M. Scholz[2,8], M. Witzenrath[1,9], G. Nouailles[1,9], H. Kirsten[2]

[1]Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Infectious Diseases, Respiratory Medicine and Critical Care, Berlin, Germany, [2]University of Leipzig, Institute for Medical Informatics, Statistics, and Epidemiology, Leipzig, Germany, [3]Center for Scalable Data Analytics and Artificial Intelligence (ScaDS.AI), Leipzig, Germany, [4]Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany, [5]Cyprus International University, Faculty of Medicine, Nicosia, Cyprus, [6]Faculty of Medicine, Institute of Molecular Infection Biology (IMIB), University of Würzburg, Würzburg, Germany, [7]Helmholtz Institute for RNA-based Infection Research (HIRI), Helmholtz Center for Infection Research (HZI), Würzburg, Germany, [8]University of Leipzig, Faculty of Mathematics and Computer Science, Leipzig, Germany, [9]German Center for Lung Research (DZL), Berlin, Germany

Pneumonia significantly increases the risk of cardiovascular events, yet the molecular mechanisms driving this association remain poorly understood. In this study, we investigate the interplay between pneumonia and atherosclerosis by employing single-cell RNA sequencing (scRNA-seq) following murine modeling of bacterial pneumonia alongside human patient data. ApoE-deficient mice on a high-fat diet were infected with *Streptococcus pneumoniae*, and immune and vascular cell responses were profiled at acute (48 hours) and recovery (35 days) timepoints post-infection. This approach allowed us to map cell-type specific transcriptional signatures, identify key pathways dysregulated during infection and convalescence, identify biomarkers and model disease progression.

Preliminary results suggest that pneumonia induces persistent tissue-specific cellular stress responses and inflammatory signatures that may contribute to chronic vascular remodeling and heightened cardiovascular risk. To bridge the gap between mouse and human data, we are analyzing peripheral blood mononuclear cells (PBMCs) of patients from the LIFE-Heart cohort with a documented history of pneumonia and subsequent cardiovascular events. scRNA-seq of human PBMCs is used to uncover immunological mediators that could link respiratory infections to vascular pathology. This integrative, cross-species approach combines expertise in infectious disease, cardiovascular biology, and computational analysis to uncover molecular mechanisms by which pneumonia may trigger or aggravate cardiovascular disease. The insights gained could pave the way for the development of diagnostic biomarkers and targeted therapies for individuals at increased risk following a pulmonary infection.

408

Effect of hyperglycemia and pneumolysin-stimulation on expression of angiopoietin-like-4 protein in human alveolar epithelial cells

A. Sahin[1], S. Hammerschmidt[2], M. Witzenrath[1,3], **B. Gutbier**[1]

[1]Charité – Universitätsmedizin Berlin, Department of Infectious Diseases, Respiratory Medicine and Critical Care, Berlin, Germany, [2]University of Greifswald, Department of Molecular Genetics and Infection Biology, Interfaculty Institute for Genetics and Functional Genomics, Center for Functional Genomics of Microbes,, Greifswald, Germany, [3]German Center for Lung Research (DZL), Berlin, Germany

Pneumonia is the most common infectious disease worldwide with high lethality and *Streptococcus pneumoniae* (*S. pn.*) is one of the main pathogens. Influence of the pathogen and/or an uncontrolled immune reaction can lead to alveolocapillary barrier failure and result in acute lung injury.
Comorbidities, such as diabetes mellitus, are risk factors for pneumonia. Diabetes increases incidence and lethality of severe pneumonia, but contributing pathomechanisms are still unknown. The metabolic regulator angiopoietin-like-4 (Angptl-4) influences junctional proteins, Angptl-4 inhibition had a protective effect on pulmonary barrier function in murine influenza pneumonia and Angptl-4 is increased in diabetes.
The aim of our study is to characterize the effect of hyperglycemia and/or pneumolysin (PLY) -main pathogenic factor of *S. pn*.- on Angptl-4 expression in human alveolar epithelial cells (hAepiC). This first investigation, can help to assess if Angptl-4 could be a target for new therapeutic option in pneumonia induced barrier disruption.
Angptl-4 mRNA expression was determined in cultured hAepiC 4, 8, 12 and 24 hours (h) after PLY stimulation (0.25 µg/ml) via qPCR. Cells were pretreated with „high glucose“ (HGM), normal glucose (NGM) or control medium (CM), for 24 h before PLY stimulation. Angptl-4 protein levels were measured via ELISA in cell culture supernatant.
We observed 4h after PLY stimulation only a slight increase of Angptl-4 expression, and further enhanced by combination of PLY with HGB. No differences between the experimental groups were seen in the ELISA analysis.
The results currently indicate that no clear effect of hyperglycemia in combination with PLY-stimulation, on angiopoietin-like-4 expression of hAepiC can be observed. Further investigations are necessary.

Interstitial Lung Diseases: 501 - 529

501

Establishing an ex vivo Model for the Functional Analysis of AT2 cells

N. Artysh[1,2], J. Ruwisch[2,3], B. Bartkute[2,3], H. Wang[4], C. Werlein[5], K. Sewald[1,2], J. Gote-Schniering[4], A. Prasse[1,2,3,6]
[1]Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany, [2]Deutsches Zentrum für Lungenforschung (DZL), BREATH, Hannover, Germany, [3]Hannover Medical School, Department of Respiratory Medicine, Hannover, Germany, [4]University of Bern, Department for BioMedical Research (DBMR), Bern, Switzerland, [5]Hannover Medical School, Department of Pathology, Hannover, Germany, [6]University Hospital Basel, Pneumology, Basel, Switzerland

Introduction: Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, and fatal interstitial lung disease marked by excessive matrix deposition and lung remodeling. Alveoli, the sites of gas exchange, contain type 1 (AT1) cells for gas exchange and type 2 (AT2) cells for AT1 renewal and surfactant production. In IPF, persistent injury causes AT2 cell exhaustion, senescence, and apoptosis, impairing gas exchange. AT2 cells are therefore promising therapeutic targets. To better study their function and test compounds, we developed an ex vivo platform using human Precision Cut Lung Slices (PCLS) and successfully validated it with a candidate drug.
Methods: Lung explant tissues were filled with agarose. Subsequently, cores were drilled out of the stiff tissue blocks. Then, the cores were cut into 300-µm thick slices and cultured for 28 days. Slices were either cultivated untreated for 28 days or treated with compound X for two weeks. Medium exchange was done every second day. Slices were harvested in formalin and stored in ethanol for further experiments. Fixed slices underwent RNA-Scope® (ACD a Bio-Techne Brand) and Single Nuclei Sequencing (10x Genomics) according to manufacturer.
Results: RNA-Scope® was performed on formalin-fixed slices to unveil the dynamics of cellular composition during PCLS culture. Baseline cellular composition differed between fibrotic and control PCLS, with fewer AT2 cells and AT1 cells in fibrotic PCLS. Over the culture period, both systems lost AT2 cells. Treatment of fibrotic PCLS with compound X for two weeks reduced the loss of AT2 cells, compared to untreated samples. Single-nulcei sequencing of untreated samples confirmed the reduction in AT2 cell numbers during cultivation in both fibrotic and control PCLS.
Conclusion: This study represents the first development of a human long-term PCLS model for studying AT2 cell functions and testing potential therapeutics.

502

Dysfunctional phagolysosome maturation and consecutive giant cell formation in sarcoidosis

B. Bartkute[1,2], C. Ehlers[2,3], J. Ruwisch[1,2], T. Graalmann[2,3], A. Prasse[1,2,4]

[1]Hannover Medical School, Clinic for Respiratory Medicine and Infectious Diseases, Hannover, Germany, [2]BREATH, Hannover, Germany, [3]Translational Immunology, TWINCORE, Centre for Experimental and Clinical Infection Research, Hannover, Germany, [4]University Hospital Basel, Clinic of Respiratory Medicine, Basel, Switzerland

Introduction. Sarcoidosis is a granulomatous disease in which several aspects in regards to etiology remain unclear. Multiple evidence points towards a role of genetic susceptibility, environmental factors, and autoimmune mechanism in the etiopathogenesis of sarcoidosis. Our study aims to investigate the role of giant cells and phagolysosomes formation in sarcoidosis patients.

Methods. To test granuloma formation we modified the already established *in vitro* granuloma model by Eliott Crouser`s group. Stimulated and non-stimulated PBMCs from sarcoidosis (n = 6) and healthy volunteers (n = 3) were used to form granuloma and evolution of multinucleated giant cells was detected. Granuloma formation was observed after 0, 3, and 7 days using bright light microscopy. The evolution of multinucleated giant cells, phagosome, lysosome and phagolysome structures within giant cells was monitored by confocal laser microscopy.

Results. Our *in vitro* granuloma model showed statistically significant difference in spontaneous and trehalose 6`mycolate (TDM) induced granuloma formation comparing sarcoidosis patients versus healthy volunteers. In addition, spontaneous and induced multinucleated giant cells formation was highly increased in sarcoidosis patients compared to healthy volunteers. Furthermore, we detected increased and persistent phagolysosome formation in multinucleated giant cells of sarcoidosis patients. Following stimulation with TDM phagolysosome formation was not terminated in sarcoidosis patients in contrast to healthy volunteers after 14 days of cell culture.

Conclusion. Our obtained results suggest that phagolysosome maturation is dysfunctional in sarcoidosis patients and linked to giant cell formation in sarcoidosis patients. Further studies delineating the underlying mechanisms are ongoing.

ORAL PRESENTATION

503

Nutritional drivers of lung aging: western diet reprograms alveolar epithelium toward fibrosis

A. Chahin[1,2], M. C. Melo-Narváez[1,2,3], G. Boosarpu[1,2], E. Fousekis-Papakonstantinou[1,2], M. M. Götz[1], A. L. Jung[1,4], M. A. Alejandre Alcázar[2,5], S. Miguel da Silva[6], M. Schmitt[6], M. Lehmann[1,2,3]

[1]Institute for Lung Research, Universities of Giessen and Marburg Lung Center, Philipps University Marburg, German Center for Lung Research (DZL), Marburg, Germany, [2]Institute for Lung Health (ILH), Giessen, Germany, [3]Comprehensive Pneumology Center (CPC), Institute of Lung Health and Immunity (LHI), Helmholtz Zentrum München, German Center for Lung Research (DZL), Munich, Germany, [4]Core Facility Flow Cytometry - Bacterial Vesicles, Philipps-University Marburg, Marburg, Germany, [5]Cologne Excellence Cluster for Aging and Aging-Associated Diseases (CECAD), Cologne, Germany, [6]Pharmakologisches Institut, Philipps University Marburg, Marburg, Germany

Dietary habits are increasingly recognized as modifiers of chronic lung disease (CLD) susceptibility and progression. We investigated how a Western diet (WD) shapes and promotes cellular senescence programs, changes in lung physiology and architecture. Using mouse models exposed to New Western Diet (NWD1) versus standard diet (AIN76A), we combined histology, immunofluorescence, lung organoid assays, and bulk RNA-seq to assess alveolar epithelial cell fate, regenerative capacity, and transcriptional remodeling. NWD1-fed mice displayed aberrant alveolar structure, increased mean linear intercept, and reduced expression of AT2/AT1 markers (Sftpc, Abca3, Nkx2-1,Ager), with enrichment of transitional Krt8$^+$ epithelial cells. Organoids derived from NWD1 lungs showed decreased colony-forming efficiency, reduced size, elevated ROS and senescence markers (SPiDER-β-gal), indicating impaired AT2-mediated repair. RNA-seq of EpCAM$^+$ cells revealed a diet-driven reprogramming, with suppression of surfactant/metabolic pathways and upregulation of ECM remodeling and pro-fibrotic genes (Fn1, Col1a1, Timp2). Pathway analysis confirmed activation of inflammatory cascades (cytokine signaling) alongside impaired repair and metabolic programs. Profiling of extracellular vesicles (EVs) and their specific surface proteins from BAL fluid further supported systemic WD-induced alterations, including pro-inflammatory and pro-fibrotic signaling. Together, these data show that NWD1 accelerates alveolar epithelial senescence, compromises regenerative capacity, and induces a pro-fibrotic transcriptome thereby promoting a lung environment permissive to ageing. Notably, EVs undergo significant alterations in response to a Western diet, suggesting their potential role in mediating diet-induced reprogramming. This work establishes dietary modulation as a critical life-style factor in lung ageing, providing a platform for therapeutic strategies targeting metabolism–senescence interactions.

