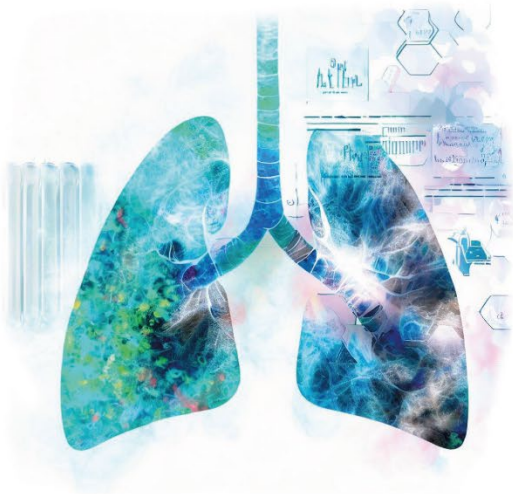

19 - 21 June 2024 | Groningen | The Netherlands

BRONCHITIS XI
International Symposium

Heterogeneity of lung disease in a changing environment

Bronchitis XI Book of Abstracts

Content

Preface

Dear Colleagues and Friends,

Welcome to Bronchitis XI! We are proud to welcome 150 scientists, students, health professionals and representatives of the biotech and pharmaceutical industry from 15 different countries all over the world, to join us for the 11th BRONCHITIS conference, that have been held every 5 years, since the sixties in Groningen.

BRONCHITIS-XI '"Heterogeneity of lung disease in a changing environment"' promises to be exciting and cutting edge. A large range of topics will be covered including climate change and chronic lung disease, epigenetics and environment in obstructive lung disease, novel medical devices for emphysema, Genetics of Asthma and COPD, AI in lung imaging of obstructive lung disease.
These topics will be presented and discussed by renowned researchers in these areas, and there will be an emphasis on discussion and interaction between all participants.

Ample space and time will also be dedicated to the 39 abstracts that we received, which will be presented by young researchers as an oral presentation or poster presentation.

During the social program, participants will have extensive time and opportunity to interact, share personal views and discuss possible collaborations.

We are grateful to the financial support from our sponsors, which made the organization of this conference possible.

We, at the Groningen Research Institute of Asthma and COPD (GRIAC), are excited and are very much looking forward to welcoming you on Wednesday 19th June in Groningen!

Yours Sincerely,

On behalf of the Bronchitis XI Organizing Committee,
Corry-Anke Brandsma, Department Pathology and Medical Biology (chair)
Irene Heijink, Department Pathology and Medical Biology
Machteld Hylkema, Department Pathology and Medical Biology
Dirk-Jan Slebos, Head department of Pulmonary Diseases and Tuberculosis
Maaike de Vries, Department Epidemiology

Faculty

Jonathan **Baker**, Department of Immunobiology, King's College London (UK)
Maarten **van den Berge**, Department of Pulmonary Diseases and Tuberculosis, University Medical Center Groningen (NL)
Guy **Brusselle**, Head of Clinic, Department of Respiratory Medicine, Ghent University Hospital, (BE)
Michael **Cho**, Channing Division of Network Medicine and Division of Pulmonary and Critical Care Medicine, Harvard Medical School (USA)
Rosa **Faner**, Biomedicine department, Immunology unit, University of Barcelona (ES)
Frits **Franssen**, Department of Respiratory Medicine at Maastricht UMC (NL)
Bram **van Ginneken**, Medical Image Analysis, Radboud UMC (NL)
Tillie **Hackett**, The University of British Columbia (CAN)
Huib **Kerstjens**, Pulmonary Diseases, University Medical Center Groningen (NL)
Gerard **Koppelman**, Department Head of Pediatric Pulmonology and Pediatric Allergy, University Medical Center Groningen (NL)
Mareike **Lehmann**, Institute for Lung Research, Helmholtz Munich (DE)
Marcus **Mall**, Charité Universitätsmedizin Berlin (DE)
Fernando D. **Martinez**, Director- Asthma & Airway Disease Research Center, University of Arizona (USA)
Erik **Melén**, Department of Clinical Sciences, Karolinska Institutet (S)
Barbro **Melgert**, Department of Molecular Pharmacology, University Medical Center Groningen (NL)
Alexander **Misharin**, Northwestern University, Feinberg School of Medicine, (USA)
Prof. dr. Wiro **Niessen**, Board of Directors, dean University Medical Center Groningen (NL)
Morten **Scheibye-Knudsen**, Department of Cellular and Molecular Medicine, University of Copenhagen (DK)
Pallav **Shah**, Royal Brompton Hospital and Imperial College, London (UK)
Dirk-Jan **Slebos**, Department Head, Pulmonary Medicine and Tuberculosis Pulmonary Diseases, University Medical Center Groningen (NL)
Lidwien **Smit**, IRAS, Utrecht University (NL)
Jakob **Stokholm**, University of Copenhagen (DK)
Giovanni **Viegi**, CNR Institute of Clinical Physiology (IFC), (IT)
Maaike **de Vries**, Department of Epidemiology, University Medical Center Groningen (NL)
Daniel **Weiss**, Department of Medicine, University of Vermont, (USA)

Program Bronchitis XI

Wednesday 19 June 2024

08.15-08.50 Registration and coffee

08.45-09.00 Opening
Corry-Anke Brandsma, chair (NL)
Wiro Niessen (NL)

Heterogeneity of Chronic Lung Disease

Moderator; Alen Faiz and Alaina Ammit

09.00-09.20 AI in lung imaging of obstructive lung disease
Bram van Ginneken (NL)
09.20-09.30 Discussion

09.30-09.50 Spatial Imaging of small airway remodeling in Asthma and COPD
Tillie Hackett (CAN)
09.50-10.00 Discussion

10.00-10.45 Coffee break

10.45-11.05 Exacerbations in Asthma and COPD
Maarten van den Berge (NL)
11.05-11.15 Discussion

11.15-11.35 Endotyping of Asthma and COPD
Rosa Faner (Spain)
11.35-11.45 Discussion

11.45-12.05 Genetics of Asthma and COPD
Michael Cho (USA)
12.05-12.15 Discussion

12.15-13.15 Lunch

Environmental changes with impact on lung disease

Moderators; Hermelijn Smits and Rosa Faner

13.15-13.35 Climate change and chronic lung disease
Giovanni Viegi (Italy)
13.35-13.45 Discussion

13.45-14.05 Air pollution and lung diseases
Lidwien Smit (NL)
14.05-14.15 Discussion

14.15-15.15 Poster session

15.15-15.45 ***Tea break***

15.45-16.05 Microplastics exposure and chronic lung disease
Barbro Melgert (NL)
16.05-16.15 Discussion

16.15-16.35 The airway and gut microbiome in lung disease
Jakob Stokholm (Denmark)
16.35-16.45 Discussion

16.45-17.05 Epigenetics and environment in obstructive lung disease
Erik Melén (Sweden)
17.05-17.15 Discussion

17.15-17.30 Short break

17.30-18.30 Industry Session

19.00 **Reception and Buffet**
Personnel restaurant ground floor UMCG

Thursday 20 June 2024

08.00-08.30 Registration and coffee

The Aging lung

Moderators; Roy Woldhuis and Ali Önder Yildirim

08.30-08.50 Single cell sequencing and ageing
Alexander Misharin (USA)
08.50-09.00 Discussion

09.00-09.20 Epigenetic clocks and their relevance to COPD
Maaike de Vries (NL)
09.20-09.30 Discussion

09.30-09.50 The application of deep learning and spatial approaches to define aging and senescence
Morten Scheibye-Knudsen (DK)
09.50-10.00 Discussion

10.00-10.30 ***Coffee break***

10.30-10.50 Impaired regeneration in the ageing lung
Mareike Lehmann (DE)
10.50-11.00 Discussion

11.00-11.20 The future of anti-ageing strategies in lung disease
Jonathan Baker (UK)
11.20-11.30 Discussion

Short communications from selected abstracts

Moderators; Daan Pouwels and Anne van der Does

11.30-11.45 Mimecan and a functional fragment hereof as a novel regenerative agent for COPD
Luke van der Koog (NL)

11.45-12.00 Interference with inflammatory responses in a mouse model of pollutant-aggravated allergic asthma
Joyceline De Volder (BE)

12.00-12.15 The Gut-Airways Microbiome axis in COPD
Julieta Viglino (Spain)

12.15-12.25 Lunch to go

12.25-12.30 Walk to main entrance UMCG

12.30 -20.30 Social Program

Friday 21 June 2024

08.30-09.00 Registration and coffee

Bronchitis

Moderators; Dirk-Jan Slebos and Irene Heijink

09.00-09.20 Interview with patient
Huib Kerstjens (NL)
09.20-09.30 Questions

09.30-09.50 Muco-obstructive lung disease
Marcus Mall (DE)
09.50-10.00 Discussion

10.00-10.20 Early origins of different forms of chronic lung disease
Fernando D. Martinez (USA)
10.20-10.30 Discussion

10.30-11.00 Coffee break

11.00-11.20 Bronchitis treatment; N2-cryospray ablation and rheoplasty
Pallav Shah (UK)
11.20-11.30 Discussion

Short communications from selected abstracts

Moderators; Elin Kersten and Marieke Duiverman

11.30-11.45 The protective role of testosterone and its precursors in asthma and asthma-related symptoms: cross-sectional findings from the Rotterdam Study
Sebastian Riemann (BE)

11.45-12.00 Endobronchial valve treatment improves diaphragm function in severe emphysema patients
Else ter Haar (NL).

12.00-12.15 Bronchitis XI group photo (Blauwe Patio)

12.15-13.15 Lunch

Innovative therapy

Moderators; Huib Kerstjens and Wim Timens

13.15-13.35 Personalized treatment and biologicals in asthma; current and future perspectives
Gerard Koppelman (NL)

13.35-13.45 Discussion

13.45-14.05 Personalized treatment and biologicals in COPD; current and future perspectives
Guy Brusselle (BE)

14.05-14.15 Discussion

14.15-14.35 Treatable traits in obstructive lung disease
Frits Franssen (NL)

14.35-14.45 Discussion

14.45-15.15 Tea break

15.15-15.35 Stem cell therapy for lung disease: where are we now?
Daniel Weiss (USA)

15.35-15.45 Discussion

15.45-16.05 Novel medical devices for emphysema
Dirk-Jan Slebos (NL)

16.05-16.15 Discussion

16.15-16.45 Awards ceremony and closure

16.45-17.15 Drinks and farewell (Blauwe Patio)

Short communications

Abstract no: 1

Mimecan and a functional fragment hereof as a novel regenerative agent for COPD

L. van der Koog[1,2], M.E. Woest[3], I.C. Gorter[3], M.L.K. Ngassie[2,4], Deepesh Dhakad[5], Y.S. Prakash[6] C.A. Brandsma[2,4], A.Ö. Yildirim[5,7], H.W. Frijlink[8], A. Nagelkerke[9], R. Gosens[1,2]
[1]Department of Molecular Pharmacology, Groningen Research Institute of Pharmacy, University of Groningen, Groningen, the Netherlands [2]GRIAC, Groningen Research Institute for Asthma and COPD, University Medical Center Groningen, Groningen, The Netherlands, [3]Aquilo BV, Groningen, The Netherlands [4]Department of Pathology and Medical Biology, University Medical Center Groningen, Groningen, the Netherlands, [5]Comprehensive Pneumology Center (CPC), Institute of Lung Health and Immunity (LHI), Helmholtz Zentrum München, Member of the German Center for Lung Research (DZL), Munich, Germany, [6]Department of Anasthesiology and Perioperative Medicine, Mayo Clinic, Rochester, Minnesota, United States [7]Institute of Experimental Pneumology, University Hospital, Ludwig-Maximilians University (LMU), Munich, Germany [8]Department of Pharmaceutical Technology and Biopharmacy, Groningen Research Institute of Pharmacy, University of Groningen, Groningen, the Netherlands [9]Department of Pharmaceutical Analysis, Groningen Research Institute of Pharmacy, University of Groningen, Groningen, the Netherlands.

Introduction: COPD is characterized by progressive airflow limitation and emphysema development, associated with enhanced tissue destruction and defective repair. Currently, there is no pharmacological treatment that reactivates lung repair. Here, we aimed to identify proteins within the secretome of lung fibroblasts that can promote regeneration in alveolar epithelial progenitors.
Methods: Proteomics analysis was conducted on MRC5 lung fibroblast secretomes. Lung organoids were generated by co-culturing epithelial progenitors ($CD31^-/CD45^-/Epcam^+$) obtained from mice or human COPD IV patients with lung fibroblasts in Matrigel. After 14 days, organoid number and size was determined, as was the number of differentiated alveolar organoids. Precision-cut lung slices (PCLS) were obtained from naive mice and treated with 2.5 µg/mL elastase for 16h to induce lung injury. Single-cell RNA-sequencing (scRNA-seq) was performed on mice lungs exposed to increasing periods of cigarette smoke. Immunostaining was performed to quantify mimecan expression in human lung tissue from non-, current, and ex-smokers.
Results: We found 41 distinct growth factors and cytokines within the MRC5 secretome. Next, we screened these for proteins possessing a signalling sequence and interaction with receptors expressed in alveolar epithelium, resulting in identification of 12 potential drug targets. These candidates were subsequently tested for regenerative potential in murine lung organoids. Among the tested ligands, mimecan (MC001) exhibited an exceptionally potent regenerative effect compared to the other recombinant proteins. Both MC001 and MC002, a functional fragment of mimecan, induced organoid formation in a concentration dependent manner in murine organoids, in the absence and presence of 5% cigarette smoke extract (CSE) or 0.5 ng/mL transforming growth factor-ß (TGF-ß). Furthermore, exposure to CSE and TGF-ß lowered the percentage of alveolar organoids, which was counteracted by the addition of MC001 or MC002. Treatment with MC001 or MC002 also improved organoid count from epithelial cells obtained from COPD IV patients. In PCLS, elastase treatment induced a 1.6-fold increase in mean linear intercept (MLI). Concurrent treatment with MC001 for 40h prevented this increase significantly. Furthermore, scRNA-seq revealed that extended cigarette smoke exposure leads to diminished mimecan expression in mice lungs, with fibroblasts being the primary endogenous source. Immunostaining showed

that mimecan expression was significantly reduced in the parenchyma and whole lung tissue of current and ex-smokers, compared to non-smoker controls.
Conclusion: Using a proteomics-guided drug discovery strategy based on the secretome of lung fibroblasts, we identified mimecan and a functional fragment hereof as novel regenerative agents for COPD.

Abstract no: 16

Interference with inflammatory responses in a mouse model of pollutant-aggravated allergic asthma

Joyceline De Volder[1], Annelies Bontinck[1], Valerie Haelterman[1], Louis Boon[2], Guy G Brusselle[1], Guy F Joos[1], Tania Maes[1]
[1]Department of Respiratory Medicine, Laboratory for Translational Research in Obstructive Pulmonary Diseases, Ghent University Hospital, Ghent, Belgium
[2]JJP Biologics, Warsaw, Poland

Introduction: Diesel exhaust particles (DEP) have been proven to aggravate asthma pathogenesis. We previously demonstrated that combined exposure to house dust mite (HDM) and DEP in mice increases both eosinophils and neutrophils in bronchoalveolar lavage fluid (BALF) compared to sole exposures and also results in higher levels of neutrophil-recruiting chemokines and neutrophil extracellular trap (NET) formation (PMID: 37105460). We therefore aimed to unravel whether neutrophils modulate the DEP-aggravated eosinophilic airway inflammation.
Material & methods: For the subacute pollutant-aggravated allergic asthma mouse model, isoflurane anesthetized female C57BL6 mice (8/group) were intranasally exposed to saline or the combination of 1 µg HDM and 25 µg DEP on days 1, 8 and 15. First, we monitored the airway inflammatory cell dynamics by analyses at multiple time points (24, 48 and 72 hours) after last exposure. To evaluate whether there is a role for neutrophils in inducing type 2 eosinophilic responses, we interfered with neutrophils and neutrophil elastase in our murine HDM+DEP model by intraperitoneal anti-Ly6G and sivelestat administration, respectively, on days 14, 15 and 16. Moreover, we evaluated whether administration of anti-IL-5 on days 8 and 15 would be effective in the mixed granulocytic phenotype of our subacute HDM+DEP model. For the interference experiments, mice were sacrificed 2 days after last HDM+DEP exposure. Flow cytometric analysis, immunohistochemistry, RT-qPCR and ELISA were used to evaluate airway inflammation.
Results: BALF neutrophils were highest at 24 hours after last HDM+DEP exposure and decreased at later time points, while BALF eosinophils tended to increase with time. This early neutrophilic response associated with high expression of neutrophil-attracting chemokines (CXCL1, 2 and 5) and NET formation. Neutrophils were significantly reduced -but not completely absent- after anti-Ly6G administration in BALF, lung and blood without affecting the eosinophilic inflammation upon HDM+DEP exposure. Sivelestat treatment tended to decrease BALF inflammation, including eosinophils, upon HDM+DEP exposure, but did not affect eosinophilic inflammation in lung tissue. The administration of anti-IL-5 antibodies significantly decreased eosinophilic responses upon subacute HDM+DEP exposure in BALF, lung and blood while BALF neutrophils remained present. Notably, both homeostatic and inflammatory eosinophils were affected by anti-IL-5 treatment.
Conclusion: Inhibition of IL-5 signalling -but not neutrophil interference- does significantly attenuate eosinophilic inflammation in a mouse model of mixed granulocytic asthma, elicited by air pollution exposure.

Abstract no: 35

The protective role of testosterone and its precursors in asthma and asthma-related symptoms: cross-sectional findings from the Rotterdam Study

Riemann S (1,2), Chaker L (3), Brusselle G (1,2,3)
(1) Department of Respiratory Medicine (Ghent University Hospital) - Ghent (Belgium)
(2) Department of Internal Medicine and Pediatrics (Ghent University) - Ghent (Belgium)
(3) Department of Epidemiology (Erasmus MC) - Rotterdam (Netherlands)

Background: Two thirds of adult patients with asthma are female, suggesting an immune modulatory effect of sex hormones in asthma. In a limited number of studies, testosterone has been linked to a protective effect against asthma.
Methods: The Rotterdam Study (RS) is a prospective population-based cohort study in Rotterdam, the Netherlands. Since 1990, 18926 participants have been enrolled in 4 longitudinal RS subcohorts in all of which asthma cases have been validated based on medical records. Serum levels of circulating sex steroids (testosterone and its precursors androstenedione, dehydroepiandrosterone(-sulphate) (DHEA(S)); and cortisol) have been measured in a subset of 4533 participants. Questionnaire-based respiratory symptoms were registered in a subset of 1729 participants.
Results: The total analysis included 485 participants with (mild-to-moderate) asthma and 4048 non-asthmatic controls (table 1, values displayed as mean). Oral corticosteroid use was reported in 12 (2,3%) and 36 (0,9%) of these participants respectively and was not found to impact these results.

	MEN			WOMEN		
	No asthma	Asthma	p	No Asthma	Asthma	p
N	1703	147		2345	338	
AGE (YEARS)	63.6	62.4		65.3	62.0	
TESTOSTERONE (NG/DL)	505.25	473.89	0.03	27.57	23.97	<0.001
DHEA (NMOL/L)	12.14	10.76	0.04	12.41	12.07	0.49
DHEAS (NMOL/L)	3185.15	2618.93	<0.001	2060.08	2060.68	0.99
ANDROSTENEDIONE (NG/DL)	95.89	88.81	0.05	77.38	75.37	0.39
CORTISOL (NMOL/L)	350.32	353.46	0.73	367.02	359.13	0.29

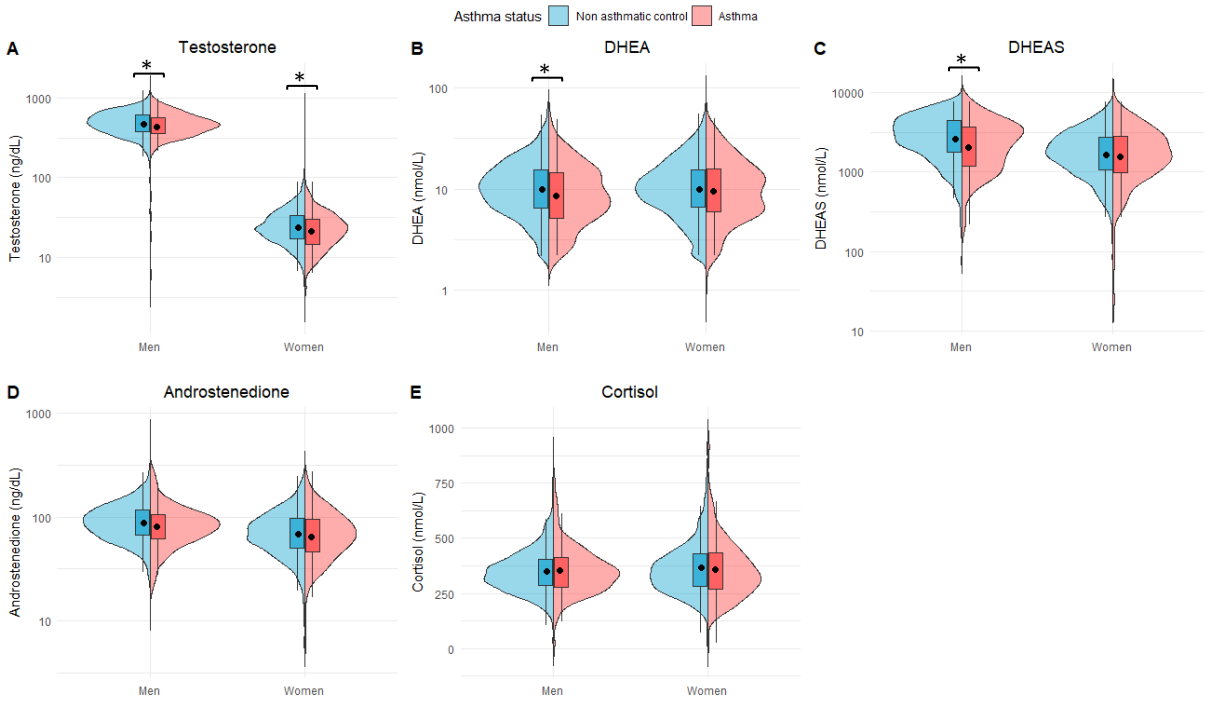

Figure 1: sex-stratified comparison of testosterone (A), DHEA (B), DHEAS (C), androstenedione (D), and cortisol (E) in participants with (red) and without (blue) asthma

Testosterone was significantly decreased in asthmatic females compared to non-asthmatic females (p<0.001), whereas levels of androstenedione, DHEA, DHEAS and cortisol were not significantly different between these groups. In men, there was a significantly lower level of testosterone precursors DHEA (p=0.04) and DHEAS (p<0.001) in asthmatic compared with non-asthmatic subjects, and of testosterone after adjustment for age. Cortisol levels were not significantly different between asthmatics and non-asthmatics.

A subanalysis was performed in 219 asthmatics and 1510 controls with available data on respiratory symptoms (cough, dyspnea). This revealed an association between low testosterone levels and current symptoms in asthmatic females, and between both low DHEA levels and androstenedione and symptoms in asthmatic males.

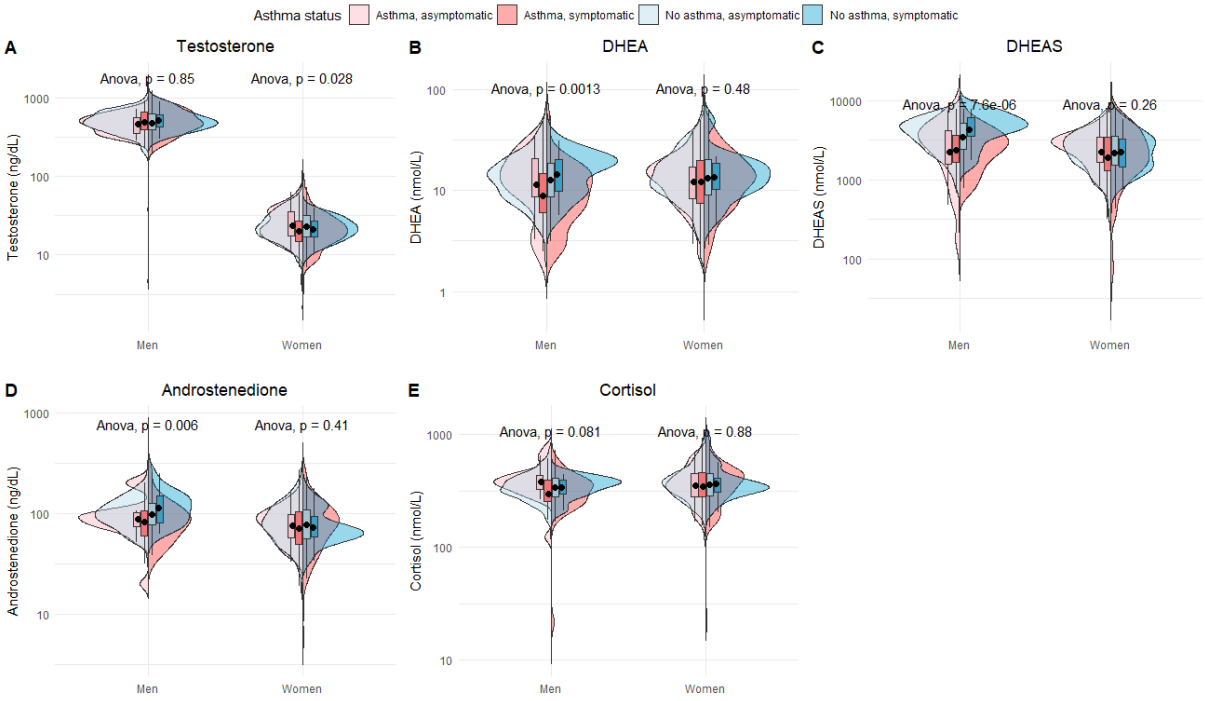

Figure 2: sex-stratified comparison of testosterone (A), DHEA (B), DHEAS (C), androstenedione (D), and cortisol (E), substratified by presence or absence of respiratory symptoms

In a large population-based study we demonstrate decreased levels of testosterone in patients with asthma (both sexes) and testosterone precursors in males. Our findings suggest a protective effect of testosterone with regards to asthma and asthma related symptoms, encouraging further mechanistic studies. Currently, we are investigating complementary urine metabolomics in the Rotterdam Study.