ORAL PRESENTATION

504

Aerosol-Cell Exposure System for Microliter-Scale In Vitro Drug Testing at the Air–Liquid Interface conditions: Application to Antisense Oligonucleotide Therapy in Idiopathic Pulmonary Fibrosis (IPF)

A. Chakraborty[1], P. Kumar[1], E. Richter[1], L. Yang[1], A. Schröppel[1], M. Coralie[2,3], R. Vanbever[4], L. Plantier[2,3,5], N. Heuzé-Vourch[2,3], O. Schmid[1]

[1]Institute of Lung Health and Immunity, and Comprehensive Pneumology Center with the CPC-M bioArchive, Ludwig-Maximilians-Universität München and Helmholtz Zentrum München, Munich, Germany, Member of the German Center for Lung Research (DZL)., Neuherberg, Germany, [2]Centre d'Etudes des Pathologies Respiratoires / Inserm UMR1100, Tours, France, [3]Université de Tours, Tours, France, [4]Université catholique de Louvain, Louvain Drug Research Institute, Advanced Drug Delivery & Biomaterials, Louvain, Belgium, [5]CHRU de Tours, Service de Pneumologie et Explorations Fonctionnelles Respiratoires, Tours, France

Unlike traditional submerged cell cultures, aerosolized drug delivery to air-liquid interface (ALI)-cultured lung cells provides a more physiologic and clinically more relevant route for *in vitro* testing of inhalable drugs. However, current aerosolized drug delivery systems are limited with respect to efficient use of often expensive experimental drugs. So, we introduce the ALICE Cloud (Alpha) MAX technology, made commercially available by VITROCELL Systems, characterize its dosimetry performance, and apply it to in vitro toxicity, efficacy and biokinetics testing of a novel inhalable 3rd-generation antisense oligonucleotide (ASO) for gene editing. The Cloud MAX technology delivered 33 ± 7% (mean ± 2SD) of 3 to 20 µl nebulized ASO suspension to ALI-cultured cells on up to six 6-well transwell inserts, i.e., as little as 1µl/insert (=0.2µl/cm^2). As a proof-of-concept study for aerosolized drug testing with the Cloud MAX technology, we demonstrated target engagement of a LIMCH1-ASO for silencing the LIM and Calponin Homology domain 1 (LIMCH1) in primary lung fibroblasts from IPF patients "shielded" by an alveolar epithelial cell layer (A549) mimicking the conditions during inhalation therapy. Target engagement of the LIMCH1-ASO in fibroblasts cultured underneath alveolar epithelial cells was found to be effective (>5-fold downregulation) on both transcript and protein level even for the lowest tested dose (0.64 nmol /cm^2). As nebulization of ASO did neither inhibit drug efficacy nor exert any additional cytotoxic effects (for less than 3.21 nmol /cm^2), this 3rd generation ASO appears suitable for aerosolized application via inhalation therapy. Using larger liquid volume for ASO delivery (as commonly done for non-aerosol based drug testing), the drug delivery kinetics was reduced proportionally to the liquid volume, but the observed 4h delay in peak drug dose reaching the fibroblasts did not affect drug efficacy measured at 72h. This study demonstrates that the Cloud MAX technology is a promising substance-efficient platform for clinically relevant *in vitro* pharmacokinetics/-dynamics testing of low volumes of inhalable drugs for targeted pulmonary therapies.

505

Biomimetic Multifunctional siRNA Nanoplatform for Macrophage-Targeted SPP1 Suppression in Pulmonary Fibrosis

P. Chen[1], L. Yang[1], A. Dietrich[2], M. Rehberg[1], O. Schmid[1]

[1]Institute of Lung Health and Immunity, Helmholtz Munich, Comprehensive Pneumology Center (CPC-M), Member of the German Center for Lung Research (DZL), Munich, Germany, Munich, Germany, [2]Walther Straub Institute of Pharmacology and Toxicology, Munich, Germany

Idiopathic Pulmonary Fibrosis (IPF) is a major global health concern, affecting approximately 3 million people worldwide, with prevalence increasing with age. Although Pirfenidone and Nintedanib can alleviate symptoms and slow disease progression, a definitive cure remains unavailable. Emerging evidence indicates that SPP1 plays a critical role in TGF-β1 activation and extracellular matrix (ECM) deposition in IPF. Data from the Human Lung Cell Atlas show that SPP1 is highly expressed in macrophages from IPF patients, suggesting macrophage-derived SPP1 as a promising therapeutic target. However, pathological alterations in lung structure and physiology present significant challenges for effective pulmonary drug delivery.

Here, we report a biomimetic siRNA delivery system designed to enhance macrophage targeting and mucus penetration. Apoptotic cell membranes were fused with lipid nanoparticles (LNPs) to load SPP1 siRNA. Surface proteins on the membranes, including **calreticulin** (an “eat-me” signal) and **TGF-β receptor II** (scavenging free TGF-β1), enable macrophage targeting and cytokine neutralization. The membrane coating also facilitates mucus penetration and reduces non-specific uptake, as evidenced by slightly lower internalization by normal cells compared to unmodified LNPs. Upon macrophage internalization, ionizable LNPs escape lysosomes via charge-tunable mechanisms, releasing SPP1 siRNA to silence SPP1 expression, thereby reducing TGF-β1 activation and ECM deposition.

In artificial mucus penetration models, our system achieved a double higher penetration rate at 4 hours compared to unmodified LNPs. In vitro, it exhibited high macrophage targeting efficiency (84%) and substantial TGF-β1 clearance (62%). In a murine precision-cut lung slice (PCLS) model, the system effectively silenced SPP1, decreased TGF-β1 and ECM markers (collagen I, fibronectin, α-SMA), and significantly alleviated IPF-like pathology.

In summary, this study presents an efficient siRNA nanoplatform that targets macrophages, penetrates mucus, and silences SPP1, thereby attenuating fibrotic signaling and offering a promising strategy for IPF therapy.

506

AI-Driven Immunofluorescence Quantification to Complement Spatial Transcriptomics in Sarcoidosis

L. Christian*[1,2], J. Ruwisch*[1,2], L. Giercke[1], J. M. Hohlfeld[1,2,3], J.-C. Kamp[1,2,4], D. D. Jonigk[3,4,5], M. M. Hoeper[2,6], N. Kaminski[7], C. Falk[8,9], Y. Li[10,11], B. Seeliger[2,6], S. Traidl[12,13], L. Neubert*[3,4], J. C. Schupp*[2,3,6,7]

[1]Hannover Medical School (MHH), Department of Respiratory Medicine and Infectious Diseases, Hannover, Germany, [2]Biomedical Research in End-Stage and Obstructive Lung Disease (BREATH), Hannover Medical School (MHH), German Center for Lung Research (DZL), Hannover, Germany, [3]Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Hannover, Germany, [4]Institute of Pathology, Hannover Medical School (MHH), Hannover, Germany, [5]University Clinic of the RWTH Aachen, Aachen, Germany, [6]Hannover Medical School (MHH), Department of Respiratory Medicine and Infectious Diseases, Hannover, Germany, [7]Pulmonary, Critical Care, and Sleep Medicine, Yale University, New Haven, United States of America, [8]Institute of Transplant Immunology, Hannover Medical School (MHH), Hannover, Germany, [9]German Center for Infectious Diseases (DZIF), TTU Infection of the Immunocompromised Host, Partner Site Hannover-Braunschweig, Braunschweig, Germany, [10]Centre for Individualised Infection Medicine (CiiM) and TWINCORE, a joint venture between the Helmholtz-Centre for Infection Research (HZI) and the Hannover Medical School (MHH),, Hannover, Germany, [11]Helmholtz-Centre for Infection Research (HZI) and the Hannover Medical School (MHH), Hannover, Germany, [12]Department of Dermatology and Allergy, Hannover Medical School (MHH), Hannover, Germany, [13]Cluster of Excellence RESIST (EXC 2155), Hannover Medical School (MHH), Hannover, Germany

*Contributed equally

Background: Non-caseating granulomas in the lungs and other tissues are the histopathological hallmark of sarcoidosis. Spatial transcriptomic methods are increasingly applied to investigate the spatial organization of structures such as granuloma in tissue. Yet, this method requires similarly powerful orthogonal validation approaches.

Aim: To evaluate AI-based immunofluorescence image quantification using digital pathology analysis as a complementary method to spatial transcriptomics in sarcoidosis granulomas.

Methods: In a prior study, spatial transcriptomics (10x Visium) was performed on n=9 granuloma-containing lung explants from chronic sarcoidosis patients. Sections from n=4 patients underwent immunohistofluorescence (IHF) protein staining and RNAscope RNA in situ hybridization (ISH) to validate spatial transcriptomic data. Image quantification was performed using StarDist for nuclei segmentation and QuPath for AI-based automated cell classification. Independent fluorescence intensity quantification using ImageJ was employed as a reference.

Results: Gene expression patterns identified through spatial transcriptomics in a previous study were validated at the protein level using immunofluorescence and RNA in situ hybridization (ISH), confirming the spatial localization of key transcripts. AI-based image analysis enabled precise single-cell quantification, revealing that approximately 43 ± 19% of granuloma cells expressed IFN-γ, while 24 ± 22% were positive for SPP1. These findings were consistent with the spatial transcriptomic data. In addition, QuPath facilitated the classification of distinct molecular niches within granulomas, notably highlighting a central enrichment of macrophage-associated gene signatures. Complementary quantification using ImageJ yielded comparable fluorescence intensity distributions, further validating the robustness of the image analysis pipeline.

Conclusion: AI-based digital image analysis provides a complementary approach for quantitative analysis of immunofluorescence images. The results correlate well with spatial transcriptomic data, supporting the use as accessible tool for validating complex spatial gene expression profiles.

507

The roles of distinct alveolar epithelial type 2 (AT2) cell subpopulations in normal and aberrant lung development

S. D'Agostino[1,2], A. Rahadian[1,2], V. Rickert-Zacharias[1,2], R. E. Morty[1,2], I. Mizik[1,2]
[1]Heidelberg University Hospital, Department of Translational Pulmonology and Translational Lung Research Center Heidelberg, Heidelberg, Germany, [2]German Center for Lung Research (DZL), Heidelberg, Germany

Bronchopulmonary dysplasia (BPD) is the most common disease of prematurity and a major contributor to neonatal mortality. Main postnatal contributing factors include mechanical ventilation, oxygen supplementation, inflammation and infection. Both, prematurity and BPD were shown to be associated with changes in pulmonary epithelium, particularly in the distal alveolar region, leads to a difficult environment for gas exchange. However, the key epithelial population involved remain unidentified. A recent single-cell study from our team identified a dual nature of AT2 cells in postnatally developing lung. The two subpopulations are differentiated by the expression of *Lyz1*. Here we present a gene and protein expression profile at five different developmental timepoints from postnatal day (P)3 to P14. We show, that *Lyz1* gene expression and *Lyz1*$^+$ AT2 cells number gradually decrease after hyperoxia exposure. *Lyz1*$^+$ AT2 cell population can be further delineated by the expression of *Lcn2* and *Lrg1*, whose gene and protein expression conversely increases. Notably, hyperoxia exposure results in gradual shift towards the *Lrg1*$^+$ phenotype within the AT2 *Lyz1*$^+$ population. Importantly, *Lyz1*, *Lcn2* and *Lrg1* expression is associated with "activated" or "primed" AT2 cells. Further, we have employed the fluorescent *in situ* hybridization to co-localize and quantify the expression of *Lyz1*/ *Lcn2*/ *Lrg1* by AT2 cells in the lungs from normally-developing, as well as hyperoxia-exposed mouse pups at P14. Our data suggest that *Lyz1*$^+$ AT2 cell population might play a distinct role in lung homeostasis and in BPD pathogenesis, providing the basis for further investigation and intervention.

508

Targeted uptake of pirfenidone with lung surfactant enhances macrophage-epithelial anti-inflammatory cross-talk

M. Dukic[1], M. Ochs[2], E. Lopez-Rodriguez[3]

[1]Charité - Universitätsmedizin Berlin, Institut für Funktionelle Anatomie, Berlin, Germany, [2]Charité - Universitätsmedizin Berilin, Institut für Funktionelle Anatomie, Berlin, Germany, [3]Charité – Universitätsmedizin Berlin, Institut für Funktionelle Anatomie, Berlin, Germany

In the alveolar region, a continuous epithelium made of alveolar epithelial type I and type II (ATI and ATII) cells is covered by a thin alveolar lining layer (ALL) in which alveolar macrophages reside. The main component of the ALL is lung surfactant (LS).

We hypothesized that LS vesicles can be internalized by alveolar macrophage-like cells and primary alveolar epithelial cells, and that the combination of anti-fibrotic and anti-inflammatory drug pirfenidone (PFD) with LS vesicles enhance its anti-inflammatory effects and cellular cross-talk compared with the free drug. To test this hypothesis, we established mono- and co-culture *in vitro* models of the alveolar region using differentiated THP-1 (dTHP-1) macrophage-like cells and human primary alveolar epithelial cells (hPAEpCs).

Our results using flow cytometry and confocal microscopy showed that dTHP-1 cells uptake more fluorescently labelled LS than hPAEpCs, but this uptake is impaired by LPS. In addition, the internalization of LS by dTHP-1 cells was demonstrated to be mostly through endocytosis. Even though using lipid hydration method PFD was not successfully encapsulated in LS vesicles, the anti-inflammatory effects were observed in mono- and co-culture conditions compared to the free drug. Furthermore, it was observed that anti-inflammatory effects in co-culture are not a simple sum of released cytokines from monocultures. Incubating dTHP-1 in monoculture with conditioned media from hPAEpCs monoculture showed increased release of IL-1β and TNF-α in dTHP-1, demonstrating paracrine cross-talking between both cell types, while co-culture conditions showed cell-cell contact dependent reduction of IL-1β and TNF-α. These findings highlight the differential behavior of cells in monoculture versus co-culture systems, emphasizing the importance of using physiologically relevant models for future drug testing in pulmonary disease.