Abstract no: 14

Endobronchial valve treatment improves diaphragm function in severe emphysema patients

ter Haar EAMD[1,2], Slebos DJ[1,2], Augustijn SWS[1], Dijkstra LJ[1], Hartman JE[1,2]

[1.]Department of Pulmonary Diseases, University of Groningen, University Medical Centre Groningen, Groningen, The Netherlands
[2.]Groningen Research Institute for Asthma and COPD, University of Groningen, University Medical Centre Groningen, Groningen, The Netherlands

Introduction: Diaphragm dysfunction is common in severe emphysema patients, primarily due to hyperinflation, placing the diaphragm at a mechanical disadvantage. Endobronchial valve (EBV) treatment is a bronchoscopic treatment modality to reduce hyperinflation in this patient population. Our aim was to investigate the effect of EBV treatment on diaphragm function.
Material & methods: We performed a prospective study in which 25 patients were included. Diaphragm function was measured at baseline and 6 weeks after EBV treatment by the following tests: maximal inspiratory pressure (MIP), maximal expiratory pressure (MEP), ultrasound to assess diaphragm motion during tidal breathing and maximal inspiration, and surface electromyography to quantify diaphragm activity during tidal breathing, maximal inspiration and sniff manoeuvre. The ratio of MIP and diaphragm activity, reflecting the amount of output in kilopascal (kPa) per unit of input in microvolt (µV), was used to assess diaphragm efficiency. Paired-Samples T Test or Wilcoxon Signed Ranks Test in case of non-normal distribution was performed to test whether there were differences between baseline and 6 week follow-up measurements.
Results: Patients had a mean age of 64 ± 7 years and were predominantly female (76%). Forced expiratory volume in 1 second (+0.24Liter, SD 0.17, P<0.001) increased significantly, whereas residual volume (-0.85Liter, SD 0.49, P<0.001), and the Modified Medical Research Council Dyspnoea Scale (-1point, range -2–0, P<0.001) decreased significantly 6 weeks post EBV treatment. MIP (+0.6kPa, range -1.2–2.8, P=0.001) and MEP (+0.4kPa, range -1.6–4.7, P=0.035) increased significantly 6 weeks post EBV treatment. Diaphragm motion during tidal breathing (-3.3mm, range -24.7–14.6, P=0.023), as well as the ratio tidal breathing/maximal inspiration (-0.12, range -0.45–0.19, P=0.001) decreased significantly. Moreover, diaphragm activity for tidal breathing (-1.6µV, range -8.8–5.8, P=0.007), maximal inspiration (-3.3µV, range -42.3–16.0, P=0.008), and sniff manoeuvre (-5.9µV, range -42.2–24.2, P=0.045) significantly decreased. At last, diaphragm efficiency (+0.07kPa/µV, range -0.40–0.37, P=0.001) improved significantly as well.
Conclusions: Our results show that diaphragm function significantly improves after EBV treatment in severe emphysema patients. The decrease in diaphragm activity suggests less respiratory effort by the patient post treatment. This could be one of the underlying mechanisms contributing to reduced dyspnoea severity following EBV treatment, as an elevated neural drive to the diaphragm, leading to increased activity, enhances dyspnoea sensation.

Poster presentations overview 19 June 2024

POSTER BOARD	ABSTR NO	NAME	TITLE
Exposures ***Moderators: Hermelijn Smits and Lidwien Smit***			
1	10	P.A. Saputra	Cigarette Smoke Exposure Activates the cGAS-Sting Pathway and Downstream Interferon Signaling in Mice
2	31	G.F. Vasse	Polyvinylchloride and polypropylene microplastics affect outgrowth of murine lung organoids
3	17	R. Fuentes-Mateos	Chronic exposure to cigarette smoke distorts epithelial progenitor cell differentiation
4	18	S. Qian	TRAPping the effects of smoking: the regulation of ACP5 expression in lung tissue
5	19	V. Violi	Cigarette smoke affects differentiation potential of lung epithelial progenitors by altering the WNT pathway
6	21	A. Faiz	Thymic Stromal Lymphopoietin (TSLP) Expression Is Restricted To Resting Basal Cells And Is Low In Current Smokers
Exposures ***Moderators: Ali Önder Yildirim and Anne van der Does***			
7	23	A. Dehghani	Pregnancy exacerbates neutrophil responses in murine lungs after cigarette smoke exposure
8	34	A. Dehghani	Th2-biased immune response in offspring induced by maternal smoking exposure
9	25	R. Elferink	The lung fibroblast secretome supports alveolar organoid formation in the presence of cigarette smoke extract
10	5	R. Post	Nylon microplastics induce CXCL2 and CCL3 secretion in murine alveolar macrophages
11	16	J. De Volder	Interference with inflammatory responses in a mouse model of pollutant-aggravated allergic asthma
Ageing ***Moderators: Mareike Lehmann and Jonathan Baker***			
12	20	R. Woldhuis	Senescence-induced and COPD-derived fibroblasts hamper human alveolar organoid formation
13	26	J. Viglino	Epigenetic age acceleration and severity of airflow limitation on blood and lung tissue in COPD patients
14	15	J. Viglino	The Gut-Airways Microbiome axis in COPD
15	28	N.J. Bekker	Differential miRNA expression in SEO-COPD is associated with altered lysosome, vesicular transport and ECM-related pathways
16	30	J. Tjepkema	Altered TGF-β Induced Repair Response of Senescent Lung Fibroblasts

POSTER BOARD	ABSTR NO	NAME	TITLE
Asthma ***Moderators: Daan Pouwels and Elin Kersten***			
17	6	H. Wen	The nose mirrors asthma-associated gene expression profiles in the lower airway
18	8	T. Karp	Nasal gene expression in T2-low asthma
19	9	I. Mommers	Identifying comorbidities associated with switching patterns of asthma treatment: a real-world drug utilization study using the parametric g-formula
20	24	C.S. Koster	Sensory Neurons Support Mast Cell Differentiation In An In Vitro Co-culture Model
21	32	T. Kole	Nasal periostin expression is higher in asthma patients with versus without persistent airflow limitation
22	36	J. Vlasma	Interleukin-13 represses epithelial cell differentiation in primary bronchial epithelial cells from patients with asthma
23	39	H.J.L. Koefoed	Serum levels and genetic variants of CC16: Associations with asthma and lung function development in childhood and adolescence
24	35	S. Riemann	The protective role of testosterone and its precursors in asthma and asthma-related symptoms: cross-sectional findings from the Rotterdam Study
ECM ***Moderators: Tillie Hackett and Reinoud Gosens***			
25	3	Y. Liu	In lung fibrosis osteoprotegerin, fibulin-1 and latent TGFβ binding protein 1 form a complex in the extracellular environment
26	11	Y.W. Fan	Collagen type VI α chain 1 levels were higher in the lung parenchyma of critically ill patients with persistent Acute Respiratory Distress Syndrome
27	27	L. Wang	Improving the 3D microenvironment of mesenchymal stromal cells for lung tissue repair in COPD
28	29	M.M. Joglekar	Chronic obstructive pulmonary disease extracellular matrix promotes tissue repair responses in fibroblasts

POSTER BOARD	ABSTR NO	NAME	TITLE
COPD ***Moderators: Rosa Faner and Huib Kerstjens***			
29	4	P. Pineda Villegas	MEOX2-Mediated Epigenetic Imbalance of PRC2-EZH2 Versus TRX-SNF5 Modulates Severity in COPD and Progression to Lung Malignancy
30	14	E.A.M.D. ter Haar	Endobronchial valve treatment improves diaphragm function in severe emphysema patients
31	12	E. Geervliet	Breathing Life into Innovation: Developing an Advanced COPD Lung Model for Therapeutic Discovery
32	13	D.F. Nugraha	Bone Morphogenetic Protein 6 Deficiency Distorts Wnt and Oxidative Stress Signaling in Lung Tissue
33	22	R. Wadhwa	Inhibition of succinate dehydrogenase reduces oxidative stress in lungs and ameliorates the pathogenesis of COPD in mice
COPD ***Moderators: Barbro Melgert and Janette Burgess***			
34	33	W. Krimsky	Evaluation of Airway Mucosal Biopsy in Symptomatic Chronic Bronchitis Patients with Preserved Lung Function
35	37	S. Geirnaert	Increased presence of ferroptosis features in lungs of patients with Chronic Obstructive Pulmonary Disease (COPD)
36	40	A.L. Manzano-Covarrubias	Respiratory viral infections cause lipid peroxidation in lung epithelial cells
37	7	J. Fang	Extracellular vesicles from bronchoalveolar lavage fluid provide insights into the ICS treatment response in COPD
38	41	D. Li	TNF signaling plays a role in lipopolysaccharide (LPS) induced lung epithelial repair response

Poster presentation

Abstract no: 3

In lung fibrosis osteoprotegerin, fibulin-1 and latent TGFβ binding protein 1 form a complex in the extracellular environment

Y Liu[1,2], H.Habibie[2,3,4], Theo Borghuis[1,2], Gang Liu[5], Philip M Hansbro[5] ,B.N.Melgert[2,3], J.K. Burgess[1,2]

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Rationale: Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive lung disease with an unclear cause and no cure. Osteoprotegerin (OPG), a well-known regulator of bone extracellular matrix (ECM), is increased in pulmonary fibrosis and is associated with lower lung function and IPF progression. Fibulin-1c (Fbln1c), a secreted glycoprotein, binds to latent TGF-β–binding protein 1 (LTBP1) inducing activation of TGF-β, thereby contributing to fibrogenesis. Preliminary data have shown a positive correlation between the expression of OPG and Fbln1c in IPF, but if and how they interact is unclear. This study aimed to investigate interactions between OPG/Fbln1c/LTBP1 in lung fibrosis.
Methods: Human lung tissue was obtained from patients with IPF or patients undergoing surgical resection for carcinoma and was used for isolation of lung fibroblasts, immunofluorescence analysis, and proximity ligation assay (PLA) of OPG, Fbln1c, and LTBP1. OPG and Fbln1c were also silenced using small interfering RNAs in primary human lung fibroblasts. Levels of mRNA and protein expression of OPG and Fbln1c were quantified by quantitative PCR, western blot, and ELISA.
Results and conclusions: OPG, Fbln1, and LTBP1 were detected in lung tissue using immunofluorescence, with apparent overlap between the three proteins in both IPF and control tissues. Subsequently, PLA analyses showed close proximity of OPG to Fbln1 and also Fbln1 and LTBP1 but not OPG and LTBP1, particularly in the interstitial regions of both control and IPF lung tissues. In human lung fibroblasts silencing of OPG mRNA and protein expression did not impact mRNA levels of Fbln1c and similarly, silencing of Fbln1c did not alter OPG mRNA levels. This suggests that neither OPG nor Fbln1c were dependent on each other for the regulation of their expression. OPG mRNA expression levels increased in the presence of TGFβ in fibroblasts derived from both Fbln1c knockout (KO) and control mice, confirming this independent regulation. However, OPG protein deposition was reduced in lung tissues of Fbln1c KO mice compared to controls. In conclusion, OPG, Fbln1 and LTBP1 may form a complex in the extracellular environment that plays an important regulatory role during the development of lung fibrosis. Interfering with this complex may be a new therapeutic avenue for IPF treatment.

Abstract no: 4

MEOX2-Mediated Epigenetic Imbalance of PRC2-EZH2 Versus TRX-SNF5 Modulates Severity in COPD and Progression to Lung Malignancy

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Introduction: Impaired lung function is predictive of mortality and key diagnostic for chronic obstructive pulmonary disease (COPD). COPD is also a significant risk factor for lung cancer and both diseases share a common risk factor: cigarette smoke (CS). Susceptibility to CS is associated to genetic mutations[1] and epigenetic changes as chromatin remodeling [3]. Polycomb and Trithorax[4] are epigenetic regulators of histone modifications (H3K27me3/H3K4me3), promoting chromatin remodelling and aberrant expression of epithelial repair genes implicated in COPD and lung cancer as CHD1, the gene encoding E-cadherin[10,11]. MEOX2 also modulates histone marks and genes of epithelial repair. Nevertheless, its still un known if and how MEOX2 regulates genes of epithelial repair in COPD and lung cancer. Our aim was study the association of MEOX2 to mortality and E-cadherin expression in lung cancer patients with and without COPD and assess chromatin remodelling *in vitro* in airway epithelial cells.
Materials and methods: RNA-seq data and overall survival(OS) were analysed in a cohort of lung cancer patients with (FEV1/FVC<0.7) or without(FEV1/FVC≥0.7) COPD. Airway epithelial cells (AEC) were derived from tracheobronchial tissue of donor lungs (control) or transplanted lungs from COPD patients (n=3/group). Additionally, human alveolar lung carcinoma A549 and non-cancerous human bronchial epithelial BEAS-2B cells were used. MEOX2 expression was silenced using siRNA and effects on Polycomb (EZH2-H3K27me3), Trithorax (SNF5-H3k4me3) and E-cadherin were assessed in the presence and absence of CS extract (CSE, 24h).
Results: The RNA-seq indicates that MEOX2 negatively correlates with both, OS and CDH1 expression, only in lung cancer patients with COPD but positively correlated with packyears. In vitro, we observed a trend towards higher MEOX2 in AECs from COPD patients compared to control. CSE tended to increase H3K27me3 and EZH2 and reduce SNF5 in AECs from COPD patients, without alterations in control. Preliminary data suggest that MEOX2 silencing in AECs from COPD patients and A549 cells results in a reduction in EZH2/H3K27me3 and increase in E-cadherin levels, which was not observed in control and BEAS-2B cells.
Conclusions: Our results suggest that CSE may induce MEOX2 expression, particularly in airway epithelium from COPD patients, resulting in gain of H3K27me3 and a reduction in E-cadherin, and that in vivo this may be related to higher mortality in patients with lung cancer and COPD, contributing to impaired epithelial repair. The molecular mechanism still to be confirmed, but we propose that MEOX2 modifies the epigenetic landscape upon smoking modulating the Polycomb and Trithorax (H3K27me3/H3K4me3) balance.

Abstract no: 5

Nylon microplastics induce CXCL2 and CCL3 secretion in murine alveolar macrophages

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Introduction: Humans are nowadays continuously exposed to microplastics through food, water and air. Airborne microplastics can be inhaled and have been found deep in the lungs, but possible harmful effects of ambient microplastic exposure on lung health remain largely unknown. Our recent research revealed that nylon (polyamide 6,6) microplastics are detrimental to developing airway organoids and that chemicals leaching from nylon are responsible for this effect. Yet, the first line of defense against pollutants in the lung, the alveolar macrophages, are not represented in this model. The objective of the present study was therefore to investigate whether nylon microplastics also impact the viability and behavior of murine alveolar macrophages.
Materials & methods: Murine fetal alveolar-like macrophages and primary adult alveolar macrophages were exposed to different size ranges (1-5 µm or 5-10 µm) and concentrations (1, 10 or 100 µg/mL) of nylon microplastics or to nylon leachate. In fetal alveolar-like macrophages, cytotoxicity was assessed using an MTS assay and an exploratory array for 40 cytokines and chemokines was employed to screen for differentially expressed cytokines and chemokines in culture supernatant. Differential secretion of particular cytokines and chemokines by nylon microplastics was subsequently verified in primary adult alveolar macrophages using ELISA analyses. Production of reactive oxygen species (ROS) was assessed using fluorescence microscopy.
Results: Nylon microplastics were phagocytosed by both fetal alveolar-like macrophages and adult primary alveolar macrophages in a dose-dependent manner. In fetal alveolar-like macrophages, the highest dose of 100 µg/mL was cytotoxic. Cytokine array analysis revealed higher secretion of especially CXCL2 and CCL3, and to a lesser extent CCL2 and IL-1α, by macrophages treated with nylon microplastics. The secretion of classic pro-inflammatory cytokines and chemokines, such as TNFα, IL-1β and IL-6 was not induced by nylon microplastics. In adult primary alveolar macrophages, dose-dependent CXCL2 and CCL3 secretion as well as ROS production was observed. The effects on chemokine secretion were shown to be particle-based, as nylon leachate did not have similar effects.
Conclusions: Our findings indicate that nylon microplastics can induce cytotoxicity, ROS production and the secretion of a limited number of chemokines in murine alveolar macrophages. The lack of impact of nylon leachate suggests that the higher secretion of CXCL2 and CCL3 is particle-based and linked to phagocytosis. Furthermore, the chemoattractant properties of CXCL2 and CCL3 imply that other circulating immune cells are also important for a coordinated response to microplastics *in vivo*.

Abstract no: 6

The nose mirrors asthma-associated gene expression profiles in the lower airway

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Introduction: Transcriptomic analysis of bronchial brush samples has provided new insights into the mechanisms of different clinically relevant asthma endotypes. However, the invasive nature limits its use. Based on the "united airways" hypothesis, the upper airways (nose) have been put forward as a non-invasive proxy for the lower airways, offering a potential solution to overcome this issue for clinical and research purposes. We aim to assess if and to what extent the nose reflects the differential expressed genes (DEGs) in lower airways between asthma patients and healthy controls.
Methods: We analyzed both ARMS bronchial and nasal brush RNA-seq data, which included 26 asthmatic patients (mean age 54 years, mean pre-bronchodilator forced expiratory volume in 1s (FEV_1) 2.93 L) and 28 healthy controls (mean age 55 years, mean pre-bronchodilator FEV_1 3.74 L). DEG analyses were performed using EdgeR (version 4.0.2) to detect consistent DEGs in bronchial and nasal brushes, comparing asthma and healthy controls. The ARMS DEGs results were subsequently replicated in an independent cohort of 427 nasal brush samples (i.e., the ATLANTIS study). In DEGs analyses, Low-expressed genes were filtered out using the edgeR's built-in filterByExpr function. DEG analyses were conducted using quasi-likelihood negative binomial generalized log-linear models adjusting for covariables such as age and sex for the ARMS cohort and smoking status as the additional covariable for the ATLANTIS cohort. The quasi-likelihood (QL) F-test was applied to test the contrast between asthmatics and healthy controls. The Benjamini-Hochberg false discovery rate (FDR) method was used for multiple testing corrections. For bronchial brushes, genes with FDR < 0.05 were deemed as differentially expressed, while for nasal brushes, genes with P-value < 0.05 were considered as differentially expressed.
Results: Comparing asthmatic groups to healthy control groups, 51 DEGs were identified in the ARMS Bronchial brush transcriptome dataset, comprising 40 up-regulated and 11 down-regulated genes. By intersecting these DEGs with those identified in nasal brushes bulk RNA-seq data, seven genes (e.g., CLCA1, FETUB, CST1, NTRK2, TPSAB1, CDH26, DHX35) were associated with asthma in both ARMS bronchial and nasal brush samples and also replicated in the ATLANTIS nasal brushes cohort.
Discussions: Comparing asthma and healthy controls, we found seven specific up-regulated DEGs in nasal brushes reflecting those in the airways. Utilizing the nasal transcriptome as a proxy can enhance the efficiency and safety of asthma research for patients.

Abstract no: 7

Extracellular vesicles from bronchoalveolar lavage fluid provide insights into the ICS treatment response in COPD

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Introduction: Chronic obstructive pulmonary disease (COPD) remains a major global health challenge, and there is increasing interest in using inhaled corticosteroids (ICS) as a treatment option. ICS treatment helps reduce airway inflammation as well as the frequency and severity of COPD exacerbations, contributing to the alleviation of symptoms and improving overall quality of life in COPD patients. However, not all COPD patients benefit from ICS treatment, and some patients may experience little to no improvement in symptoms or lung function. Recent research indicates the involvement of extracellular vesicles (EVs) in various lung diseases. In addition, circulating plasma-derived EVs have been shown to have great potential to be used for the diagnosis, prognosis and therapeutics of lung diseases such as COPD.
Aim: To investigate the effect of ICS treatment on the protein content of EVs isolated from bronchoalveolar lavage fluid (BALF) in patients with COPD.
Methods: BALF-derived EVs were obtained from 58 COPD patients of the Groningen Leiden Universities Corticosteroids in Obstructive Lung Disease (GLUCOLD) study, using ICS or placebo during 6-month follow-up. Samples were obtained at baseline and after 6 months treatment with ICS or placebo. Protein levels were determined using discovery-based proteomics (using label-free quantification) for relative protein concentrations.
Results: Proteomics analysis showed a higher abundance of 31 proteins and a lower abundance of 20 proteins in EVs from ICS-treated patients (n=15) compared to the placebo group (n=7). GO pathway analysis indicated that these proteins primarily contribute to 'modulation of the immune response', 'inflammation', and 'apoptotic cell clearance'. Ingenuity pathway analysis predicted ICS treatment to decrease the inflammatory responses, including neutrophil degranulation, which was previously reported to be activated in patients with COPD. Gene Set Enrichment Analysis (GSEA) unveiled that ICS treatment appeared to 'mitigate oxidative stress', 'enhance reactive oxygen species (ROS) clearance', and 'activate the growth factor signaling pathway', possibly explaining the ICS treatment-induced lung function improvement, previously described for this cohort. Interestingly, we also observed an enhancement in the negative regulation of coagulation in the context of ICS therapy. These results shed light on the multifaceted effects of ICS treatment on COPD pathophysiology, highlighting the intricate interplay between EVs, cellular pathways, and therapeutic responses in COPD treatment.
Conclusion: BALF-derived EVs well reflect the anti-inflammatory effect of ICS use and may serve as a diagnostic and predictive biomarker in COPD.

Abstract no: 8

Nasal gene expression in T2-low asthma

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Introduction: Nasal gene expression in T2-low asthma
characterised by absence of T2 inflammation and resistance to inhaled corticosteroids with unknown molecular mechanisms and limited treatment options. Our prior work demonstrated altered nasal brush transcriptome in asthma reflecting disease-relevant biology. This study aims to explore nasal gene expression related to T2-low asthma in ATLANTIS study[1].
Methods: We compared nasal RNA-seq data from 82 T2-low and 63 T2-high (male 24% and 46%; FEV_1 %pred 89 and 80; BMI: 26 and 26 respectively) asthma patients to 57 healthy controls. T2-low asthma was defined as blood eosinophils (BE) < $0.15*10^9$/L; FeNO < 25 ppb and T2-high as BE > $0.3*10^9$/L; FeNO > 25 ppb. The remaining 194 patients were classified as undetermined and not included in the current analysis. CIBERSORTx cellular deconvolution algorithm was used to predict cell types in bulk RNA-seq samples.
Results: T2-low asthma patients were more often female and allergic and had more small airways disease compared to healthy controls (male 24% vs 47%, p = 0.008; positive PHADRES 72% vs 42%, p = 0.002; RV/TLC %pred: 98 vs 89, p = 0.014; FEF_{50} %pred: 77 vs 100, p < 0. 001). Although strong gene expression differences were found in T2-high asthma, T2-low asthma showed no genome-wide differentially expressed genes compared to healthy controls. We found higher predicted club cell proportions in T2-low asthma compared to controls (median [IQR]: 0.03 [0, 0.12] vs 0 [0, 0.04], FDR = 0.017). Weighted Gene Coexpression Network Analysis identified two gene modules linked to T2-low asthma, involved in T-cell immunity and ribosomal RNA biology, with the HLA-DPB1 and RPL23 being the hub genes.
Conclusion: Although we did not detect genome-wide significant gene expression differences in T2-low asthma, we did observe higher club cell proportions and suggestive evidence of T-cell mediated immune responses, possibly contributing to T2-low asthma pathogenesis.
Literature:
1.Postma DS, Brightling C, Baldi S, *et al.* Exploring the relevance and extent of small airways dysfunction in asthma (ATLANTIS): baseline data from a prospective cohort study. The Lancet Respiratory Medicine 2019;**7**(5):402–16.

Abstract no: 9

Identifying comorbidities associated with switching patterns of asthma treatment: a real-world drug utilization study using the parametric g-formula

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Introduction: Comorbidities can aggravate asthma, influencing treatment needed to control symptoms, and leading to under- or overtreatment when causing asthma-like symptoms. However, little is known about how the presence of comorbidities affects asthma treatment trajectories in clinical practice. This study aims to investigate whether the parametric g-formula can approximate observed asthma treatment trajectories, and predict subgroup differences within switching patterns of treatment for asthma.
Materials and methods: This retrospective inception cohort study used the IADB.nl community pharmacy dispensing database. Individuals aged 16 to 45, who initiated inhaled asthma medication between 1994 and 2021 in the Netherlands were included. Switching is defined as a change of treatment step. Parametric micro-simulations using the g-formula predicted the association of age, sex, arthritis, atopic diseases, cardiovascular diseases, diabetes, use of immunosuppressants, mental health problems, gastroesophageal reflux disease, and thyroid disease, separately, with switching patterns within two years after initiation.
Results: The g-formula predicted 67.5% out of 24 506 individuals to switch, compared to 68.2% observed in the real data. Among switchers, the time until the first switch was 8.7 months (SD: 5.5) predicted vs. 8.4 (SD: 5.5) observed, and the average number of switches was 2.2 (SD: 1.3) predicted vs. 2.1 (SD: 1.3) observed. Switchers aged 45 years switched earlier compared to 16-year-olds (8.7 vs. 9.0 months; p=0.04) and more often (2.3 vs. 2.1 times; p<0.01). Fewer individuals with atopic diseases or mental health problems switched (both 66 vs 68%; p=<0.01), and after longer treatment duration (both 9.0 vs. 8.8 months; p=0.03 and p=0.04, respectively), compared to those without. Individuals with gastroesophageal reflux disease switched later compared to those without (9.1 vs. 8.8 months; p<0.01). For other subgroups, no evidence was found for differences in switching patterns after initiation.
Conclusion: The parametric g-formula closely approximated the empirical distribution of treatment trajectories in general and within subgroups. It is best used when analysing dynamic processes, or when interested in full trajectories instead of only one point in time. While comorbidities can significantly impact the burden of asthma, their individual impact on differential treatment switching patterns after initiation seems limited. This might indicate that in clinical practice, comorbidities are either possibly not sufficiently taken into account when treating and monitoring asthma patients, or treatment trajectory alteration in response to comorbidities is not sufficiently uniform among treating physicians.