509

Distinct Immune Signatures in ARDS of Bacterial and Non-Infectious Origin A Comparative Cytokine Analysis in Serum and BALF

M. Elbalkini[1,2], E. J. Winter[3], T. Pape[3], B. Nalbant[3], J. Ruwisch[1,3], K. Stahl[4], S. David[5], T. Welte[3], U. Kalinke[6], B. Seeliger[1,3], T. Graalmann[1,2,7]

[1]Biomedical Research in End-Stage and Obstructive Lung Disease (BREATH), Hannover Medical School (MHH), German Center for Lung Research (DZL), Hannover, Germany, [2]Junior Research Group for Translational Immunology, TWINCORE, Centre for Experimental and Clinical Infection Research, Hannover, Germany, [3]Department of Respiratory Medicine, Hannover Medical School, Hannover, Germany, [4]Department of Gastroenterology, Hepatology, Endocrinology and Infectious Diseases, Hannover Medical School,, Hannover, Germany, [5]Institute of Intensive Care Medicine, University Hospital Zurich, Zurich, Eswatini, [6]Institute for Experimental Infection Research, TWINCORE, Centre for Experimental and Clinical Infection Research, a joint venture between the Helmholtz Centre for Infection Research and the Hannover Medical School, Hannover, Germany, [7]Department for Rheumatology and Immunology, Hannover Medical School, Hannover, Germany

Background: Acute respiratory distress syndrome (ARDS) is a life-threatening respiratory failure. It can be triggered by infections such as bacterial pneumonia or by conditions like acute pancreatitis. Although clinical symptoms are similar, immune responses vary depending on etiology. Understanding these immune signatures is critical for guiding personalized ARDS treatments.

Methods: Peripheral blood was collected at ICU admission from ARDS patients with Legionella (n=9), Streptococcus pyogenes (n=9), S. pneumoniae (n=14), or pancreatitis (n=7). BALF samples were available from Legionella (n=7), S. pyogenes (n=3), S. pneumoniae (n=7), and pancreatitis (n=5). Serum and BALF were analyzed for 30 cytokines using LEGENDplex

Results: The mean age was 57 years; 22.5% were female. All patients required mechanical ventilation, with some on VV-ECMO. Immune signatures varied by ARDS cause. In serum, S. pneumoniae infection showed broad inflammation with high IL-2, IL-4, IL-8, IL-12p70, IL-13, TNF-α, LT-α, CCL3, and CCL5. Legionella triggered interferon driven responses (CXCL9-11, IFN-γ, CCL2, CCL20), while S. pyogenes induced a Th17 profile (IL-6, IL-10, IL-17A, leptin, resistin, CCL17). Patients with pancreatitis showed milder systemic inflammation, marked by CCL5, APRIL, and CCL11.

In BALF, cytokine profiles also differed: S. pneumoniae increased IL-17A, IL-4, and IL-13; Legionella elevated BAFF, CCL5, and IFN-γ; S. pyogenes showed moderate IL-6, IL-10, and IL-17A; pancreatitis showed minimal cytokine activity. All ARDS types had local chemokine expression (IL-8, CXCL1, and CXCL10) in the lungs.

Conclusion: ARDS etiology drives spatially distinct immune responses in blood and lungs. Systemic and local immune patterns may help guide precise immunotherapies

510

Differentiation of commercial human epithelial cells to produce human lung surfactant

Z. Fan, M. Dukic, M. Ochs, E. Lopez Rodriguez
Charité Universitätsmedizin Berlin, Institut für Funktionelle Anatomie, Berlin, Germany

Lung surfactant (LS) is vertical to neonatal medicine as its deficiency cause neonatal respiratory distress syndrome (NRDS). Commercial lung surfactants are available for the clinical treatment of NRDS. Nowadays, it is still used as prophylactic therapy in NRDS. However, most of these products are still derived from animal sources, such as Curosurf™ (porcine), Infasurf™ (ovine) or Survanta™ (bovine). In order to solve the problem of its rarity and potential species cross reaction, it is necessary to develop a model with human alveolar epithelial cells that could produce a species-specific human LS (hLS). Alveolar epithelial type II (AEII) cells synthesize and secret the LS that is critical for the decrease of surface tension to prevent collapse of the alveoli during expiration.

Therefore, we hypothesized that both A549 and commercially available human primary alveolar epithelial cells (hPAEpCs) have the ability to differentiate into AT2 cell progenitors and we can induce their maturation to produce hLS. For this purpose, we propose different strategies including various culturing conditionss to promote differentiation and maturation.

We found that changing the standard culture medium of the cells by CnT-PR medium and adding CHIR99021, A549 and hPAEpC could differentiate into AT2 cell progenitors with the ability to produce lung surfactant. Secondly, we exposed the cells to phorbol 12-myristate 13-acetate (PMA) combined with adenosine triphosphate (ATP,) or ferric ammonium citrate (FAC,) to stimulate the production of hLS in A549 and hPAEpC.We found that incubation of A549 cells with FAC was optimal to stimulate hLS production, while cells exposed to PMA combined with ATP was the optimal treatment for secretion of hLS.

These findings suggest that stimulating differentiated A459 with FAC to produce hLS then expose them to PMA combined with ATP will help A549 to secret hLS.

Taking all together, in the near future, we will be able to produce sufficient hLS using commercially available cells for the clinical applications, especially for adult patients.

511

High pressure freezing and freeze substitution-transmission electron microscopy of the mouse and human alveolar epithelial glycocalyx.

V. Gluhovic*[1], E. Lopez-Rodriguez*[1,2], S. Timm[1], M. Vukovic[1], A. Löwa[3], A. C. Hocke[3], W. Kübler[2,4,5,6,7], M. Ochs*[1,2,8], **S. G. Twamley***[1,5]

[1]Charité - Universitätsmedizin Berlin, Institute of Functional Anatomy, Berlin, Germany, [2]German Center for Lung Research (DZL), Berlin, Germany, [3]Charité - Universitätsmedizin Berlin, Department of Infectious Diseases, Respiratory Medicine and Critical Care, Berlin, Germany, [4]Charité - Universitätsmedizin Berlin, Institute of Physiology, Berlin, Germany, [5]German Center for Cardiovascular Research (DZHK), Berlin, Germany, [6]St. Michael´s Hospital, University, Keenan Research Centre, Toronto, Canada, [7]University of Toronto, Departments of Surgery and Physiology, Toronto, Canada, [8]Charité - Universitätsmedizin Berlin, Core Facility Electron Microscopy, Berlin, Germany

*Contributed equally

Alveolar epithelial cells (AEC) are covered apically by the alveolar lining layer (ALL). AEC contain an apical glycocalyx (GCX) which is part of the ALL. This alveolar epithelial glycocalyx (AEGCX) consists of a mixture of glycoproteins, proteoglycans and glycosaminoglycans. In comparison to the endothelial GCX, much less is known about the AEGCX although it is involved in many cellular processes. Given that aldehyde fixation alone incompletely preserves the fragile GCX, we aimed to improve the preservation of the ultrastructure of the AEGCX using high pressure freezing and freeze substitution (HPF/FS) for transmission electron microscopy (TEM) analysis. HPF/FS is a type of cryopreservation that uses both liquid nitrogen and high pressure. By using HPF/FS to preserve human primary AEC, human alveolar organoids, and *ex vivo* tissue from both mice and human lungs, we observed two distinct regions on the apical surface of AEC via TEM: an inner layer along the cell membrane and a loosely attached outer layer. Our data suggests that the inner layer corresponds to the AEGCX. To minimize freeze induced damage during HPF, a protein-rich cryoprotectant was introduced to stabilize the sample. By processing control samples of protein-rich cryoprotectants lacking cells, we found that HPF/FS preserves components of the cryoprotectant, as confirmed by TEM analysis. This implies that HPF/FS preserves not only cellular derived constituents but also components found in the cryoprotectant which may interfere with interpretation of the outer surface layer.

512

Longitudinal molecular profiling identifies a temporal sequence of pathological signaling in human and experimental BPD lungs

H. Huang*[1,2,3], O. Ibáñez-Solé*[2,3], C. Kuiper-Makris*[1,2,3], S. Stephan[4], S. Danopoulos[5], D. Hirani[1,2,3], J. Selle[1,2,3], I. Mizik[6], C. Vohlen[1,2,3], G. Pryhuber[7], W. Seeger[8], J.-W. Lackmann[3], D. Al Alam[5], J. Dötsch[9], B. Schumacher[2,3,10], M. A. A. Alcazar[1,2,3,11]

[1]University of Cologne, Faculty of Medicine and University Hospital Cologne, Translational Experimental Pediatrics - Experimental Pulmonology, Department of Pediatric and Adolescent Medicine, Köln, Germany, [2]Center for Molecular Medicine Cologne (CMMC), Köln, Germany, [3]Cologne Excellence Cluster for Aging and Aging-Associated Diseases (CECAD), Köln, Germany, [4]Justus Liebig University Giessen, Center for Infection and Genomics of the Lung (CIGL), Giessen, Germany, [5]Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, Torrance, United States of America, [6]University Hospital Heidelberg, Heidelberg, Germany, [7]University of Rochester Medical Center, Division of Neonatology, Department of Pediatrics, New York, United States of America, [8]Universities of Giessen and Marburg Lung Center (UGMLC), Institute for Lung Health (ILH), Cardio-Pulmonary Institute (CPI), Member of the German Center for Lung Research (DZL), Giessen, Germany, [9]University of Cologne, Faculty of Medicine and University Hospital Cologne, Department of Pediatric and Adolescent Medicine, Köln, Germany, [10]University of Cologne, Faculty of Medicine and University Hospital Cologne, Institute for Genome Stability in Aging and Disease, Köln, Germany, [11]University of Giessen and Marburg Lung Center (UGMLC), Institute for Lung Health (ILH), Cardio-Pulmonary Institute (CPI), Member of the German Center for Lung Research (DZL), Giessen, Germany

*Contributed equally

Background: Prematurity and oxygen supplementation disrupt physiological lung morphogenesis, leading to bronchopulmonary dysplasia (BPD), that predisposes for chronic lung disease (CLD). While oxidative stress–induced lung growth arrest is central in the pathogenesis of BPD, the molecular dynamics underlying its progression into CLD remain elusive. We therefore performed longitudinal molecular profiling in both hyperoxia-based experimental BPD and lungs of infants with BPD.

Methods: First, we performed RNA sequencing of 71 human lungs (29 BPD and 42 age-matched controls), ranging from newborn to 8 y/o. Samples were classified in stages based on postnatal age: acute (37 weeks gestational age - 1 y/o) and chronic (>1 y/o). Second, we performed proteomic analysis of newborn mice exposed to either hyperoxia (85% O_2) or normoxia (21% O_2) for 14 days, at postnatal days (P)14, 28, and 70. Differential expression analysis, GSEA and GO-term enrichment were performed.

Results: First, differential expression analysis revealed a downregulation of pathways involved in inflammation and DNA damage and repair in the acute phase of BPD in human, whereas the chronic phase showed significant upregulation of DNA damage and repair systems as well as extracellular matrix (ECM) organization. Moreover, comparing acute-to-chronic BPD development with healthy lung development revealed a lack of ECM organization pathways in BPD. Second, proteomic profiling identified age-specific patterns as well: mitochondrial-related pathways are significantly altered at P14 (acute phase), followed by downregulation of genes in DNA repair pathways and upregulated ECM components at P28 and P70, respectively. The temporal-specific dynamics of molecular pathways across the lifespan were confirmed with immunostaining and structural analysis.

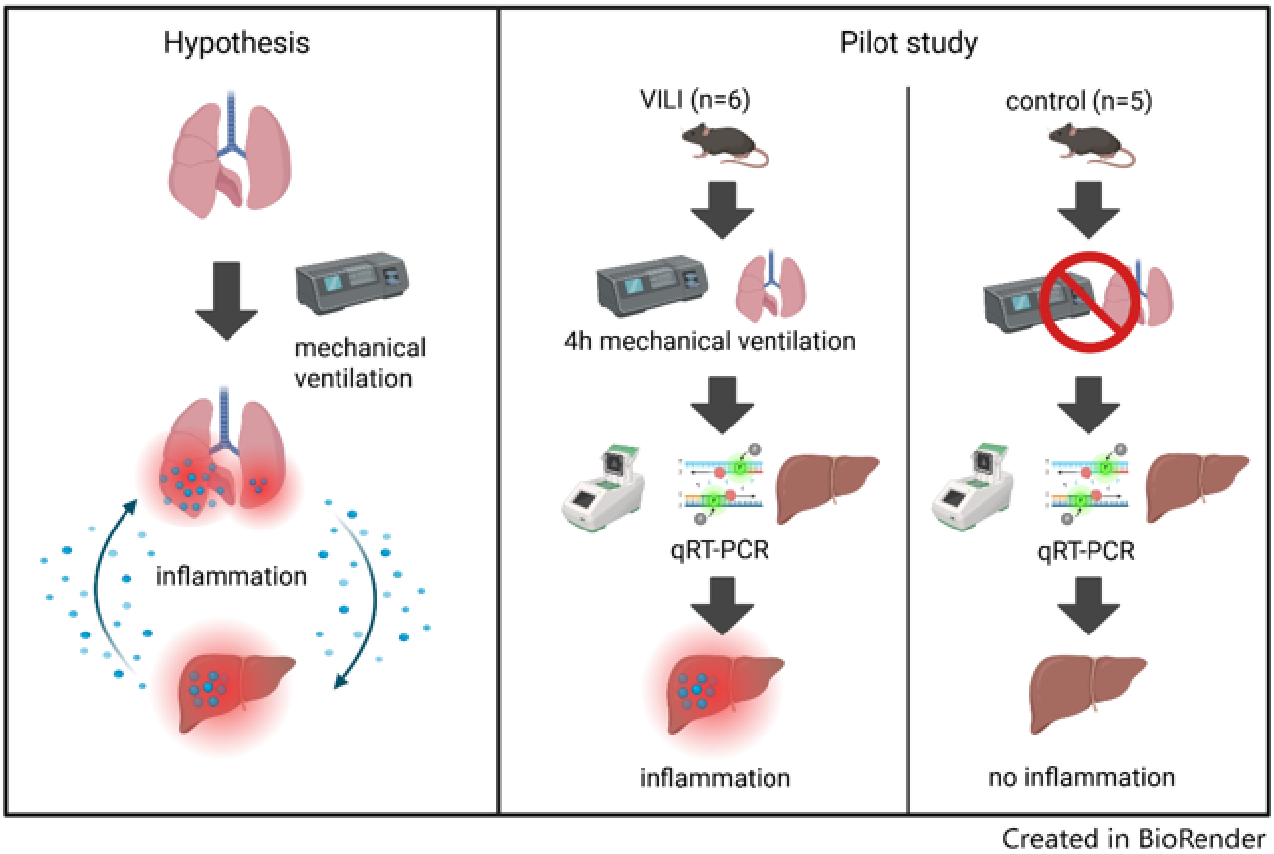
Conclusion: Here, we identified a temporal sequence of pathological processes in clinical and experimental BPD, with oxidative-stress driven genomic instability and long-term lung matrix remodeling. Collectively, these processes could contribute to an increased susceptibility to CLD later in life in infants with BPD, and could offer new avenues for preventive strategies.