Abstract no: 10

Cigarette Smoke Exposure Activates the cGAS-Sting Pathway and Downstream Interferon Signaling in Mice

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Introduction: Chronic Obstructive Pulmonary Disease (COPD) is the third cause of death and the sixth cause of disability for all ages globally. It is widely known that environmental insults, such as cigarette smoke, are the main risk factors of COPD development. However, the underlying molecular mechanism is still not fully understood.
Several studies show that interferon (IFN) signaling is defective in COPD. However, there is still little information on how IFN signaling become disrupted after cigarette smoke exposure. cGAS-STING pathway is one of several pathways that can activate Interferon signaling.
The present study aimed to investigate the effect of smoke exposure on IFN pathways and it potential activators pathways using a multi-omics approach.
Materials & methods: Mice were exposed to smoke (n=8) or to air (n=8) twice a day for six weeks. 16 hours after the last exposure, mice were sacrificed, lungs were collected and $CD31^-/CD45^-$/Epcam+ epithelial cells and macrophages were isolated. The freshly isolated epithelial cells were subsequently used for ATAC-sequencing, RNA-sequencing, and proteomics assays while macrophages were used for RNA-sequencing. The omics data analyses were performed using R (version 4.3.2) in RStudio (version 2023.12.1) with *DESeq2* and *Fgsea* packages.
Results: Pathway enrichment analysis of the RNA-Seq data showed that the *Reactome DDX58/IFIH1-mediated induction of interferon-alpha/beta* was significantly enriched, with a normalized enrichment score of 1.8 (p.adj: 0.017). Intriguingly, no IFN mRNA transcripts were observed in both the RNA-Seq or in the proteomics data from epithelial cells and IFNs are not differentially expressed in macrophages. Further analysis of IFN pathway member genes showed significantly higher expression of *Zbp1* (3.6 $\log_2$ fold change (lfc), p.adj: 9.5×10^{-33}), and *Sting1* (0.768 lfc, p.adj: 8.7×10^{-9}) in CS-exposed cells compared to control cells . Both Zbp1 and Sting1 are members of cGAS-STING signalling pathway, with Zbp1 being known to be overexpressed after the activation of the pathway. Interestingly, the proteome analysis revealed that Sting1 expression was significantly higher by 0.96 lfc (p.adj: 0.002) in CS-exposed epithelial cells compared to control epithelial cells.
Conclusion: In conclusion, our data show that smoke exposure leads to more gene and protein expression of components of the cGAS-STING pathway. This could explain the elevated IFN signalling observed in the epithelial cells in the absence of IFN gene expression. Follow-up studies will investigate if the cGAS-STING pathway is the key players that disrupt the IFN pathway in epithelial cells after smoke exposure.

Abstract no: 11

Collagen type VI α chain 1 levels were higher in the lung parenchyma of critically ill patients with persistent Acute Respiratory Distress Syndrome

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Introduction: Acute respiratory distress syndrome (ARDS), characterized by diffuse alveolar damage and reduced lung diffusion, is associated with high morbidity and mortality. Whereas injury to the alveolo-capillary barrier plays a main role in initiating pulmonary injury in ARDS, remodelling of the extracellular matrix (ECM), which is pivotal to tissue repair and organ recovery may play a large role in persistent ARDS. Collagen type VI (ColVI), which establishes a link between basement membrane and interstitial matrix, was previously shown to be increased in the respiratory diseases. In this study, we investigated structural changes, particularly focusing on ColVI,in the parenchymal tissue of ARDS patients.
Material & methods: Paraffin embedded lung sections collected during autopsy or following lung transplantation were selected from ARDS patients (N=26) admitted to the Intensive Care Unit (ICU) of the University Medical Center Groningen between 2010-2020. Sections were divided into 3 groups based on ARDS duration (group 1<7 days, group 2 7-14 days, group 3 >14 days). Sections were stained with Haematoxylin & Eosin (HE), Masson's trichrome (MT), and immunohistochemically with Collagen type VI α chain 1 (ColVIa1). Ten independent observers, blinded to the grouping, scored the degree of ColVIα1 present in the sections. Data are reported as the median and interquartile ranges.
Results: No differences were observed in the age range among the patient groups: group 1 (age 55 [45-60] years, N=9), group 2 (age 57 [50.5-67.5] years, N=7), and group 3 (age 47 [34.5-60.5] years, N=10). The cause of ARDS was of pulmonary origin in 20 (76.9%) patients.
HE and MT staining revealed exsudative changes, including intra-alveolar oedema and inflammatory cell infiltration, predominantly observed in the first 14 days. Furthermore, fibrotic alterations, characterized by ECM deposition within alveolar septa, were predominantly evident in patients from groups 2 and 3. ColVIa1 was present throughout the alveolar walls in all patients, with extensive concentrated ColVIa1 bundles observed particularly in group 3. Visual semi-quantitative scoring revealed a tendency toward a greater amount of ColVIα1 present in parenchyma in group 3 (2.4 [2.2, 2.7]), compared to groups 1 and 2 (2.1 [1.6-2.6] and 1.9 [1.7-2.7], respectively).
Conclusions: Increased collagen deposition and higher levels of ColVIa1 were observed in the alveolar tissue of patients with persistent ARDS (group 3). This increased Col VI deposition, along with other ECM molecules, within the alveolar wall may impact impaired gas exchange and respiratory mechanics, thereby contributing to persistent respiratory failure.
Impact statement for Dutch Lung Congress Acute Respiratory Distress Syndrome (ARDS) is a severe form of lung injury, often seen in intensive care patients. ARDS which continues can lead to increased lung stiffness because of extra protein buildup which stops the lungs from working properly. At the moment we do not know which proteins change and how they change in ARDS. Our research investigates these lung protein changes, to help us understand what goes wrong in the lungs in patients with ARDS.

Abstract no: 12

Breathing Life into Innovation: Developing an Advanced COPD Lung Model for Therapeutic Discovery

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Introduction: While chronic obstructive pulmonary disease (COPD) ranks as the third leading cause of death worldwide, the currently available treatments only offer support. The introduction of new therapeutics for COPD has been limited over the past four decades, primarily attributed to the absence of dependable lung models capable of facilitating rapid, cost-effective, and high-throughput testing. In this study, we formulated a healthy and COPD *in vitro* lung mimic that incorporates (1) microcirculation to emulate physiologically relevant conditions, (2) extracellular matrix (ECM) to replicate biochemical aspects, and (3) a diseased COPD primary cell line to mimic a COPD lung. To asses the reproducibility and reliability of our model we created an extensive quality control (QC) assessing barrier integrity and physical and chemical characteristics.
Methods: (1) To replicate the dynamic flow conditions in human lungs, a microfluidic 3D system using CNBio's PhysioMimix™ was created, maintaining cells in a unidirectional flow throughout the entire culture duration. (2) Considering the impact of ECM on cellular activity and responses, two prevalent ECM components, namely collagen type I and fibronectin, were employed to functionalize the substrates. (3) a co-culture of normal human bronchial epithelial cells (NHBEs) with normal human lung fibroblasts (NHLFs) was compared to a co culture of diseased human bronchial/ tracheal epithelial cells (DHBEs) with HNLF to create a COPD lung mimic. In assessing the reproducibility and reliability of the lung models, we devised a comprehensive QC strategy that incorporated real-time and end-point quantitative and qualitative measurements, encompassing light microscopy, scanning electron microscopy (SEM), impedance spectroscopy, immunofluorescence (IF) staining and cytokine secretion profiling.
Results: (1) Dynamic culture conditions show higher heterogeneity of phenotype (light microscopy) and hairy-like structures of ciliated cells (SEM) indicating improved barrier protection and mucociliary clearance. (2) Impedance spectroscopy indicated that collagen-I improved cell adhesion, growth, and differentiation more than fibronectin. (3) DHBE exhibited damage barrier integrity as assessed by transepithelial electrical resistance (TEER) (impedance spectroscopy), hypertrophy of goblet cells (IF), reduction of cilia (SEM) and an inflammatory phenotype (cytokine secretion profiling).
Conclusion: In summary, our study underscored the significance of technological and methodological elements in crafting lung models that effectively mimic human pathophysiology. In addition a

representable COPD model incorporating all QC assessments was made that can be used in the discovery of COPD therapeutics.
Acknowledgement: WCH acknowledges Wenner-Gren Foundations and Medical Advances Without Animals (MAWA) for the financial support.

Abstract no: 13

Bone Morphogenetic Protein 6 Deficiency Distorts Wnt and Oxidative Stress Signaling in Lung Tissue

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Introduction: Chronic Obstructive Pulmonary Disease (COPD) is a progressive lung disease that leads to an irreversible decrease in the lung function, being associated with tissue destruction and defective lung tissue repair. Previously, we identified endothelial cell-derived Bone Morphogenetic Protein 6 (BMP6) as a key factor for communication with alveolar epithelial cells, driving their proliferation. We found reduced BMP6 expression in lung tissue of COPD patients and smokers compared to non-smokers. We also observed a decreased *Bmp6* expression in mouse models after cigarette smoke exposure. In this study, we aimed to characterize the role of Bmp6 further.
Material & methods: We subjected lung tissue samples from mice after 24 weeks of exposure to either air or cigarette smoke (CS) of both wild type (WT) or *Bmp6*$^{-/-}$ mice. Subsequently, mRNA was isolated and subjected to RNA sequencing. Data were processed using R/RStudio, employing Deseq2 to identify differentially expressed genes. Gene Set Enrichment Analysis (GSEA) was carried out using fgsea. Cellular deconvolution was conducted using the reference dataset GSE124872, utilizing the Multi-subject Single Cell Deconvolution method.
Results: Deseq2 analysis showed that 243 genes to be significantly downregulated and 333 genes upregulated in *Bmp6*$^{-/-}$ compared to WT mice exposed to air. Exposure to CS led to 232 downregulated genes and 310 upregulated genes in *Bmp6*$^{-/-}$ compared to WT mice. GSEA of all *Bmp6*$^{-/-}$ mice (both air and CS-exposed) showed upregulation of genes involved in *Oxidative stress and redox pathway* such as *Gclc*, *Gsta3*, *Gstm2*, and *Pxd6*. In contrast, genes related to iron metabolism, such as *Hamp*, *Fth1*, and *Tfrc* were downregulated in *Bmp6*$^{-/-}$ mice. In the air exposed mice, GSEA results showed that genes involved in the *Wnt signaling pathway and pluripotency*, including *Wnt10b* and *Fzd1* were downregulated in *Bmp6*$^{-/-}$ compared to WT mice. Additionally, among the groups exposed to CS, we found that *Wnt7a*, *Fzd2*, *Fzd7*, and *Jun* were downregulated in *Bmp6*$^{-/-}$ compared to WT mice. Further analysis revealed no interaction between exposure and genotype in the mentioned genes. Cellular deconvolution analysis predicted that the proportion of capillary endothelial cells was significantly reduced in lung tissue from *Bmp6*$^{-/-}$ mice.
Conclusions: *Bmp6*$^{-/-}$ mice exhibit a predicted reduction in pulmonary capillary endothelial cells, lower Wnt signaling pathway and pluripotency gene expression, and increased oxidative stress and redox pathway gene expression in both the air and CS exposed groups. These results extend our prior data, indicating a potential beneficial role for Bmp6 in lung repair.

Abstract no: 15

The Gut-Airways Microbiome axis in COPD

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Background: In Chronic Obstructive Pulmonary Disease (COPD) alterations in the airways microbiome (dysbiosis) have been related to the severity of airflow limitation and frequency of exacerbations. No previous study, however, has characterized simultaneously the airway microbiome of the upper and lower airways as well as of the gut.

Methods: Prospective, multicentre, cross-sectional study including clinically stable COPD patients (n=60) and healthy volunteers (n=30) as controls in whom we *1)* determined the microbiome composition in oropharyngeal (OP) swabs, sputum, bronchoalveolar lavage fluid (BALF) and stool samples using 16s rRNA-seq and 2) applied weighted gene co-expression network analysis (WGCNA) to identify groups of highly co-abundant bacteria (modules) and explore their association with FEV1, exacerbations, eosinophilia and inhaled steroids use (ICS).

Results: We found that: 1) the microbiome was different in COPD patients *vs.* controls ($p<0.05$) and across different compartments. Interestingly, sputum and OP swabs presented similar bacterial composition, while stool presented the most different one; *2)* WGCNA identified 8 to 12 modules in each sample type. In OP swabs and sputum half of the modules were associated with FEV1, dyspnea, exacerbations and ICS use. In BALF, 4 modules were linked to FEV1 and dyspnea but only 2 to ICS use. In stool, 1 module was related to FEV1 but 5 to ICS use.

Conclusion: In stable COPD patients the microbiome varies across different body locations. However, different bacterial co-expressed communities can be found across body locations, and they are associated with relevant clinical features, supporting a role of microbiome dysbiosis in COPD.