513

Lung-liver-interaction in ventilated mice – a pilot study

T. Kloock[1,2], F. Roeder[1,2], L. Knudsen[1,2], J. Schipke[1,2], C. Mühlfeld[1,2]

[1]Institute of Functional and Applied Anatomy, Hannover Medical School, Hannover, Germany, [2]Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH), Member of the German Center for Lung Research (DZL), Hannover, Germany



Hypothesis and pilot study

Lung and liver share embryological, architectural and cell biological similarities and are known to interact with each other. Here, we hypothesized that lung injury is accompanied by an inflammatory response of the liver.

Ventilation induced lung injury (VILI) was induced in 13 weeks old female mice (n=6) by mechanical ventilation at PEEP 2 (n=3) or 8 (n=3) for 4 hours. Afterwards, mice were sacrificed and liver tissue was frozen for mRNA analysis. Healthy sex and age-matched unventilated mice served as controls (n=5) (Fig. 1). The expression of interleukin 1β (IL-1β) and 6 (IL-6), tumor-necrosis-factor-α (TNF-α), monocyte chemotactic protein 1 (MCP-1), C-X-C motif chemokine ligand 1 (CXCL-1) and 2 (CXCL-2) and serum amyloid A (SAA) was measured with quantitative real-time PCR.

Compared to unventilated controls, several inflammatory markers (IL-1ß, MCP-1, CXCL-1 and SAA) were significantly increased in livers of mice with VILI.

In conclusion, these data point to a hepatic inflammatory response associated with VILI. These promising results indicate an interaction between lung and liver. Further and more detailed studies with a larger study cohort are necessary to investigate the underlying mechanisms and functional significance of the lung-liver-axis.

514

Investigating mechanisms of lung injury caused by nanoparticle-triggered virus reactivation

N. Kuruppu[1,2], E. Günther[1,2], H. Adler[2,3], T. Stöger[1,2]

[1]Comprehensive Pneumology Center/ Helmholtz Zentrum München, Institute of Lung Health and Immunity, Neuherberg, Germany, [2]German Center of Lung Research (DZL), München, Germany, [3]Ludwig-Maximilians-University Munich/ Walther-Straub Institute of Pharmacology and Toxicology, München, Germany

Carbonaceous nanoparticles (CNPs), increasingly omnipresent due to biomass combustion and industrial emissions, have emerged as exacerbators of chronic lung diseases [1]. Studies have shown that CNPs act as a "second hit", reactivating latent murine γ-herpesvirus 68 (MHV-68), a model for human herpesviruses endemic in all populations. In the lungs, CNPs were seen to activate MAPK signalling in $CD11b^{+}$ macrophages, creating a permissive environment for viral reactivation [2]. Additionally, repeated pulmonary CNP exposure promoted $CD8^{+}$ T cell recruitment, T-cell proliferation and IFN-γ release following viral reactivation, with persistent T-cell activity. CNPs were also seen to cause epithelial injury, disrupting the alveolar barriers [3].

However, the mechanisms by which CNP exposure promotes macrophage-dependent T cell activation, driving cytotoxic epithelial injury through viral reactivation is poorly understood. To address this, we combine bulk RNA-seq analysis, immunohistochemistry, cytokine assays, and, in vitro co-cultures. These approaches will enable us to profile T cell activation and differentiation during CNP exposure and viral reactivation, map cytokine production and T cell–macrophage interactions in lung tissue, examine molecular signalling and reactivation mechanisms, assess tissue damage and inflammation, and determine how T cell depletion influences immune responses and lung pathology.

This work aims to elucidate interactions between environmental nanoparticles and latent viruses in chronic lung injury, potentially uncovering therapeutic targets for virus-driven exacerbations of lung diseases.

References:

[1] Islam N, Saikia BK, (2022), An overview on atmospheric carbonaceous particulate matter into carbon nanomaterials: A new approach for air pollution mitigation. , Chemosphere, 135027, Volume 303, Part 2, https://pubmed.ncbi.nlm.nih.gov/35623423/, 2025-09-09

[2] Sattler C, Moritz F, Chen S, Steer B, Kutschke D, Irmler M, Beckers J, Eickelberg O, Schmitt-Kopplin P, Adler H, Stoeger T, (2017), Nanoparticle exposure reactivates latent herpesvirus and restores a signature of acute infection, Particle and Fibre Toxicology, Volume 14, https://pubmed.ncbi.nlm.nih.gov/28069010/, 2025-09-09

[3] Han L, Haefner V, Yu Y, Han B, Ren H, Irmler M, Beckers J, Liu Q, Feuchtinger A, Yildirim A, Adler H, Stoeger T, (2023), Nanoparticle-Exposure-Triggered Virus Reactivation Induces Lung Emphysema in Mice, ACS Nano, 21056 - 21072, Volume 17, https://pubs.acs.org/doi/10.1021/acsnano.3c04111, 2025-09-09

515

Murine lung organoids as new in vitro approach to investigate cell circuits of injury and regeneration induced by airborne pollution

Q. Lei[1], O. Huzain[2], L. Han[2], T. Stoeger[2], C. Voss[3]

[1]Hannover Medical School, Clinic for Cardiac, Thoracic, Transplantation and Vascular Surgery, Leibniz Research Laboratories for Biotechnology and Artificial Organs, Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH), Member of the German Center for Lung Research (DZL), HANNOVER, Germany, [2]Institute of Lung Health and Immunity (LHI), Helmholtz Munich, Comprehensive Pneumology Center, Munich, Germany, [3]Hannover Medical School, Clinic for Cardiac, Thoracic, Transplantation and Vascular Surgery, Leibniz Research Laboratories for Biotechnology and Artificial Organs, Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH), Institute of Lung Health and Immunity (LHI), Helmholtz Munich, Comprehensive Pneumology Center (CPC-M), Hannover, Germany

Objective: We aim to recapitulate key cell circuits underlying lung inflammation initiated by sterile stimuli, such as inhaled nanomaterials (NM) at the in vitro level, which remains a major challenge to date and requires validated in vitro models for predictive, respiratory toxicity
Methods: Epithelial lung organoids will be established by co-culturing murine fibroblast cell line (CCL206) with primary adult stem cells to form lung organoids. This model will be treated with carbon-based NM or conditioned medium from exposed alveolar macrophages for 12h or 6d. Mitochondrial activity (MTT) and cell death (TUNEL) will be measured to assess viability of the organoids. Gene expression analyses will elucidate temporal dynamics of cellular responses and help to evaluate the in vivo relevance of this in vitro model. Immunofluorescence staining will analyze cellular characteristics, focusing on activated and transitional epithelial cell states (Lcn2, Krt8). scRNA-seq data integration into in vivo data will assess the system's reliability in recapitulating pulmonary responses, with downstream analysis focusing on material-specific cell circuits and molecular mechanisms.
Outlook: This study seeks to reveal critical NM-induced inflammatory cell circuits in murine lung organoids, supporting formulation of a reliable new approach methodology.

516

Monocyte-to-Macrophage Differentiation and Extracellular Matrix Interactions in Idiopathic Pulmonary Fibrosis

S. Li
Helmholtz Zentrum Müchen, Munich, Germany

Abstract

Idiopathic pulmonary fibrosis (IPF) is characterized by aberrant extracellular matrix (ECM) remodeling and dysregulated monocyte–macrophage responses, yet the cellular and molecular mechanisms driving these processes remain poorly understood.

Objective: We aimed to dissect how circulating monocytes and their macrophage progeny in IPF patients interact with healthy versus fibrotic ECM, and to identify transcriptional and signaling pathways that mediate their pro-fibrotic activity.

Methods: Peripheral blood monocytes from IPF patients and healthy controls were isolated, differentiated, and polarized into macrophages at defined timepoints (day 0–8). ECM substrates derived from healthy or IPF lung fibroblasts were used to model cell–matrix interactions. Functional assays included QuantiGene multiplex profiling to validate polarization dynamics, bulk RNA-sequencing of IPF monocytes to derive disease-specific transcriptional signatures, and integrative single-cell RNA-seq analyses to map developmental trajectories from circulating monocytes to lung macrophages. Serum proteomics and ELISA were performed to identify candidate circulating factors driving pro-fibrotic differentiation, while cytokine stimulation and CRISPR/siRNA knock-down experiments tested key interferon-related pathways (e.g., IFITM3/WARS axis).

Results: Distinct monocyte clusters enriched in rapidly progressive IPF patients were identified, with transcriptional signatures indicative of interferon activation and ECM-remodeling potential. In vitro, IPF serum and ECM promoted a skewing of monocyte–macrophage differentiation toward a profibrotic phenotype, characterized by altered cytokine responses and enhanced modulation of fibroblast-derived ECM. Functional perturbation of interferon signaling attenuated these effects, highlighting its central role in IPF monocyte plasticity.

Conclusion: Our findings establish that IPF-specific monocyte subsets and their interaction with fibrotic ECM are key drivers of macrophage polarization and profibrotic activity. Targeting interferon-dependent pathways may represent a promising therapeutic strategy to modulate monocyte–macrophage dynamics in pulmonary fibrosis.

517

The role of Muc5b in the pathogenesis of pulmonary fibrosis in adult Nedd4-2$^{-/-}$ mice

J. C. Meyer auf der Heyde[1,2,3], N. Smyzcek[1,2,3], D. H. W. Leitz[1,2,3], A. González[1,2,3], C. M. Evans[4], M. A. Mall[1,2,3], J. Duerr[1,2,3]

[1]Charite - Universitätsmedizin Berlin, Berlin, Germany, [2]German Center for Lung Research (DZL), associated partner site, Berlin, Germany, [3]German Center for Child and Adolescent Health (DZKJ), Partner Site, Berlin, Germany, [4]Department of Medicine, University of Colorado, Aurora, CO, United States of America

Several studies have identified the airway mucin MUC5B as a strong genetic risk factor for pulmonary fibrosis in patients. However, the exact mechanisms of how MUC5B contributes to pulmonary fibrosis are not yet fully understood. By conditional deletion of the ubiquitin ligase *Nedd4-2* (*Nedd4-2*$^{-/-}$) in lung epithelial cells of mice, we recently generated a new mouse model sharing clinical and histological key features with pulmonary fibrosis. To investigate the role of Muc5b in the pathogenesis, we genetically delete Muc5b in the lung epithelial cells of conditional *Nedd4-2*$^{-/-}$ (*Nedd4-2*$^{-/-}$/ *Muc5b*$^{-/-}$) mice.

We compared the pulmonary phenotype of adult *Nedd4-2*$^{-/-}$ mice to *Muc5b*$^{-/-}$, *Nedd4-2*$^{-/-}$/*Muc5b*$^{-/-}$ and control mice, by assessing survival, oxygen saturation, weight, inflammation in bronchoalveolar lavage (BAL) and lung histology.

Since the MUC5B expression is elevated in patients, our hypothesis was that a knock-out of *Muc5b* might be beneficial for the pulmonary phenotype of conditional *Nedd4-2*$^{-/-}$ mice. However, our initial results show a slightly higher mortality in *Nedd4-2*$^{-/-}$/ *Muc5b*$^{-/-}$ as opposed to *Nedd4-2*$^{-/-}$ mice. Furthermore, *Nedd4-2*$^{-/-}$/ *Muc5b*$^{-/-}$ mice display an elevated amount of inflammatory cells in the BAL. Both *Nedd4-2*$^{-/-}$/ *Muc5b*$^{-/-}$ and *Nedd4-2*$^{-/-}$ mice show a similarly reduced oxygen saturation when compared to *Muc5b*$^{-/-}$ and control mice.

Taken together, our preliminary data suggest that genetic deletion of Muc5b does not confer a protective effect on the pulmonary phenotype of adult *Nedd4-2*$^{-/-}$ mice and instead indicates the important function of Muc5b in the maintenance of mucociliary clearance.

518

Investigating epithelial cell response to Mtb antigens and failure in epithelial repair using a Human alveolar organoid model.

M. Neyazi, R. Mishra, H. Abendroth, V. Thacker
UKHD, Heidelbeg, Germany

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), primarily targets the lungs, where infection begins in the alveolar space through uptake of bacilli by resident cells such as macrophages. Beyond these immune cells, the alveolar epithelium is increasingly recognised as an active participant in TB pathogenesis. Mtb secretes several protein antigens that promote virulence and immune evasion, including the antigen 85 (Ag85) complex, which comprises Ag85A, Ag85B, and Ag85C and plays an essential role in mycobacterial cell wall synthesis. Recent studies have identified Mtb mRNA and Ag85 protein within alveolar epithelial cells in infected human tissues. Ag85B, together with Mtb transcripts, accumulates not only in lymphoid cells within granulomas but also in epithelial cells, suggesting that epithelial infection and antigen sensing may influence disease progression. These findings highlight the need to investigate epithelial responses to Mtb antigens, particularly Ag85, and their contribution to impaired repair and regeneration.
The alveolar epithelium is composed of type 1 (AT1) cells, responsible for gas exchange, and type 2 (AT2) cells, which act as progenitors to replenish the epithelium. Conventional TB models, including mice, non-human primates, and immortalised cell lines, do not adequately capture human epithelial regeneration or chronic injury phenotypes. Advances in organoid technology provide physiologically relevant systems to investigate epithelial–pathogen interactions. We have established primary human alveolar organoids that can be maintained beyond 20 days post-seeding and propagated across passages while retaining a stable AT2 population. Gene expression analysis confirmed upregulation of SFTPB and SFTPC, and immunofluorescence demonstrated the presence of HT1-56^{+} AT1 and HT2-280^{+} AT2 cells.
By integrating organoid-derived monolayers at the air–liquid interface and lung-on-chip approaches, we aim to dissect interferon signalling, epithelial barrier function, and repair dynamics in response to Mtb infection and Ag85 stimulation. These studies will generate new mechanistic insights into epithelial dysfunction in TB and post-TB lung disease.

ORAL PRESENTATION

519

The Tie2 agonist PMC-403 might reduce endothelial barrier dysfunction and increased susceptibility to LPS after exposure to nicotine containing e-cigarette vapor

T. Pape*[1,2], O. Pak*[3,4], T. Graalmann[5], C. Ehlers[5], J. Deutscher[3,4], P. Horn[6], K. Stahl[7], M. M. Hoeper[1], S. David[8], N. Sommer*[3,4], B. Seeliger*[1,2]

[1]Hannover Medical School, Department of Respiratory Medicine and Infectious Diseases, Hannover, Germany, [2]German Center for Lung Research (DZL), Hannover, Germany, [3]Justus Liebig University Giessen, Excellence Cluster Cardio-Pulmonary Institute, Giessen, Germany, [4]German Center for Lung Research (DZL), Giessen, Germany, [5]Twincore, Center for experimental and clinical infection research, Hannover, Germany, [6]Charité Berlin, Medical Clinic for Hepatology and Gastroenterology, Hannover, Germany, [7]Hannover Medical School, Department of Gastroenterology, Hepatology, Infectious Diseases and Endocrinology, Hannover, Germany, [8]University Hospital Zurich, Institute for Intensive Care Medicine, Zurich, Switzerland

*Contributed equally

Background: The use of e-cigarettes is increasing in the European population. In some individuals, e-cigarettes can cause acute lung injury (EVALI) with the need for invasive ventilation. Pathophysiologically, a capillary leak seems to be involved. Currently, no causal therapy exists.
The effects of e-cigarette vapor extracts (ECVE) on the integrity of the endothelial barrier were investigated *in vitro* and *ex vivo*, as well as the possible anti-permeability agent PMC403 (Tie2 agonist).

Methods: HUVECs were cultured with different ECVEs and/or LPS and endothelial activation was determined *in vitro* by measuring secreted protein and mRNA levels of Angpt-2 and soluble Tie2 (sTie2). Barrier dysfunction was analyzed by continuous transendothelial electrical resistance (TER). An *ex vivo* isolated mouse lung model was used and endothelial permeability and edema formation were analyzed. The Tie2 agonist PMC403 was applied *in vitro* and *ex vivo* and its effect on endothelial permeability was determined.

Results: We observed a significant increase in Angpt-2 secretion in HUVECs after 24 h stimulation with nicotine-containing (Nic) ECVE and, to a lesser extent, without Nic. Preincubation with ECVE significantly increased Angpt-2 secretion after subsequent LPS stimulation by 20%. The Angpt-2/Tie2 mRNA ratio increased significantly with nicotine exposure and was increased even more after pre-incubation with nic ECVE and LPS stimulation. Functionally, nic ECVE resulted in greater LPS-induced loss of endothelial barrier integrity (TER measurement). In *ex vivo* isolated, ventilated and perfused mouse lungs intratracheal ECVE application or LPS perfusion increased endothelial permeability and lung edema. The novel Tie2 agonist PMC-403 decreased the LPS-induced loss of endothelial barrier function *in vitro* and was able to decrease ECVE- or LPS-induced endothelial barrier dysfunction *ex vivo*.

Conclusions: Our data suggests that e-cigarette vapor induces endothelial permeability and subsequent lung edema. Susceptibility to LPS stimulation might be increased. The anti-permeability agent PMC403 (Tie2 agonist) was identified as a potential therapeutic agent of EVALI.

ORAL PRESENTATION

520

Immune Cell Dynamics Orchestrating Environmental Barrier Injury and Regeneration

N. Patlolla[1], L. Czichon[1], A. Schmidt[1], C. Barro[2,3,4,5], T. Stöger[2], R. Olmer[1], C. Voss[1,2]

[1]Hannover Medical School, Clinic for Cardiac, Thoracic, Transplantation and Vascular Surgery, Leibniz Research Laboratories for Biotechnology and Artificial Organs, Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH), Member of the German Center for Lung Research (DZL), Hannover, Germany, [2]Institute of Lung Health and Immunity (LHI), Comprehensive Pneumology Center, Helmholtz Zentrum München, German Research Center for Environmental Health, and Member of the German Center of Lung Research (DZL), Munich, Germany, [3]Translational Immunology in Environmental Medicine, School of Medicine and Health, Technical University of Munich, Munich, Germany, [4]Translational Immunology, Faculty of Medicine, University of Augsburg, Augsburg, Germany, [5]Department of Pediatrics, Klinikum rechts der Isar, School of Medicine and Health, Technical University of Munich, Munich, Germany

Objective: Airborne pollutants are a leading cause of chronic lung diseases such as COPD, fibrosis, asthma, & lung cancer, contributing to millions of premature deaths worldwide. Early inflammatory responses following exposure to environmental stressors like particles, gases, or viruses are thought to shape long-term respiratory outcomes, but the underlying mechanisms remain poorly understood. Lung organoids, which closely mimic the structure and function of the distal human lung, offer a promising alternative to traditional cell lines by enabling detailed studies of injury, inflammation, & regeneration. This project aims to develop a dynamic, vascularized lung organoid-on-a-chip model to investigate early immune responses & tissue repair processes following environmental lung injury.

Methods: A microfluidic platform (Mimetas) will be used to generate a vascularized lung model by integrating human iPSC-derived lung organoids with an endothelial cell network. Angiogenic factors will support vascular growth & the formation of a functional air-blood barrier. Epithelial damage will be induced using selected pollutants prior to organoid integration, allowing targeted analysis of epithelial stress responses. Organoid viability, inflammatory markers, & activated cell states will be assessed through various methods. Circulating immune cells, specifically monocytes, will then be introduced to study infiltration dynamics & immune-epithelial interactions.

Conclusion: The vascularized, organoid-on-a-chip system will enable the study of immune cell recruitment, injury, & regeneration in the human lung. This model aims to uncover the impact of early inflammation after environmental exposure on respiratory health, while evaluating regenerative capacity on the air-blood barrier.

ORAL PRESENTATION

521

N23Ps as a Novel Antifibrotic Therapy: Insights from 3D Human Fibrosis Model and Single-Cell Profiling

D. L. Porras-Gonzalez[1], X. Wei[1], K. Merchant[1], A. K. Verma[1], M. Kratochwil[2], N. J. Lang[3], A. Agami[3], H. Ma[2], J. F. Jäger[1], R. Aufmkolk[1], J. Merl-Pham[4], S. Hauck[4], M.-G. Stoleriu[1,5], N. Kneidinger[1,6,7], J. Behr[6], M. Lehmann[1,8], H. A. Schiller[3,9], A.-Ö. Yildirim[1,9], O. Plettenburg[2], M. Gerckens[1,6], G. Burgstaller[1]

[1]Institute of Lung Health and Immunity (LHI), Comprehensive Pneumology Center (CPC) with the CPC-M bioArchive, Helmholtz Munich, Member of the German Center for Lung Research (DZL), Munich, Germany, [2]Institute of Organic Chemistry and Center for Biomolecular Drug Research, Leibniz Universität Hannover, Hannover, Germany, [3]Research Unit for Precision Regenerative Medicine, Helmholtz Centre Munich, Munich, Germany, [4]Metabolomics and Proteomics Core, Helmholtz Zentrum Munich, Munich, Germany, [5]CPC-M bioArchive/Helmholtz Centre Munich and Asklepios Fachkliniken Munich-Gauting, Munich, Germany, [6]Internal Medicine V, University of Munich, LMU University Hospital, Ludwig-Maximilians University (LMU), Munich, Germany, [7]Universitätsprofessor für Innere Medizin und Pneumologie Medizinische Universität Graz, Graz, Austria, [8]Institute for Lung Research, Philipps-University Marburg, Marburg, Germany, [9]Institute of Experimental Pneumology, LMU University Hospital, Ludwig-Maximilians University (LMU), Munich, Germany

Idiopathic pulmonary fibrosis (IPF) is a fatal lung disease marked by irreversible tissue scarring and fibroblast activation, driven largely by TGFβ1. Current treatments, Pirfenidone and Nintedanib, only delay progression, underscoring the urgent need for new therapies. Through high-throughput screening and medicinal chemistry, we developed N23Ps, a novel class of antifibrotic N-(2-butoxyphenyl)-acrylamides derived from the anti-allergic drug Tranilast. Contrary to Tranilast, N23Ps had a much higher potency in inhibiting ECM deposition and induced a distinct cytoskeletal remodeling in TGFβ1-treated IPF fibroblasts, revealed through morphology profiling by comprehensive imageOmics. Previously, by using human precision-cut lung slices (PCLS) and scRNAseq, we validated this ex-vivo model's fidelity to mimic early IPF features—including aberrant cell types and dysfunctional communication routes. Here, new scRNAseq analyses showed that N23Ps reversed profibrotic programs in CTHRC1+ (myo)-fibroblasts and attenuated aberrant basaloid–stromal signaling, while secretome profiling confirmed reduced secretion of collagens, matricellular proteins, and TGFβ effectors. Together, these convergent datasets position N23Ps as disruptors of CTHRC1+ (myo)-fibroblast-driven fibrogenic signaling. Importantly, our data suggest that this disruption of microtubules might occur through a regulated process other than direct binding to tubulins. Conclusively, our findings introduce N23Ps as a potential first-in-class microtubule-directed antifibrotics, offering a mechanistically novel strategy to counter IPF progression and improve therapeutic outcomes. By integrating single-cell, secretome, and phenotypic profiling in human PCLS, this work not only establishes N23Ps' efficacy but also provides a translational framework for accelerating antifibrotic drug discovery.

ORAL PRESENTATION

522

Investigating the role of Marco in hyperoxia-induced pulmonary macrophages in pathogenesis of Bronchopulmonary dysplasia (BPD)

A. S. Rahadian[1], S. D'Agostino[1], V. Rickert-Zacharias[1,2], R. Morty[1], I. Mižik[1]

[1]Heidelberg University Hospital/ Institute of Translational Pulmonology, Heidelberg, Germany, [2]Institute of Human Genetics Heidelberg University Hospital, Heidelberg, Germany

In developed countries, Bronchopulmonary dysplasia (BPD) is among the most common and severe complications of preterm birth and the most common chronic lung disease. BPD is not only contributing to high number of mortalities in preterm born infants, but also causing long-term consequences to those affected and survived the infancy. This disease is characterized by simplification of alveolar structure and dysregulation of immune development. However, the immune cell-specific changes during pathogenesis of BPD are still not well-understood.
Recent single-cell study from our team using a hyperoxia-induced murine BPD model suggests the presence of a distinct population of hyperoxia-induced macrophages (HIMs), identified primarily by the expression of MARCO. Here we present a gene and protein expression profile of MARCO$^+$ HIMs throughout the postnatal developmental period of the lung from postnatal day (P)3 to P14. We showed, that both gene and protein expression of *Marco*, as well as the number of MARCO$^+$ HIMs are steadily increased in the lungs of BPD mice, peaking at P7. Next, we wanted to decipher whether the presence of MARCO$^+$ HIMs has protective or detrimental role in pathogenesis of BPD. To address this, we employed a treatment with a competitive MARCO inhibitor polyguanylic acid (PolyG) and also by establishing MARCO knock-out mice model. Our findings suggest the MARCO$^+$ HIMs play a complex role in lung homeostasis and in preservation of lung development on the background of hyperoxia injury.