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Abstract no: 17

Chronic exposure to cigarette smoke distorts epithelial progenitor cell differentiation

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Rationale: Chronic obstructive pulmonary disease (COPD) is a prevalent disease marked by persistent bronchitis, emphysema, and impaired repair, with smoking as a major risk factor due to increased oxidants and ongoing inflammation
Cigarette smoke (CS) contains oxidants that directly damage cells and tissues, deplete antioxidants, and induce inflammation. Moreover, inflammation can persist after smoking cessation, owing to changes in immunity, retained particles, and epigenetic changes triggered by oxidative stress.
Using a mix of *in vivo* and *in vitro* approaches, we sought to determine the influence of chronic inflammation induced by CS on the regenerative capacity of epithelial progenitors when subjected to second-hit inflammatory stimuli.
Methods: Lung organoids were generated by co-culturing Epcam+ cells with CCL206 lung fibroblast in Matrigel®. For the *in vitro* approach, organoids were pre-exposed to CS extract, replated, and cultured in the presence of a second-hit (Poly (I:C), LPS, COPD cytokine cocktail containing IL-1β, IL-6, KC, and TNFα, or IFNγ). For the *in vivo* approach, mice were exposed to CS or fresh air for 6 weeks. Lung function was evaluated with Flexivent and Epcam+ cells were isolated. Organoids were generated and cultured in the presence of a second hit. Additionally, proteomic analysis was performed on Epcam+ cells.
Results: *In vitro* CS extract exposure reduced organoid formation capacity upon replating. The addition of the inflammatory second-hit enhanced organoid growth in control organoids but had less impact on CS extract-pre-exposed cells.
In vivo CS exposure led to impaired lung function in mice. Interestingly, organoid formation efficiency remained unaffected in Epcam+ isolated from CS-exposed mice, but organoid size increased. Notably, both CS and air-exposed lung organoids showed similar formation efficiency when treated with Poly(I:C), LPS, or the COPD cytokine cocktail. However, the response to IFNγ differed significantly, showing a detrimental effect on progenitor cells from control mice but unaffected organoid growth in CS-exposed animals. Furthermore, organoids derived from CS-exposed mice exhibited reduced alveolar differentiation as observed through immunofluorescence staining and reduced protein expression of differentiation markers in the proteomics analysis.
Conclusions: Taken together, while *in vitro* CS exposure significantly distorted organoid formation, chronic *in vivo* CS exposure did not affect organoid formation but rather the differentiation process. Notably, our findings also suggest a paradoxical response to IFNγ in CS-exposed progenitor cells compared to other second-hit inflammatory stimuli. Thus, our data gives insights into the complex interaction between cigarette smoke-induced chronic inflammation and the regenerative capacity of lung epithelial progenitors.

Abstract no: 18

TRAPping the effects of smoking: the regulation of ACP5 expression in lung tissue

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Tartrate-resistant acid phosphatase (TRAP) is an enzyme with unknown function in lung tissue Its expression in lung macrophages of smokers and COPD patients was shown to be higher compared to nonsmoking controls, suggesting a role in smoke damage. This is underscored by the fact that the proteoform TRAP5a can be cleaved into the more active TRAP5b by enzymes released by smoke-activated macrophages, such as cathepsins and matrix metalloproteins. In this study we explored the function of TRAP and regulation of its different mRNA transcripts in lung tissue exposed to cigarette smoke.
We found that expression of TRAP mRNA (*Acp5*) in lung tissue after smoking was mainly driven by transcripts *Acp5-201* and *Acp5-202*, with expression of *Acp5-202* originating from macrophages. Expression of *Acp5-202* correlated with expression of macrophage-specific transcription factors such as early growth response 2 (*Egr2*) and basic helix-loop-helix e40 (*Bhlhe40*). As these transcription factors were previously found to influence proliferation of macrophages, we assessed the role of TRAP in macrophage proliferation using fetal liver-derived alveolar macrophages lacking *Acp5* expression (*Acp5*$^{-/-}$). We found significant loss of proliferation compared to wild type macrophages.
With respect to protein expression, almost all macrophages expressed TRAP protein at significantly higher levels than other cells present in lung tissue of mice. Smoke exposure resulted in higher expression of total TRAP, but not of TRAP5a, suggesting higher TRAP5b expression. This finding was confirmed when we found more TRAP activity in lung parenchyma of smoke-exposed mice compared to air-exposed mice.
In conclusion, TRAP appears to influence proliferation of alveolar macrophages via an unknown pathway. Smoke exposure may increase TRAP5b expression in macrophages and we postulate that this may be a compensatory response to increased apoptosis of macrophages exposed to smoke. By promoting proliferation, TRAP may help maintain the population of macrophages after smoke exposure.

Abstract no: 19

Cigarette smoke affects differentiation potential of lung epithelial progenitors by altering the WNT pathway

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Introduction: Cigarette-smoke (CS) exposure is the most important etiological factor in the development of chronic obstructive pulmonary disease (COPD). The progression of the pathology leads to tissue destruction in combination with abnormal tissue repair. Consequently, the disease is characterized by airflow obstruction that is not fully reversible and subsequent lung function loss.
Lung epithelial progenitor cells are crucial for lung tissue maintenance and regeneration as they play a key role in responding to lung injuries in chronic lung diseases. Little is known on how CS affects epithelial proliferation and differentiation capacity. The canonical WNT pathway aids tissue renewal via β-catenin, while non-canonical pathways regulate cell polarity and morphogenesis and counteract the canonical WNT pathway. For this reason, the study aimed to investigate in detail how the WNT pathway is affected in lung epithelial cells following CS exposure, using a multi-omics approach.
Materials and methods: Mice were exposed to either CS (n=8) or air (n=8) twice a day for either 1 or 6 weeks. 16 hours after last smoke exposure, lungs were collected and $CD45^-/CD31^-/Epcam^+$ cells were isolated. $Epcam^+$ cells were further processed for RNA-sequencing (1 and 6 weeks) and for ATAC-sequencing (6 weeks only). Following analyses were conducted using the DeSeq2 package in R and results were filtered to include only significant hits adjusted p-value <0.05 and absolute log_2FC >0.5. Additionally, lung organoids were generated by co-culturing the isolated $Epcam^+$ cells with CCL206 lung fibroblasts.
Results: We found that in vivo CS exposure in mice led to less AT2 differentiation of epithelial cells in ex vivo lung organoids compared to air exposure, along with disrupted signaling pathways related to proliferation and tissue repair. Specifically, the WNT signaling pathway was differentially expressed in epithelial cells from CS-exposed mice compared with control mice, and this effect was more pronounced at 6 weeks than at 1 week. Pathway enrichment analysis indicated negative enrichment of genes involved in canonical WNT/β-catenin signaling, whereas genes involved in non-canonical WNT signaling were positively enriched. Examples of downregulated genes include Wnt3a and Lgr5, whereas Rac2 was upregulated. The ATAC-seq data showed that among the genes exhibiting less chromatin accessibility, the canonical WNT signaling pathway was enriched, most notably the canonical WNT signaling effector Lef1.
Conclusions: Our findings suggest significant downregulation of the WNT/β-catenin pathway in epithelial cells derived from CS-exposed mouse lungs. We propose that chronic CS exposure may impair the differentiation potential of epithelial progenitor cells through alterations in the WNT/β-catenin pathway.

Abstract no: 20

Senescence-induced and COPD-derived fibroblasts hamper human alveolar organoid formation

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Introduction: Higher levels of senescence have been demonstrated in COPD-derived lung tissue and structural cells, including fibroblasts and especially in severe early onset (SEO-)COPD. The higher level of senescence in these fibroblasts was associated with higher secretion of a pro-inflammatory senescence-associated secretory phenotype (SASP) and extracellular matrix (ECM) dysregulation. As both chronic inflammation and ECM dysregulation can contribute to impaired alveolar repair in COPD, we hypothesized that senescent fibroblasts hamper alveolar repair. Therefore, we investigated the effect of senescence-induced and SEO-COPD-derived fibroblasts on human alveolar epithelial organoid formation as a model for alveolar repair.
Material and methods: A human alveolar organoid model was used with the human alveolar epithelial cell line H441 in combination with primary human parenchymal lung fibroblasts. We included fibroblasts from 9 SEO-COPD patients and senescence-induced (using paraquat) fibroblasts from 10 non-COPD patients and compared the effects with untreated fibroblasts from the same non-COPD controls. H441 cells and fibroblasts were cultured with a 1:1 mix in Matrigel on inserts (mixed) with medium containing 1% FCS (n=9-10). To assess the paracrine effects of fibroblasts, H441 cells were cultured in Matrigel on inserts separately from fibroblasts, which were cultured in the basolateral compartment (n=5-6). The numbers and size of organoids were assessed after 7 and 14 days of organoid culture.
Results: The number of alveolar organoids was significantly reduced in the mixed organoid cultures with senescence-induced compared to untreated fibroblasts after 7 and 14 days (7% and 14% median decrease respectively). We observed a similar trend (p= 0.06) for the mixed organoids with SEO-COPD compared to control-derived fibroblasts after 7 days (35% median decrease). The size of alveolar organoids was reduced in the mixed organoid cultures with SEO-COPD fibroblasts after 7 days, while no effect on size was observed in the mixed organoids at day 14 or with senescence-induced fibroblasts. In line with the effects of direct interaction, a trend (p= 0.06) towards reduced numbers of organoids was found in the paracrine model with senescence-induced fibroblasts after 14 days (13% median decrease). No significant differences were observed in the paracrine model with SEO-COPD fibroblasts.
Conclusions: Senescence-induced and SEO-COPD-derived fibroblasts reduced alveolar organoid formation compared to untreated non-COPD fibroblasts, which may partly be caused by a paracrine effect. These findings support our hypothesis that senescent fibroblasts hamper alveolar repair in COPD. This study opens new avenues towards developing senescence targeting strategies to improve alveolar repair in COPD.

Abstract no: 21

Thymic Stromal Lymphopoietin (TSLP) Expression Is Restricted To Resting Basal Cells And Is Low In Current Smokers

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Introduction: Thymic Stromal Lymphopoietin (TSLP) is a well-known alarmin primarily secreted by airway epithelial cells upon exposure to insults like viruses and cigarette smoke. Currently, anti-TSLP, which has been successful in suppressing symptoms of asthma, is being trailed in COPD. Previously, our group has shown that another alarmin IL-33 expression was restricted to a subpopulation of basal cells (resting basal cells), and this subpopulation was decreased during smoke exposure (Faiz et al., 2023). Clinical studies have shown that anti-IL33 biologic therapies are beneficial for ex-smokers compared to current smokers. It is currently unclear whether TLSP has a similar expression profile as IL-33 and whether it is influenced by current smoking.
Methods: Single-cell RNA-Seq expression of TSLP was investigated in the integrated human lung cell atlas (HLCA) and two Air Liquid Interface (ALI) culture datasets of primary bronchial epithelial cells of healthy never and COPD current smokers (n=7 and n=6, respectively). Trajectory analysis (using the r package monocle 2) was conducted to investigate the TSLP expression shift throughout the differentiation process. Cellular deconvolution was conducted to investigate the correlation between TSLP expression and basal cell proportions in GLUCOLD (current (n=33) vs ex-smokers(n=46)), NORM (Current(n=37) vs never-smokers (n=40)) bronchial biopsy bulk sequencing data and a microarray data of COPD Current (n=30) vs ex-smokers (n=57) cohorts. All the analyses were done in R statistical software.
Results: TSLP expression was found to be specific to resting basal cell population in the HLCA and in ALI cultures. Trajectory analysis of ALI shows that TSLP expression is lost following the differentiation of resting basal cells into suprabasal cells. Cellular deconvolution of RNA-Seq and microarray datasets from bronchial samples across three cohorts showed that TSLP expression was highly correlated with basal cells ($p<0.0001$). TSLP was significantly lower expressed in current smokers compared to ex-smokers in two COPD cohorts ($p=0.0176$ and $p=0.0378$) and lower expressed in current smokers compared to never-smokers in a non-COPD cohort ($p=2.56e-07$).
Conclusion: TSLP expression was found to be expressed in the resting basal cell population which is known to be decreased in current smokers. Furthermore, TSLP expression was found to be lower in current smokers thus these results suggest that clinical trials focusing on anti-TSLP therapies, should separate their patient population based on smoking status.