523

Genetic Deletion of Muc5b Reduces Interstitial Lung Disease in Neonatal Nedd4-2 Deficient Mice

N. Smyczek[1,2,3], J. C. Meyer auf der Heyde[1,2,3], D. H. W. Leitz[1,2,3], L. Schaupp[1,2,3], A. Loewe[1,2,3], M. Daniltchenko[1,2,3], A. González[1,2,3], C. M. Evans[4], M. A. Mall[1,2,3], J. Duerr[1,2,3]

[1]Department of Pediatric Respiratory Medicine, Immunology and Critical Care Medicine, Charité - Universitätsmedizin Berlin, Berlin, Germany, [2]German Center for Lung Research (DZL), associated partner site, Berlin, Germany, [3]German Center for Child and Adolescent Health (DZKJ), Partner Site, Berlin, Germany, [4]Department of Medicine, University of Colorado, Aurora, CO, United States of America

MUC5B has been implicated as a major risk factor for the development of idiopathic pulmonary fibrosis. However, insights into its role in the *in vivo* pathogenesis of interstitial lung disease (ILD) remain limited. We previously developed a mouse model for early onset ILD caused by congenital deletion of the ubiquitin ligase *Nedd4-2* (*Nedd4-2*$^{-/-}$). In this study, we introduced a lung-specific knockout of *Muc5b* (*Muc5b*$^{-/-}$) in these mice (*Nedd4-2*$^{-/-}$/*Muc5b*$^{-/-}$) to investigate the *in vivo* role of Muc5b in the pathogenesis of early onset ILD.

We compared the pulmonary phenotype of neonatal *Nedd4-2*$^{-/-}$ mice to *Muc5b*$^{-/-}$, *Nedd4-2*$^{-/-}$/*Muc5b*$^{-/-}$ and control mice during the first month of life. We assessed survival, oxygen saturation and lung mechanics. Furthermore, we evaluated inflammation in bronchoalveolar lavage (BAL) and lung histology. Muc5b expression was investigated in BAL by western blots. Finally, we applied Imaging Mass Cytometry (IMC) for multiplexed spatial protein expression analysis on a single cell level.

We found that neonatal *Nedd4-2*$^{-/-}$/*Muc5b*$^{-/-}$ show a reduced mortality ($p < 0.05$) and improved pulmonary function compared to *Nedd4-2*$^{-/-}$ mice. Western blots revealed reduced Muc5b intensities in BAL of *Nedd4-2*$^{-/-}$/*Muc5b*$^{-/-}$ versus *Nedd4-2*$^{-/-}$ mice ($p = 0.04$). Further, Muc5b deletion prevented airway mucus obstruction in *Nedd4-2*$^{-/-}$ mice which was accompanied by reduced concentrations of the inflammatory markers KC, IL-1β, IL-13, TNF-α and MIP-2 in the BAL of *Nedd4-2*$^{-/-}$/*Muc5b*$^{-/-}$ mice. Lastly, IMC analysis revealed normalized Muc5b expression in airway epithelial cells, a reduction in inflammatory cells within the lung parenchyma, and lower mesenchymal marker expression.

Taken together genetic deletion of *Muc5b* mitigates the severity of early onset ILD by preventing mucus obstruction in airways of neonatal *Nedd4-2*$^{-/-}$ mice. These data highlight the central role of Muc5b in the pathogenesis of ILD and its potential as therapeutic target in patients.

524

Altered cholesterol immunometabolism activates the NLRP3-inflammasome in lung fibrosis

M. Vaso[1], M. Dukic[1], P. Penitz[1], A. Manukyan[2], S. Collum[3], W. Lin[4], C. Winder[4], W. Dunn[4], J. Schupp[5], P. Braubach[5], M. Wygrecka[6], D. Ren[3], E. Suarez[3], H. Huang[3], R. Hussain[3], B. Patel[3], H. Karmouty-Quintana[3], A. Akalin[2], M. Landthaler[2], G. Nouailles[1], M. Ochs[1], S. Giambelluca[1], **E. Lopez-Rodriguez**[7]

[1]Charité Universitätsmedizin Berlin, Berlin, Germany, [2]Berlin Institute for Medical Systems Biology of the Max-Delbrück Center, Berlin, Germany, [3]McGovern Medical School, UTHealth Houston, Houston, United States of America, [4]University of Liverpool,, Liverpool, United Kingdom, [5]Hannover Medical School, Hannover, Germany, [6]Justus-Liebig-Universität Giessen, Giessen, Germany, [7]Charité Universitätsmedizin Berlin, Institute of Functional Anatomy, Berlin, Germany

Introduction/Rationale: Previous research has highlighted dysregulation in lipid metabolism during lung fibrosis. However, the impact of cholesterol immunometabolism during lung fibrosis progression remains unclear, but has been related to the NLRP3-inflammasome activation in cardiovascular diseases.

Objective: Identifying the link between altered cholesterol metabolism and NLRP3 inflammasome activation in fibrotic lungs.

Methods: Different pulmonary fibrosis patient cohorts (from 2 centers and publicly available datasets) and a murine model of lung fibrosis (SP-C–/–) were included. Expression of cholesterol metabolism proteins and cholesterol content were determined in lungs from patients and bronchoalveolar lavage fluid (BALF) cells of aging SP-C–/– mice, respectively by western blotting and fluorimetric assay. Cells upregulating inflammasome components were identified by flow cytometry and spatial transcriptomics. Metabolomic and lipidomic analyses were conducted in BALF and BALF cells of SP-C–/– versus wild-type (WT) mice. NLRP3 inflammasome components were assessed by RT-PCR, immunoblotting, ELISA, and immunofluorescence.

Main results: Lung samples from fibrosis patients showed higher cholesterol content, altered cholesterol metabolism and higher IL-18 levels, compared to controls. Accordingly, BALF cells of SP-C–/– mice showed alteration of their cholesterol metabolism and inflammasome activation with age. Inflammasome activation was differentially expressed in alveolar macrophages (AM) in human and mice fibrotic samples. Lipidomic analysis pointed at cholesterol esters as potential activating agent, which was confirmed with in vitro data.

Conclusion: Altered cholesterol esterification potentially activates the NLRP3 inflammasome in AM during pulmonary fibrosis in a murine model and fibrosis patients. The inflammasome activation may be the origin of IL-18 in the fibrotic lung.

525

A study of irreversible pulmonary effects after malnutrition

C. Voelz[1], S. Trinh[2], L. Blischke[2], M. de Zwaan[3], C. Mühlfeld[1]

[1]Hannover Medical School/Institute of Functional and Applied Anatomy, Hannover, Germany, [2]Uniklinik RWTH Aachen/Institute of Neuroanatomy, Aachen, Germany, [3]Hannover Medical School/Clinic for Psychosomatics and Psychotherapy, Hannover, Germany

The disease anorexia nervosa (AN) is characterized by pathologically low body weight, which leads to a number of primary and secondary damages. Long-term damage to affected organs has only been marginally investigated, and correlations between morphologic changes and the resulting molecular changes are completely unknown.
In the lung, literature shows that patients with AN have an increased risk of developing atypical lung infections, including potentially life-threatening pneumothorax, weakened respiratory muscles and structural lung remodeling – the question of "nutritional emphysema" is up for discussion.
The project presented here will identify both the acute changes caused by starvation and the non-regenerative structures after weight rehabilitation. An established animal model of AN, the activity-based anorexia model, will be used to study structural and molecular changes. Samples will be collected after a chronic phase of weight loss and after refeeding. High-end electron microscopy and spatial transcriptomics will be used to identify changes on morphological and molecular basis. Design-based stereology will be used to evaluate the number and size of alveoli, and the characteristics of alveolar epithelial type 2 cells. Spatial transcriptomics will clarify the relationship between the observed morphological alterations and molecular changes.
The combined information on structure and molecular genetics will provide a comprehensive picture of the relationships between nutritional deficiencies and chronic consequences in the lung. These findings are not only relevant for the treatment of AN, but also for other predispositions.

526

Development of Optimized N23Ps for Antifibrotic Therapy and Mechanistic Target Identification

X. Wei[1], H. Ma[2,3], D. Gonzalez[1], K. Merchant[1], M. Wögrath[1,4], N. Bach[5], A. Steinbach[5], L. Sarnova[6], M. Jirouskova[6], M. Gabriel Stoleriu[1,4,7], N. Kneidinger[4,8], J. Behr[4,7,9], M. Gregor[6], A. Ö. Yildirim[10], S. Sieber[5], O. Plettenburg[2,3], M. Gerckens[1,10], G. Burgstaller[1]

[1]Institute of Lung health and Immunity (LHI) and Comprehensive Pneumology Center (CPC),Helmholtz Zentrum München, Munich, Germany, [2]Institute of Medicinal Chemistry, German Research Center for Environmental Health (GmbH), Helmholtz Zentrum München, Munich, Germany, [3]Leibniz Universität Hannover, Institute of Organic Chemistry and Center for Biomolecular Drug Research (BMWZ), Hannover, Germany, [4]CPC-M bioArchive, Comprehensive Pneumology Center Munich DZL/CPC-M, Helmholtz Zentrum München, Munich, Germany, [5]Department of Chemistry, Center for Integrated Protein Science Munich (CIPSM), Technische Universität München, Garching, Germany, [6]Laboratory of Integrative Biology, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic, [7]Asklepios Fachkliniken Munich- Gauting, Munich, Germany, [8]Department of Internal Medicine V, Ludwig- Maximillians-University Munich, Munich, Germany, [9]Department of Internal Medicine V, Ludwig- Maximillians-University Munich, Member of the German Center for Lung Research (DZL), Munich, Germany, [10]Institute of Lung health and Immunity (LHI) and Comprehensive Pneumology Center (CPC), Helmholtz, Munich, Germany

Lung fibrosis, a progressive interstitial lung disease, remains incurable except for lung transplantation, highlighting the urgent need for novel antifibrotic therapies. Recently, we developed a phenotypic screening assay using patient-derived fibroblasts from idiopathic pulmonary fibrosis (IPF) patients, focusing on the inhibition of aberrant extracellular matrix (ECM) protein deposition. Through medicinal chemistry, we synthesized and tested over 100 derivatives of N-(2-butoxyphenyl)-3-(phenyl)acrylamides (N23Ps), establishing structure–activity relationships (SAR) and identified novel modifications that preserved or enhanced antifibrotic activity. Several derivatives also demonstrated improved metabolic stability, supporting their potential for *in vivo* pharmacokinetics.

To investigate N23Ps' molecular target, we developed a diazirine-labeled N23P affinity probe that effectively inhibited TGFβ1-induced ECM deposition. This compound allows for UV-induced crosslinking and click-chemistry-based biotinylation, enabling pull-down assays for target protein identification via mass spectrometry. Results from these chemical proteomics revealed several molecular target candidates, with some of these proteins implicated in the regulation of TGFβ1 signaling, potentially through membrane-associated GTPase activity and endocytic processes

In summary, our study presents a comprehensive chemical exploration and optimization of N23Ps, highlighting their therapeutic potential and providing active compounds for molecular target identification in the development of novel antifibrotic treatments.

528

Surfactant protein A and D regulate sialylation and barrier function of the alveolar epithelial glycocalyx in vitro

I. Yavuz[1,2], V. Gluhovic[1,2], S. Popovic[1,2], S. G. Twamley[1,2], M. Dukic[1,2], S. Giambelluca[1,2], S. Lochen[1,2], K. Fenkter[3], L. Bechtella[4,5,6], A. Zappe[3,5], C. Butnarasu[7], D. Lauster[7], P. Mertins[3], K. Pagel[4,5], G. Nouailles[8], W. M. Kuebler[2,9,10,11,12], M. Ochs[1,2], E. Lopez-Rodriguez[1,2]

[1]Charité – Universitätsmedizin, Functional Anatomy, Berlin, Germany, [2]German Center for Lung Research (DZL), Berlin, Germany, [3]Max-Delbrück-Centrum for Molecular Medicine in der Helmholtz-Gemeinschaft, Berlin, Germany, [4]Institute of Chemistry and Biochemistry, Freie Universität Berlin, Berlin, Germany, [5]Fritz Haber Institute of the Max Planck Society, Berlin, Germany, [6]Université Paris-Saclay, Univ Evry, CY Cergy Paris Université, CNRS, LAMBE, 91025, Evry-Courcouronne, France, [7]Institute of Pharmacy, Biopharmaceuticals, Freie Universität Berlin, Berlin, Germany, [8]Charité – Universitätsmedizin Berlin,, Department of Infectious Diseases, Respiratory Medicine and Critical Care, Berlin, Germany, [9]Institute of Physiology, Charité – Universitätsmedizin Berlin, Berlin, Germany, [10]German Center for Cardiovascular Research (DZHK), Berlin, Germany, [11]Keenan Research Center, St. Michael 's Hospital, University of Toronto, Ontario, Canada, [12]Departments of Surgery and Physiology, University of Toronto, Ontario, Canada

Surfactant protein A (SP-A) and D (SP-D), also known as lung collectins, are C-type lectins, calcium-dependent glycan binding proteins with a typical carbohydrate recognition domain. These proteins have been described to bind to sialic acid (SA) and inhibit neuraminidase (NA). Therefore, we hypothesized that they may play a role in the sialylation status of the alveolar epithelial glycocalyx. To test this hypothesis, an *in vitro* model of the alveolar epithelium was assessed for epithelial integrity, by trans epithelial electrical resistance and X-pert permeability assay, in the presence or absence of bacterial and/or human NA and recombinant (rec) human SP-A and SP-D. Presence of SA at the epithelial glycocalyx was detected by WGA staining after NA, PNGase F or O-Glycosidase treatment with or without recSP-A and recSP-D. In addition, free SA was measured in cell medium after the same treatments. Microscale thermophoresis was used to assess the binding affinity of recSP-A and recSP-D to NA. We found that recSP-A and recSP-D decreased SA cleavage and epithelial permeability *in vitro* by interacting with and inactivating bacterial NA. In addition, our findings raise the possibility that SP-A preferentially binds sialylated *N*-glycans at the cell borders, while SP-D binds to broadly distributed *O*-glycans protecting them from NA treatment. In conclusion, SP-A and SP-D regulate sialylation of the glycocalyx via a dual mechanism: 1) by binding to and protecting SA and 2) binding to and inhibiting NA, thus maintaining alveolar epithelial barrier integrity *in vitro*.