Abstract no: 22

Inhibition of succinate dehydrogenase reduces oxidative stress in lungs and ameliorates the pathogenesis of COPD in mice

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Introduction/Aim: Cigarette smoke (CS) exposure is the major cause of chronic obstructive pulmonary disease (COPD), which is strongly associated with aberrant immunometabolic responses leading to oxidative stress and inflammation. Activated immune cells favour glycolysis over oxidative phosphorylation pathways to meet acute increases in energy demands, leading to increased glucose influx and anaerobic glycolysis. As a result, there is accumulation of succinate, a metabolite of the Kreb's cycle. Here, we interrogated the role succinate dehydrogenase (SDH) inhibitor dimethyl malonate (DMM) on oxidative stress and the pathogenesis of COPD.
Method: We used our highly representative murine model of CS-induced COPD to examine the effects of blocking SDH action using DMM.
Results: Prophylactic administration of DMM reduced airway neutrophil numbers and partially suppressed CS-induced small airway remodelling and alveolar enlargement like emphysema. DMM treatment showed a significant improvement in some lung function parameters by decreasing transpulmonary resistance (Rrs) and trends towards decreases in hysteresis. The administration of DMM decreased the gene expression of pro-inflammatory cytokines *Il1b Cxcl1,* and *Il1a* while increased the levels of anti-inflammatory Il10 cytokine compared to vehicle treated groups with experimental COPD. Furthermore, reduced lipid peroxidation (8-Isoprostane and 4-HNE adducts) and $TUNEL^+$ cells in parenchyma (apoptosis marker) were observed *in vivo* in CS-exposed DMM treated groups.
Conclusion: Thus, we demonstrate that inhibiting succinate dehydrogenase with DMM resulted in decreased airway inflammation, pro-inflammatory cytokine expression, and restored impaired lung function parameters suggesting it as a crucial metabolic regulator with anti-inflammatory activity and prevents lung damage in COPD.
Keywords: Inflammation, Cigarette smoke, metabolism, oxidative stress
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Abstract no.: 23

Pregnancy exacerbates neutrophil responses in murine lungs after cigarette smoke exposure

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Introduction: Exposure to environmental smoke during pregnancy has detrimental effects on maternal health and contributes to the development of severe respiratory diseases. Calprotectin, a heterodimer formed by two S-100 calcium-binding cytosolic proteins, S100A8 and S100A9, has been investigated as a non-invasive biomarker for diagnosing and monitoring pulmonary inflammatory processes in serum, blood, and feces. Calprotectin is an extracellularly released multifunctional protein primarily expressed by neutrophils, mediating a broad range of physiological and pathological responses
Methods: The current preclinical study aimed to explore the impact of cigarette smoke (CS) exposure on the alterations in neutrophil migration and activation between pregnant and non-pregnant mice. Pregnant mice were subjected to either whole-body CS or air exposure throughout pregnancy and lactation. Twenty-four hours after the final CS exposure bronchoalveolar lavage (BAL) cell counts and the level of calprotectin in BAL fluid (BALF), serum, and fecal content were investigated. RNA sequencing was used to analyze the gene expression profile in lung tissue of CS-exposed pregnant dams.
Results: Pregnant mice exhibited significantly higher calprotectin levels and neutrophil influx in BALF compared to their non-pregnant counterparts. Interestingly, in both pregnant and non-pregnant mice exposed to CS, significantly elevated levels of neutrophils and calprotectin were observed compared to those exposed to air. These findings are further underscored by RNA sequencing results of the lung tissues, as CS-exposed dams, particularly during pregnancy, showed elevated expression of inflammatory genes associated with neutrophil function and migration compared to non-CS exposed groups. Similarly, in air-exposed mice, pregnancy resulted in a significant increase in serum calprotectin levels compared to non-pregnant groups, while surprisingly, this increase was suppressed in pregnant mice exposed to CS. In contrast, no significant differences in calprotectin levels were observed in fecal samples between pregnant and non-pregnant mice in both CS-exposed and air-exposed groups.
Discussions: In summary, there are distinct effects of CS on neutrophil numbers and calprotectin levels in pregnant and non-pregnant mice, in BALF and serum. These findings highlight the impact of pregnancy-induced changes in the immune system especially on the migration and activation of neutrophils. They further show that pregnancy exacerbates neutrophil inflammatory responses towards environmental noxious triggers.

Abstract no: 24

Sensory neurons support mast cell differentiation in an *in* vitro co-culture model

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Rationale: Neuroplasticity is defined as the ability of the nervous system to reorganize its structure, function and connections. In asthma this manifests as increased neuron density and neurite length as well as a lowered firing threshold, resulting in hyperresponsiveness to (a-)specific stimuli. An increase in the number of mast cells in the direct vicinity of the sensory neurons in the airways of fatal asthma patients was recently observed. However, little is known on how sensory neurons regulate mast cell function. Therefore, we aimed to study the interaction of sensory neurons with mast cells. To achieve this, we developed an *in vitro* co-culture model of human sensory neurons and mast cells to study neuroimmune interactions.
Methods: Using a 35-day differentiation protocol, H9WA09 human pluripotent stem cells (hPSCs) were differentiated into sensory neurons. After maturation of the hPSC-derived sensory neurons, a co-culture was established with mast cells (LUVA) in a 50/50-mixture of sensory neuron maturation and LUVA medium. After 5 days of co-culture, the two cell types were separated using PSA-NCAM microbeads followed by RNAseq analysis. Additionally, Ca^{2+}-measurements were performed to assess the activity of the sensory neurons with and without exposure to mast cells.
Results: The successful generation of β3-tubulin^{+}/TRPV1^{+} sensory neurons, both in the presence and absence of mast cells (FcεRI^{+}) was confirmed using immunofluorescence and flow cytometry. Additionally, co-cultures were established in the axon-guiding NeuroChip. Co-culture did not affect β3-tubulin^{+}/TRPV1^{+} sensory neuron differentiation. However, RNAseq analysis of the mast cell population showed enhanced mast cell differentiation in co-culture, as well as upregulation of 10/86 asthma susceptibility genes. GSEA analysis showed upregulation of multiple immune-related pathways. Including *INFLAMMATORY_RESPONSE*, as well as IL2*_STAT5, IL6_JAK_STAT3, TGF_BETA* and *TNFA_SIGNALING_VIA_NFKB.* Furthermore, the upregulation of several Th2-immune response-related genes was observed, such as *IL6, ARG2, ALOX5 and LTA4H*.
Ca^{2+}-measurements of the sensory neurons showed increased responsiveness of the mast cell exposed sensory neurons compared to monocultured sensory neurons, upon addition of the TRPV1 agonist capsaicin (10nM-10μM).
Conclusions: We successfully established an *in vitro* co-culture model of hPSC-derived sensory neurons and mast (LUVA) cells, which facilitates further studies of neuro-effector interactions between the two cell types. We now show induction of mast cell differentiation and immune activation upon co-culture with the sensory neurons with increased sensitivity of the sensory neurons. These findings implicate that an increased presence of mast cells in asthmatic lungs lowers the firing threshold of sensory neurons and increase sensitivity of the airways.

Abstract no: 25

The lung fibroblast secretome supports alveolar organoid formation in the presence of cigarette smoke extract

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Background: Chronic obstructive pulmonary disease (COPD) is a progressive disease, characterized by enhanced tissue destruction and impaired tissue repair. Fibroblasts in the alveolar niche have been associated with supporting the repair mechanisms of the alveolar epithelium through the secretion of extracellular vesicles (EVs) and soluble factors (SFs). Recent research has unveiled a supportive role of lung fibroblast-derived EVs and SFs on lung organoid formation in the absence of COPD-related stimuli. [1] This study aims to assess whether these factors retain their stimulatory properties in the presence of cigarette smoke extract (CSE).
Methods: EVs and SFs were isolated and purified from cultured lung fibroblasts (MRC5), utilizing ultrafiltration and size exclusion chromatography. Their regenerative potential was tested on mouse lung organoids (N=8), which were obtained by co-culturing 10,000 epithelial progenitor cells ($Epcam^+$) with 10,000 lung fibroblasts (CCL206) in Matrigel. The organoids were treated three times a week with 5% CSE and concomitantly supplemented with either 10^9 EVs/mL or 30 µg SFs/mL. On day 14, the number and size of the organoids were determined, as was the number of differentiated alveolar (surfactant protein C positive) and airway (acetylated tubulin positive) organoids.
Results: The results indicate that relative to control, CSE inhibited organoid formation by 16.61% ± 10.54%. Despite this inhibitory effect, there was a median increase of 46.70 µm in organoid size. Additionally, a rise of 27.27% ± 7.54% in the proportion of double-negative organoids was observed, indicating a reduced tendency towards alveolar and airway organoid differentiation. Conversely, co-treatment of EVs or SFs with CSE demonstrated a significant supportive effect relative to CSE, reflected in an increase in organoid count (i.e. 39.11% ± 17.80% and 60.21% ± 26.50%, respectively). Whereas the organoid size was not affected by co-treatment with either EVs or SFs. Furthermore, there was a reduction in the elevated number of double-negative organoids upon EV- and SF-treatment (i.e. 11.74% ± 10.21% and 12.9% ± 8.79%, respectively). In addition, treatment with EVs induced an upward trend in alveolar organoid differentiation, while SFs significantly supported the alveolar organoid differentiation (i.e. 20.10% ± 11.56%).
Conclusions: Taken together, it can be concluded that CSE negatively affects organoid formation and differentiation. These effects are significantly counteracted by both lung fibroblast-derived EVs and SFs, making them an interesting potential regenerative treatment to pursue for COPD.
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Abstract no: 26

Epigenetic age acceleration and severity of airflow limitation on blood and lung tissue in COPD patients

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Background: Accelerated lung ageing has been proposed as a mechanism of chronic obstructive lung disease (COPD). Yet, whether the aging markers in lung tissue and blood in COPD patients are similar or if they change with the severity of airflow limitation is unclear. This study sought to explore these questions using DNA methylation-based aging calculation algorithms (i.e. epigenetic clocks), previously developed to measure the biological age of a variety of tissues.
Methods: DNA was extracted from blood (n=168) and lung tissue (n=138) of COPD patients with different degrees of airflow limitation severity (FEV_1% ref.). Genome wide DNA methylation was assessed with EPIC arrays (Illumina), and the biological age and age acceleration was computed with 7 epigenetic clocks, and with the mDNA-TL estimator (a Telomere length shortening measure). Multivariable linear regressions adjusted for chronological age, sex, pack-year and smoking status, were used for the analysis.
Results: In blood, airflow limitation severity was associated with the biological age acceleration determined by three epigenetic clocks (BLUP, Levine, DunedinPACE) and by the mDNA-TL estimator. By contrast, in lung tissue only the mDNA-TL estimator showed a significant association with the severity of airflow limitation.
Conclusions: COPD patients with more severe airflow limitation present a higher biological epigenetic age acceleration only in blood. However, both blood and lung tissue of these patients present an accelerated Telomere shortening. These findings suggest that the pace of aging biomarkers is different in the two tissues.
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Abstract no: 27

Improving the 3D microenvironment of mesenchymal stromal cells for lung tissue repair in COPD

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Background: In COPD, the extracellular matrix (ECM) is disrupted, impacting the mechanical properties of lung tissue. Lung-resident mesenchymal stromal cells (LMSCs) secrete crucial growth factors to support alveolar epithelial repair, e.g. hepatocyte growth factor (HGF), which is impaired in COPD. We hypothesized that LMSC-ECM interactions are disturbed in COPD, and that this disbalance can be restored by altering the ECM mechanical cues from the microenvironment.
Methods: Collagen-derived gelatin methacrylate (GelMA) hydrogel (160 kDa; degree of modification 60%) were prepared by crosslinking with 0.5 mg/ml or 1 mg/ml lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP), providing 2 mechanical environments. LMSCs from COPD patients or controls (n=4/group) were cultured on plastic or 2 mm-thick GelMA hydrogels. HGF gene and protein expression was assessed and an organoid transwell model was used to co-culture human alveolar epithelial H441 cells (upper chamber) and LMSCs with/without hydrogel (lower chamber). After 14 days, organoid size was quantified.
Results: HGF gene and protein levels were lower in COPD compared to control-derived LMSCs. Culture on 0.5 mg/ml LAP GelMA hydrogel increased HGF levels in both COPD and control LMSCs. COPD-derived LMSCs induced aberrant organoid formation, with larger organoids than those supported by control-derived LMSCs. This effect was reversed when LMSCs were grown on 0.5 mg/ml LAP GelMA.
Conclusion: This study indicates that altering the ECM mechanical microenvironment has the potential to restore the reparative properties of LMSCs in COPD. This highlights a potential strategy for improving alveolar epithelial repair by normalizing LMSC function.
Keywords: COPD; LMSCs; Stromal health; Lung tissue regeneration; GelMA hydrogel

Abstract no: 28

Differential miRNA expression in SEO-COPD is associated with altered lysosome, vesicular transport and ECM-related pathways

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Introduction: Severe early-onset (SEO-)COPD manifests at early age with high disease burden; why certain individuals are more susceptible to manifesting severe COPD early is still largely unknown. Previously we identified a differential expression pattern on RNA level in SEO-COPD. Supplementing this pattern with miRNA level data gives further insight in underlying molecular mechanisms of SEO-COPD pathology.
Material & methods: Small RNA sequencing was used to determine differential miRNA expression in peripheral lung tissue samples between non-COPD (control) (n=31) and SEO-COPD subjects (n=18), followed by excluding genes that were differential between control and common COPD subjects (n=21). Both analyses were corrected for age and sex. Differential miRNAs were characterized by determining inverse correlations between the miRNAs and their targets genes, and exploring the biological pathways of the target genes.
Results: Three significantly differentially expressed miRNAs were found in SEO-COPD subjects: miR-193b-3p and miR-202-5p had higher expression and miR-331-3p had lower expression in SEO-COPD compared to control, and all three were not different between common COPD and control. MiR-193b-3p had 35 inversely correlated target genes, that were enriched for pathways related to vesicular and endosomal membranes, secretory/transport vesicles and lysosomal activity, including transmembrane proteolysis genes NCSTN and APH1A involved in Notch and Wnt signaling. MiR-331-3p had 41 inversely correlated gene targets, that were enriched for ECM organization and morphogenesis pathways, including ECM genes COL6A1, COL6A2 and ELN. No inversely correlated gene targets were identified for miR-202-5p.
Conclusions: We identified three differentially expressed miRNAs specific to SEO-COPD in lung tissue. Based on their target genes and functions, miR-193b-3p may be involved in regulation of vesicular and lysosomal activity, while miR-331-3p may be a regulator of aberrant ECM repair in SEO-COPD. By directly connecting differential miRNA expression to target gene expression we generated important new insights into the potential involvement of these miRNAs in SEO-COPD pathogenesis.