ORAL PRESENTATION

529

The Autonomous Nerve System-Lung Interface in experimental BPD: Neuropeptide Y Modulates Immune Response and Alveolar Growth in Neonatal Mice Exposed to Oxidative Stress

C. Zeitouny[1,2], C. Nies[1,3], C. Kuiper-Makris[1,3], C. Vohlen[1,3], D. Hirani[4], H. Herzog[5], J. Dötsch[3], W. Seeger[4], J. Koenig[2], M. A. Alejandre-Alcazar[1,3,4]

[1]University of Cologne, Faculty of Medicine and University Hospital Cologne, Translational Experimental Pediatrics, Department of Pediatric and Adolescent Medicine, Cologne, Germany, [2]University of Cologne, Faculty of Medicine and University Hospital Cologne, Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, Cologne, Germany, [3]University of Cologne, Faculty of Medicine and University Hospital Cologne, Department of Pediatric and Adolescent Medicine, Cologne, Germany, [4]Institute for Lung Health (ILH), University of Giessen and Marburg Lung Center (UGMLC) and Cardiopulmonary Institute (CPI), Gießen, Germany, [5]5St Vincents Centre for Applied Medical Research: Darlinghurst,, New South Wales, Australia

Oxygen supplementation triggers inflammation and disrupts alveolar and microvascular growth in preterm infants, often leading to bronchopulmonary dysplasia (BPD). The autonomic nervous system (ANS) is critical in lung tissue homeostasis and repair. The sympathetic co-neurotransmitter neuropeptide Y (NPY) emerges as a central regulator of the ANS-organ interface with immune-modulatory function. Here, we studied the activation of the sympathetic nerve system (SNS) and the contribution of NPY to a hyperoxia-based model of BPD. To this end, neonatal wild-type (WT) and NPY null mice were exposed to 85% O_2 (HYX) or 21% O_2 (NOX) from birth to postnatal day 14. Prolonged hyperoxia caused a 7-fold increase of tyrosine hydroxylase (TH), a SNS marker, and NPY (>40-fold) in neonatal WT^{NOX} lungs. Analysis of lung scRNA-seq of WT^{HYX} revealed an upregulation of NPY in alveolar macrophages. In contrast, NPY^{HYX} showed lower abundance of TH than WT^{HYX}, indicating reduced SNS activity. Quantitative histomorphometry demonstrated an aggravated hyperoxia-induced alveolar growth arrest and septal thickening in NPY^{HYX}, whereas vascular muscularization and proliferation of vascular SMC were prevented compared to WT^{HYX}. Additionally, NPY^{HYX} were protected from $CD68^{+}$ macrophage recruitment after hyperoxia, but exhibited a stronger lung-intrinsic inflammation with increased *Il6* (20 fold) and *Il1b* (4-fold) than WT^{HYX} . In summary, our data demonstrate a dual role of NPY in neonatal lungs in response to hyperoxia - preserving alveolarization while promoting immune cell recruitment and vascular muscularization, highlighting the importance of ANS-lung interface in lung maturation and injury.

Lung Cancer: 601 - 606

601

Sex-difference in immune response in NSCLC patients treated with ICIs: Interim Findings from the ALICE cohort

S. Hermann[1], M. Aykac[1], B. Resuli[1], M. Freiwan[1], M. Arredondo Lasso[1], R. Kiefl[1], C. Schneider[2], S. Kobold[3], A. Tufman[1]
[1]LMU University Hospital, Department of Internal Medicine V, Munich, Germany, [2]LMU University Hospital, Department of Surgery, Munich, Germany, [3]LMU University Hospital, Department of Clinical Pharmacology, Munich, Germany

Immune checkpoint inhibitors (ICIs) have demonstrated significant improvement in outcomes for patients with non-small cell lung cancer (NSCLC). However, their efficacy varies by sex. Emerging evidence points to sex-related variations in both immune response and ICI efficacy. This branch of the ALICE (alloimmunization and alloantibodies in male and female NSCLC patients treated with ICIs) study, is a prospective, translational cohort study, designed to examine sex-specific differences in immune response and exhaustion, with particular attention to immune cell subsets and cytokine dynamics in relation to tumor progression and therapeutic response. Peripheral blood samples of patients will be used for immunophenotyping of immune cells and for profiling inflammatory markers using a 15-plex cytokine panel. These findings will be correlated to clinical parameters such as age, disease stage, and smoking status. Progression-free survival (PFS) will be evaluated via Kaplan-Meier estimates and log-rank testing, with Cox proportional hazards modelling to explore predictors of immune response variability following ICI therapy. This first interim analysis of the ongoing prospective ALICE study includes 59 patients with histologically confirmed diagnosis of NSCLC, comprising 27 females and 32 males. The majority of patients had adenocarcinoma (n=47), while 8 had squamous cell carcinoma and 4 had other histologies, including adenosquamous carcinoma. Participant recruitment and biospecimen collection are still ongoing. Final analyses will be performed once the target enrolment of 100 patients including both male and female cohorts, has been reached to ensure sufficient power for sex specific comparisons. Insights into sex-dependent immune-inflammatory profiles may inform the development of more effective gender-based therapeutic strategies for NSCLC patient receiving ICIs.

602

Simulating pressure exerted by airway stents on a 3D in vitro model of the respiratory mucosa

M. Johrden[1], F. Wehrmann[1], K. Barbet[2], M. Schedel[2,3], C. Cornelissen[1], A. L. Thiebes[1]

[1]AME - Institute of Applied Medical Engineering, Helmholtz Institute, RWTH Aachen University, Department of Biohybrid and Medical Textiles (BioTex), Aachen, Germany, [2]University Medical Center Essen - Ruhrlandklinik, Department of Pulmonary Medicine, Essen, Germany, [3]University Medical Center Essen, Department of Pulmonology, Essen, Germany

Lung cancer is among the most prevalent cancer types. Since tracheobronchial tumours are often detected in an advanced stage, only palliative treatment by airway stents is possible to relief symptoms. However, airway stents can in turn lead to the formation of granulation tissue, again obstructing the airways. The responsible mechanisms underlying the formation of granulation tissue are not fully understood.
The pressures exerted by the airway stents may be a contributing factor to the granulation process. Therefore, the primary focus of this work is on the mechanobiology of the airway epithelium. A bioreactor was designed allowing static and dynamic compression of the tissue models. Driven by air pressure, compression of the tissue is conducted to simulate the mechanical impact of airway stents. The 3D *in vitro* model imitates the respiratory mucosa by co-culturing epithelial cells alongside endothelial and supporting cells, such as fibroblasts. Co-cultured cells were compressed comparing static and dynamic mechanical stimulation within the range of 5 to 60 kPa.
Following the compression, self-organisation of the tissue was analysed through the utilisation of histo- and immunohistochemical staining, live-cell imaging and quantitative image analysis. While the survival rate slightly decreases with increasing pressures, a sufficient number of cells survived the compression process for further analysis. We investigate epithelial mucociliary differentiation and barrier function as well as changes in gene expression.
This mechanobiological 3D model of the respiratory can serve as a tool to imitate the impact of airway stents on their surrounding tissue, with potential for further research. The implementation of such a model can contribute to reduction of *in vivo* experiments.

603

Impact of Immune-Checkpoint Blockade on Endothelial Cell States and Vascular–Immune Crosstalk in Non-Small Cell Lung Cancer

Y. Lin[1,2,3], S.-H. Wong[1,2,3], S. Schlichtner[4], F. Hofmann[1,2,3], S. Loges[1,2,3], N. Berenbrok[1,2,3]
[1]DKFZ-Hector Cancer Institute at the University Medical Center Mannheim,, Mannheim, Germany, [2]Division of Personalized Medical Oncology (A420), German Cancer Research Center (DKFZ); German Center for Lung Research (DZL), Heidelberg, Germany, [3]Department of Personalized Oncology, University Hospital Mannheim, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany, [4]Ignaz Semmelweis Institute and Division of Infectious Diseases, Translational Medical Mycology Research Unit, Host-Fungal Pathogen Interaction Group, Medical University of Graz, Graz, Austria

Background: Tumor-infiltrating immune cell populations and tumor-vasculature are intimately interconnected, with endothelial cells (EC) serving as key regulators of immune cell infiltration and activation. Clinical observations from non-small cell lung cancer (NSCLC) patients indicate enhanced sensitivity towards anti-angiogenic therapy, following immune-checkpoint inhibition (ICI) treatment. While ICI-driven changes to tumor-infiltrating immune cell populations are well characterized, it's impact on tumor-vasculature remains poorly defined.
Methods: Utilizing ICI-sensitive (MC38) and -resistant (LLC) murine tumor models, single-cell RNA sequencing and flow cytometry of purified EC $CD45^-$ $CD31^+$ and $CD45^+$ immune cell-populations were employed to profile EC subtypes and transcriptional programs, as well as their interactions with tumor infiltrating immune cell populations. *In vitro* tube-formation and immune cell co-culture assays were performed to validate the phenotypes observed *in vivo*.
Results: MC38 tumors were sensitive to ICI, showing delayed tumor growth, whereas LLC tumor growth did not respond to ICI therapy. Single-cell RNA-sequencing of EC revealed genes (CD34, LGALS9) differentially regulated upon ICI in both tumor models, suggesting a direct effect irrespective of enhanced inflammatory processes within the malignant tissue. On the other hand, only EC of MC38 ICI treated tumors showed increased expression of CD274 (PD-L1) as well as expansion of the tip-cell subpopulation, and significantly increased expression of EC activation markers, including ITGB and SELP.
Conclusion: ICI induces distinct transcriptional programs in EC of both ICI-sensitive and resistant tumors. It directly modifies tumor-vasculature, irrespective of resulting inflammatory response, however EC of ICI-sensitive tumors additionally display differential expression of genes critical to immune-cell infiltration and immunomodulation, suggesting they could serve as important contributors in efforts aiming to enhance efficacy of ICI in NSCLC.

ORAL PRESENTATION

604

Setup of a patient-derived organoid biobank from NSCLC patients

A. Motavallian[1,2,3,4], M. Ziegler[1,2,3,4], A.-K. Daum[1,2,3,4], F. Hertel[1,2,3], L. Salgueiro[1,2,3], A. L. Volckmar[5], K. Kelly[6], T. Mulholland[7], C. Wenzel[8,9], M. Schneider[3,10], F. Eichhorn[3,11], M. Wermke[9,12], J. Betge[1,7], C. Plass[3,6], H. Winter[3,11], M. Thomas[3,13], A. Stenzinger[3,5], M. Llamazares-Prada[3,6], S. Loges[1,2,3,4]

[1]DKFZ-Hector Cancer Institute at the University Medical Center Mannheim, Mannheim, Germany, [2]Division of Personalized Medical Oncology (A420), German Cancer Research Center (DKFZ), Heidelberg, Germany, [3]German German Center for Lung Research (DZL), Translational Lung Research Center Heidelberg (TLRC), Heidelberg, Germany, [4]Department of Personalized Oncology, University Hospital Mannheim, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany, [5]Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany, [6]Division of Cancer Epigenomics, German Cancer Research Center (DKFZ), Heidelberg, Germany, [7]Junior Clinical Cooperation Unit Translational Gastrointestinal Oncology and ; Preclinical Models (B440), German Cancer Research Center (DKFZ), Heidelberg, Germany, [8]Institute of Pathology, University Hospital Carl Gustav Carus, Medical Faculty, Technische Universität Dresden, Dresden, Germany, [9]National Center for Tumor Diseases (NCT/UCC), German Cancer Research Center (DKFZ), Dresden, Germany, [10]Translational Research Unit, Thoraxklinik at Heidelberg University Hospital, Heidelberg, Germany, [11]Department of Surgery, Thoraxklinik at Heidelberg University Hospital, Heidelberg, Germany, [12]Clinic for Internal Medicine, University Hospital Carl Gustav Carus, Medical Faculty, Technische Universität Dresden, Dresden, Germany, [13]Department of Thoracic Oncology, Thoraxklinik at Heidelberg University Hospital, Heidelberg, Germany

Introduction: In lung cancer patients (LCPs), patient-derived organoids (PDOs) offer a valuable platform for predicting treatment responses (1). However, establishing PDO cultures, multiomic profiling, and drug testing remain challenging due to difficulties in obtaining pure tumor organoids, requiring further optimization. (2).

Material and Method: In this study, PDOs were generated from both primary lung cancer tissues and matched non-neoplastic tissues derived from 22 LCPs. Pathological and multiomic profiling was performed to assess the concordance of genetic and epigenetic landscapes, as well as histopathological features, between primary tissues and PDOs. Furthermore, potential therapeutic vulnerabilities and resistances of PDOs were assessed through high-throughput drug screening.

Results and discussion: We generated 12 pairs of lung cancer organoids (LCOs) and matched healthy lung organoids, each validated through histopathological and molecular analyses. Histopathological and epigenetic profiling demonstrated a strong concordance between LCOs and their corresponding primary tumors. Copy number variation analysis confirmed high concordance of major mutational patterns in PDOs with primary tumors. Deconvolution of latent methylation components revealed tumor-specific epigenetic states for individual patients, which were recapitulated in the LCOs. High-throughput drug-screening proved feasible and led to the identification of novel therapeutic targets.

Conclusion: This study demonstrates that LCOs recapitulate the distinct epigenomic landscapes of primary tumors in addition to genomic features. Multiomic analyses and high-throughput drug screening enhance their use as a platform for personalized therapy in precision oncology.

References:

Ebisudani T et al., Genotype-phenotype mapping of a patient-derived lung cancer organoid biobank identifies NKX2-1-defined Wnt dependency in lung adenocarcinoma. Cell Rep. 2023; 42(3):112212.

Wang HM et al., Using patient-derived organoids to predict locally advanced or metastatic lung cancer tumor response: A real-world study. Cell Rep Med. 2023; 21; 4(2):100911.

ORAL PRESENTATION

605

Role of Sensory Innervation in Non-Small Cell Lung Cancer

S. Ravikanth[1,2,3], L. Kramm[1,2,3], J. Wang[1,2,3], M. Ling[1,2,3], R. Qadeer[1,2,3], H. Augustin[4,5], A. A. Abdul Pari[4,5], S. Loges[1,2,3], J. Engelmann[1,2,3]

[1]German Cancer Research Center (DKFZ), Division of Personalized Medical Oncology, Heidelberg, Germany, [2]University Hospital Mannheim, Medical Faculty Mannheim, University of Heidelberg, Department of Personalized Medical Oncology, Mannheim, Germany, [3]DKFZ-Hector Cancer Institute at the University Medical Center Mannheim, Mannheim, Germany, [4]German Cancer Research Center (DKFZ-ZMBH Alliance), Division of Vascular Oncology and Metastasis, Heidelberg, Germany, [5]European Center for Angioscience, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany

Lung cancer is the leading cause of cancer-related deaths globally, with non-small cell lung cancer (NSCLC) accounting for approximately 85% of cases. Recent evidence suggests that nerves within the tumor microenvironment influence cancer progression, but the specific role of sensory innervation in NSCLC is poorly understood. This study aimed to define the anatomical presence and functional role of sensory nerves in NSCLC.

To visualize sensory innervation in NSCLC we utilized the orthotopic, syngeneic Lewis Lung Carcinoma (LLC) mouse model and performed multiplex immunofluorescence staining on murine FFPE lung tissue. We found that LLC tumors are innervated by a population of nerve fibres co-expressing receptor channel transient receptor potential vanilloid 1 (TRPV1) and neuronal type III intermediate filament peripherin (PRPH), suggesting presence of sensory nerves in NSCLC tumor microenvironment.

To explore functional interaction of sensory neurons with NSCLC cancer cells *in vitro*, we utilized human-induced pluripotent stem cell (hiPSC) derived - sensory neuron cultures. We established co-culture systems with human NSCLC cell lines H460 and H1975. We found that sensory neurons enhance tumor cell proliferation, measured by BrdU incorporation and cell cycle analysis via flow cytometry, suggesting that neuron-cancer cell interaction provides pro-proliferative signals. Furthermore, sensory neurons induced chemoresistance against cisplatin *in vitro.* Mechanistically, we observed induction of intracellular LC3-positive vesicles in cancer cells, suggesting that tumor innervation might induce autophagy in NSCLC leading to chemoresistance. Overall, our study validates the presence of sensory nerves in NSCLC and provides evidence that sensory neurons are capable of regulating tumor proliferation and survival in vitro. Current efforts are focused on validating these pro-tumoral mechanisms and identifying neuron-tumor signalling axes involved in NSCLC.

ORAL PRESENTATION

606

Tumoroid-induced angiogenesis in a small cell lung cancer-on-a-chip model

E. Tsichlia[1], J. Gonzalez-Rubio[1,2], D. Schliebs[3], J. George[3], C. Cornelissen[4], A. L. Thiebes[1]

[1]AME - Applied Medical Engineering, Department of Biohybrid and Medical Textiles (BioTex), Aachen, Germany, [2]Cambridge University, Cambridge, United Kingdom, [3]University of Cologne, Cologne, Germany, [4]Clinic for Pneumology and Internal Intensive Care Medicine, RWTH Aachen University Hospital, Aachen, Germany

Small cell lung cancer (SCLC) remains one of the most aggressive and deadly cancers. Accounting for 15% of all lung cancer cases, SCLC is remarkably heterogeneous and highly metastatic. The tumors are composed of a mixture of cells with high and low neuroendocrine (NE) genetic signatures, acquiring neuronal and mesenchymal phenotypes respectively, which lead to diverse invasion patterns. Current drugs are effective in only 5% of patients, as the metastatic potential of the tumors is understudied. Traditional 2D models do not capture complex cell-microenvironment interactions, while animal models fail to reproduce disease progression.

We generated a SCLC tumor-on-chip model to recapitulate the first stage of metastasis—tumor invasion—*in vitro*. SCLC cells with either NE-high or NE-low profiles were cultured and matured into 3D aggregates. The generated tumoroids were inserted into one of the outer channels of a three-channel microfluidic chip. In the middle chamber, a bronchial fibrin gel was loaded with fibroblasts, while the other outer channel was endothelialized to mimic a vessel. In parallel, extracellular vesicles (EVs) were isolated from the conditioned media of both NE subtypes, in both normoxia and hypoxia (5% O_2). These EVs were quantified by nanoparticle tracking analysis and used to culture chips without tumors.

NE-low SCLC lines formed more compact and robust tumoroids than NE-high lines, which remained looser and more fragile. Immunofluorescent staining of the tumor-on-chip models revealed the invasive motility and penetration of the SCLC towards the fibrin hydrogel and the opposing endothelialized channel. Concurrently, endothelial cells aligned and developed sprouting vessels towards the tumor site. In the chips treated with EVs, both subtypes attracted the angiogenic sprouting of the endothelialized channel.

Our model mimics the invasion of SCLC into the 3D lung stroma, and suggests EVs as a key player in pre-metastatic angiogenesis. Future studies can use this tool to investigate the posterior cancer intravasation, gaining a mechanistic overview of the metastatic cascade and advancing our understanding of SCLC pathophysiology.

Pulmonary Hypertension: 701 - 703

ORAL PRESENTATION

701

Foxo3+ cardiac fibroblasts prevent auto-inflammatory reactions in the heart and protect from maladaptive right ventricle remodeling caused by pulmonary hypertension

G. Maroli[1], S. Nayakanti[1], S. Medrano Garcia[1], S.-E. Lemay[2], G. Hesami[1], A. Mamazhakypov[1], S. Guenther[3], B. Koyonazarov[1], M. Looso[3], T. Braun[4], S. Bonnet[2], W. Seeger[5,6], R. Savai[6], S. Savai Pullamsetti[1,5,7]

[1]University of Giessen and Marburg Lung Center, Center for Infection and Genomics of the Lung, Giessen, Germany, [2]Laval University (ULAVAL), Pulmonary Hypertension and Vascular Biology Research Group, Quebec, Canada, [3]MPI for Heart and Lung Research, Bioinformatics and Deep Sequencing Platform, Bad Nauheim, Germany, [4]MPI for Heart and Lung Research, Department of Cardiac Development and Remodeling, Bad Nauheim, Germany, [5]Member of the German Center for Lung Research (DZL), Giessen, Germany, [6]Institute of Lung Health, Giessen, Germany, [7]Cardio-Pulmonary Institute (CPI) Excellence Cluster, Bad Nauheim, Germany

Background:

Adaptation of the right heart ventricle (RV) to increased afterload plays crucial roles in determining cardiac outcomes of pulmonary arterial hypertension (PAH), but the molecular bases are not fully understood. The Forkhead box O (FOXO) transcription factor FOXO3 is involved in cardiovascular health and highly expressed in cardiac fibroblasts, where it contributes to inhibit remodeling signaling pathways. We thus tested whether FOXO3+ cardiac fibroblasts regulate heart homeostasis and maladaptive RV remodeling induced by pressure overload and PAH.

Methods:

Fibroblast-specific Foxo3 knock-out (Foxo3-FB^{KO}) mice were subjected to pulmonary artery banding (PAB) for 35 days.

Results:

FOXO3 is down-regulated in RV samples from PAH patients with decompensated RV hypertrophy, and in two different animal models of RV dysfunction (monocrotaline rats and PAB mice). Upon steady-state conditions, FOXO transcriptional activity is enriched in a subpopulation of immunomodulatory cardiac fibroblasts named injury response fibroblasts (F-IR). Conditional deletion of *Foxo3* leads to loss of F-IR markers and to activation of major histocompatibility complex type 1 (MHC-I) genes in cardiac fibroblasts. Foxo3-FB^{KO} hearts also display increased expression of MHC-II in resident macrophages, activation of pro-inflammatory gene networks in T cells and up-regulation of cytokines such as Il-1α and Il-1β. In spite of this pro-inflammatory signature, Foxo3-FB^{KO} hearts show no functional phenotype at the baseline. However, upon PAB-induced overload, Foxo3-FB^{KO} mice display maladaptive RV hypertrophy with decreased RV ejection fraction, blunted activation of the remodeling transcriptional program and reduced numbers of MHC-II+ macrophages. Finally, treatment with monoclonal antibodies directed at pro-inflammatory cytokines downstream of FOXO3 ameliorated features of maladaptive remodeling in human RV slices from PAH patients.

Conclusions:

We found that FOXO3+ cardiac fibroblasts prevent auto-inflammatory reactions under homeostatic conditions, whereas upon prolonged pressure-induced overload they serve a protective role against maladaptive remodeling.

702

Intraction of circulating immune cells with lung vascular cells in pulmonary hypertension

G. Parlato-Di Nolfo[1,2], K. Singh[1,2], J. Baldauf[1], C. Ruppert[3], S. Seegert[4], A. Krüger[4], W. Seeger[5], I. Manzini[2], R. T. Schermuly[1], A. Weiss[1]

[1]Pulmonary Pharmacotherapy, Department for internal Medicine, JLU Giessen, Germany, [2]Animal Physiology an Molecular Biomedicine, JLU Giessen, Germany, [3]Biobank UGMLC/DZL, JLU Giessen, Germany, [4]Department for Molecular Immunology, JLU Giessen, Germany, [5]Department for Internal Medicine, JLU Giessen, Germany

Pulmonary hypertension (PH) is a clinical condition characterized by an elevated blood pressure within the arteries of the lungs which is associated with physiological right-heart hypertrophy escalating into life-threatening mal-adaptations and ultimately, functional loss. In the case of pulmonary arterial hypertension (PAH), this form of hypertension specifically affects the small pulmonary arteries, which become occluded due to excessive proliferation of smooth muscle as well as endothelial cells and fibroblasts, combined with an invasion of several immune cells. As a result, the blood flow through the lung vasculature is dramatically decreased, while its pressure significantly increases. This causes an additional workload on the right heart, which will lead to heart failure if remain untreated.

The main purpose of this project is to study the cellular interaction between the side of lesion in PAH, represented by patient-derived precision-cut lung slices (PCLS), and cells of the immune system, more precisely, circulating (not resident) T cells via a co-culture model. The first objective is to determine optimal concentrations of pro-inflammatory stimulants, like IL-6 and TNF-alpha to analyze the cellular mechanisms and dynamics of the involved cell types. As a next step, we will investigate the role of Tec kinases in T cells in pulmonary hypertension (PH) in more detail. We will apply state-of-the art technologies combined with the co-culture of T cells and patient-derived PCLS: The focus will be on characterizing different T cell populations, their cytokine profile and their contribution to the pathogenesis of PH using flow cytometry as well as single RNA sequencing. In addition, inhibitors targeting T cell specific protein kinases will be applied to evaluate the potential of immune cell directed therapy. PCLS will be subjected to IF/IHC staining to visualize the status of pathological structural remodeling. These investigations will provide novel insights into T cell subset specific mediated mechanisms and Tec protein kinase function during the vascular remodeling process in PH.

703

Drug Repurposing for Pulmonary Hypertension Using Omics-Based Target Discovery

K. Singh[1], G. I. Parlato-Di Nolfo[1], J. Baldauf[1], W. Seger[2], I. Manzini[3], A. Weiß[1], M. Bartkuhn[4], R. Schermuly[1]
[1]Justus-Liebig-University, Pulmonary Pharmacotherapy, Gießen, Germany, [2]Justus-Liebig-University, Department for Internal Medicine, Gießen, Germany, [3]Justus-Liebig-University, Animal Physiology and Molecular Biomedicine, Gießen, Germany, [4]Justus-Liebig-University, Biomedical Informatics and System Medicine Science Unit for Basic and Clinical Medicine, Gießen, Germany

Drug repurposing, also known as drug repositioning or reprofiling, focuses on identifying new therapeutic uses for existing or investigational drugs. This strategy is especially valuable when conventional de novo drug development is too time-consuming, costly, or when there is an urgent need for effective treatment options. The aim is to rapidly pinpoint compounds with known safety profiles and therapeutic potential that may be effective for other indications.
The focus of this project conducted within the Drug Repurposing Hub is the identification of novel molecular targets for the treatment of pulmonary hypertension and right ventricular hypertrophy. By employing cutting-edge omics technologies, such as genomics, proteomics, transcriptomics and metabolomics, the project enables the comprehensive analysis of large datasets to uncover complex biological networks. This integrative approach supports the discovery of new biomarkers for early diagnosis, disease progression, and targeted therapeutic interventions.