Abstract no: 29

Chronic obstructive pulmonary disease extracellular matrix promotes tissue repair responses in fibroblasts

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Introduction: The lung extracellular matrix (ECM) is a dynamic micro-environment regulated by a balance between proteases and anti-proteases to maintain homeostasis[1]. Frequent insults driving cellular damage such as smoking, lead to chronic inflammation in the lung that disrupts this balance. The resultant abnormal ECM remodelling can be seen in chronic obstructive pulmonary disease (COPD). In the current study, we aimed to investigate cell-matrix and cell-cell crosstalk in healthy and COPD conditions using a 3D model of human lung ECM seeded with human lung fibroblasts.
Methods: Human lung ECM was generated by decellularising and powderizing explanted lung tissue from COPD and non-COPD control donors as described previously[2]. Proteomic content of COPD (n=1) and control (n=1) ECM powders was analysed using mass spectrometry. For subsequent experiments, COPD and control ECM powder mixes were prepared containing equal parts of ECM from each donor (n=7 donors per group). Hydrogels were generated following pepsin digestion[2] and COPD (n=6) and control (n=6) fibroblasts were encapsulated by mixing $1x10^6$ cell/mL in the pre-gels prior to gelation. Morphology of the encapsulated cells was visualized microscopically every 3 days. On day 14, the hydrogels were fixed and their widths measured to assess hydrogel contraction.
Results: In total, 681 proteins were detected in the ECM powders using mass spectrometry, with 555 proteins detected in both ECM powders and 110 unique to COPD. The biggest difference between the two groups was higher amounts of collagen type VI in COPD. COPD and control fibroblasts, encapsulated in COPD ECM hydrogels, both adopted a spindle morphology within 24 hours. However, in control ECM hydrogels the fibroblasts were viable but retained a rounded morphology even after 14 days of culture. COPD ECM hydrogels encapsulated with control fibroblasts contracted (6.4 ± 1.5 mm) and had reduced widths (p=0.012) compared to control ECM hydrogels encapsulated with control fibroblasts (9.6 ± 0.3 mm) at Day 14.
Discussion and conclusions: COPD ECM hydrogels stimulate fibroblasts to elongate and subsequently contract the hydrogels. The spindle morphology of fibroblasts and hydrogel contraction together suggest that COPD ECM stimulates a tissue repair response. Studying the differences in the composition, biomechanical properties and organization of the COPD and control ECM hydrogels will provide insight into which ECM components contribute most to the tissue repair response.

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Abstract no: 30

Altered TGF-β Induced Repair Response of Senescent Lung Fibroblasts

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Introduction: COPD is a disease of accelerated ageing, with increased cellular senescence as one of the most described ageing hallmarks. Cellular Senescence is an irreversible state of cell cycle arrest occurring in stress-induced or aged cells. Senescent cells present a distinct secretory protein profile known as the senescent-associated secretory phenotype (SASP). The SASP includes inflammatory cytokines, growth factors and proteases, which can negatively impact surrounding cells and tissue. Senescent cells and their SASP have been hypothesized to contribute to the aberrant tissue repair occurring in COPD. Previously, our group observed a link between senescent fibroblasts and extracellular matrix (ECM) dysregulation in COPD, with reduced decorin and elastin expression and higher protease levels after senescence induction. Fibroblasts are key cells involved in ECM homeostasis and lung tissue repair. TGF-β is a key growth factor involved in the activation of lung tissue repair. This project aimed to assess the TGF-β-induced tissue repair response of senescent lung fibroblasts. We hypothesised that senescent lung fibroblasts have a decreased TGF-β induced repair response.
Methods: Senescence was induced in non-COPD-derived fibroblasts by paraquat (PQ). Five days after PQ treatment, equal numbers of untreated and PQ-treated fibroblasts (n=6) were stimulated with 2.5ng/ml TGF-β to induce a tissue repair response. Gene expression of ECM proteins (COL1A1, FN1 and ACTA2) was measured. Immunohistochemical (IHC) staining for fibronectin (FN1) was performed.
Results: PQ treatment alone significantly reduced FN1 and ACTA2 expression, but not COL1A1, compared to untreated fibroblasts. TGF-β stimulation significantly induced FN1, COL1A1 and ACTA2 expression in untreated fibroblasts, with similar trends (p= 0.05-0.1) in PQ-treated fibroblasts. When comparing expression levels after TGF-β stimulation in PQ-treated vs untreated fibroblasts, we observed a trend towards lower FN1, COL1A1 and ACTA2 expression in PQ-treated fibroblasts. In addition, when comparing fold changes of TGF-β stimulation between PQ-treated and untreated fibroblasts, we observed a lower fold increase of COL1A1. FN1 gene expression results were confirmed on protein level, after TGF-β stimulation FN1 protein levels were increased in both PQ-treated and untreated fibroblasts, and FN1 protein levels were decreased after PQ-treatment alone.
Conclusion: These results suggest, that increased senescence leads to an altered TGF-β-induced repair response in lung fibroblasts, with lower induction of ECM gene expression. Further research is needed in more subjects and to identify the mechanism underlying this altered TGF-β-induced repair response in senescent fibroblasts. Altogether, this gives further insight into the repair response of senescent fibroblasts in COPD.

Abstract no: 31

Polyvinylchloride and polypropylene microplastics affect outgrowth of murine lung organoids

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Introduction: Microplastics are everywhere nowadays, including in the air we inhale and in our lungs. Epidemiological studies on occupational exposures have demonstrated higher incidences of airway and interstitial lung disease among workers in plastics and related industries. In animal studies, inhalation exposure to various microplastics has been shown to induce inflammatory responses and histological abnormalities in lung tissue. Yet, a thorough comprehension of the biological mechanisms is still lacking. We have previously shown that polyamide 6,6 (nylon) microfibers, and to a lesser extent polyethylene terephthalate (polyester) microfibers, can inhibit the development of human and murine airway organoids. Comparable inhibition was observed upon exposure to 1-5 µm or 5-10 µm nylon particles and unknown components leaching from nylon were found to be responsible for observed effects. The objective of the present study was to investigate whether microplastic particles derived from two other plastics, polypropylene and polyvinylchloride, also impact the development of murine airway and alveolar organoids.
Materials & methods: Developing murine airway and alveolar organoids derived from primary lung epithelial progenitor cells were incubated with different concentrations of <1 µm, 1-5 µm or 5-10 µm polypropylene or polyvinylchloride particles. After 14 days, the number and size of alveolar and airway organoids were determined and compared to the vehicle-treated control.
Results: We observed that all size fractions of polypropylene particles induced dose-dependent inhibition of the number of airway organoids with no difference between the sizes. The inhibitory effect on the number of alveolar organoids was minor and appeared only relevant in the highest concentration of 100 µg/ml. Polyvinylchloride particles did not affect the number of airway organoids. However, the number of alveolar organoids was slightly higher when incubated with any concentration of polyvinylchloride particles smaller than 1 µm and lower in the highest concentration of 5-10 µm particles.
Conclusions: These results suggest that polypropylene and polyvinylchloride microplastics can change developing airways and/or alveoli, emphasizing the need for further clarification of biological mechanisms and assessment of environmental exposure levels in daily life.

Abstract no: 32

Nasal periostin expression is higher in asthma patients with versus without persistent airflow limitation

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Introduction: One in three asthma patients have persistent airflow limitation (PAL), which is associated with lower quality of life and higher risk of exacerbations. In the current study, we used nasal brush transcriptome data to explore mechanisms associated with PAL in asthma patients.
Methods: In ATLANTIS, a differential gene expression (DGE) analysis of 126 previously identified asthma-associated genes (*ERJ* 2022; 60: Suppl. 66, 687) was performed using EdgeR, adjusted for age and sex. Replication was done in a cohort (ARMSTRONG) of 20 asthma patients.
Results: We included 95 asthma participants with and 204 without PAL (age 46 vs 43, male sex 51% vs 34%, FEV_1 % pred 80 vs 96, FEV_1/FVC % pred 76 vs 96, blood eos 0.25 vs 0.20 respectively) from whom nasal brush RNA-seq data were available. We found 3 higher and 5 lower expressed genes in asthma with versus without PAL (figure 1). We identified high expression of the gene *POSTN* in PAL, also replicated in nasal brushes from 9 patients with and 11 without PAL included in the ARMSTRONG study (ATLANTIS LogFC: 1.07, FDR: 0.001, AMSTRONG: logFC: 2.73, FDR: 0.03).
Conclusion: We found higher nasal periostin expression to be associated with PAL in participants with asthma. Our findings suggest that periostin, a gene associated with epithelial inflammatory responses and airway wall remodelling, is a feature of PAL in asthma.

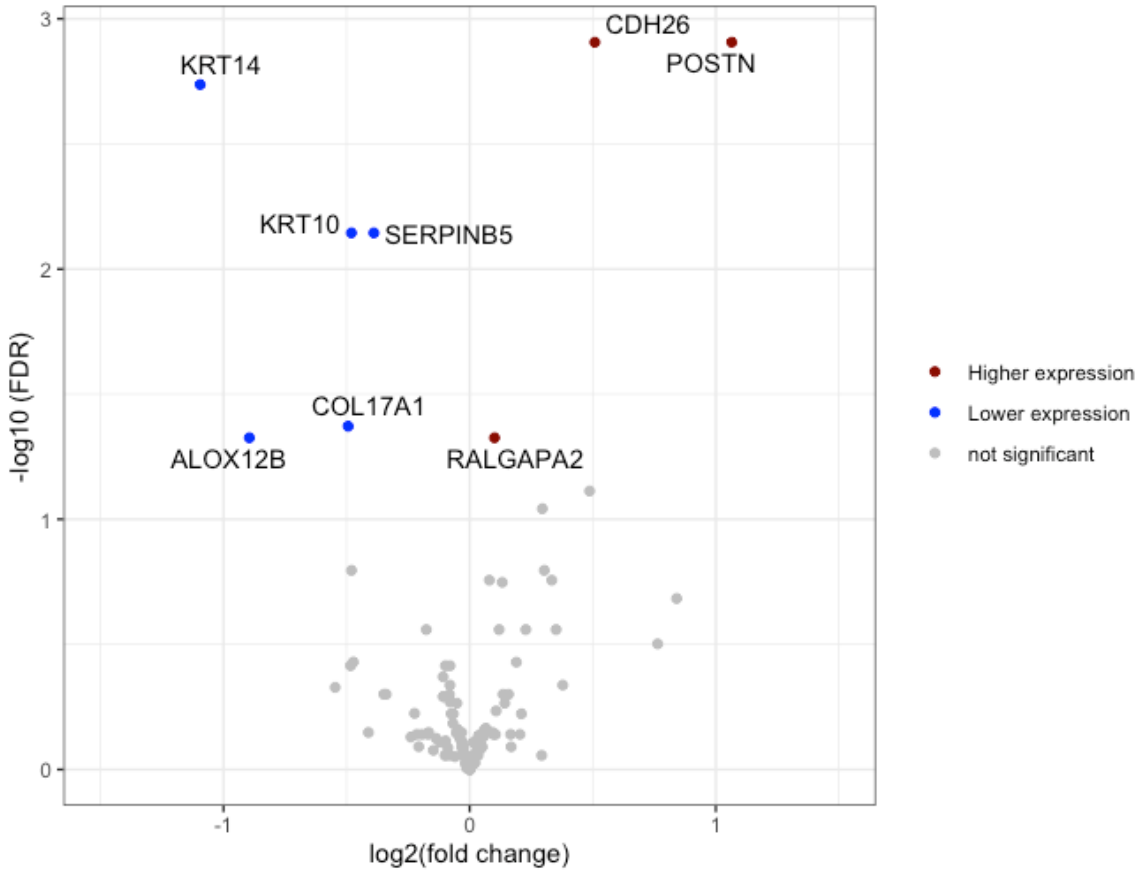

Figure 1: Results for DGE analysis in ATLANTIS

Abstract no: 33

Evaluation of Airway Mucosal Biopsy in Symptomatic Chronic Bronchitis Patients with Preserved Lung Function

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Introduction: The presence and severity of chronic obstructive disease (COPD) including the predominant and overlapping subtypes of emphysema and chronic bronchitis (CB) have traditionally been defined by spirometric testing (i.e., forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC) and FEV_1/FVC) and patient symptoms. More recently, quantitative radiographic measures of emphysema, have been established as important measures of disease severity. While these metrics are useful in evaluating disease progression over time, it is possible that chronic changes to the airway mucosa are established independent of or prior to objective functional and structural changes.
Materials and Methods: Herein, we describe a cohort of carefully selected CB patients enrolled in several Bronchial Rheoplasty (BR) feasibility studies. Patients included were former smokers, with significant symptoms based on the COPD assessment test (CAT) and minimal emphysema on CT scan. Patients underwent bilateral airway mucosal cryo-biopsy prior to each BR treatment. There were no spirometry measure requirements for study exclusion. Biopsies were assessed by a blinded pathologist for evidence of chronic alterations to the airway mucosa defined by goblet cell hyperplasia (GCH), eosinophilic infiltration, and/or chronic inflammation (Table 1).
Results: 40 patients were enrolled of whom 31 had evaluable biopsies at baseline. At study enrollment the mean (standard deviation) age was 67.2 (7.3) years, FEV_1 percent predicted was 63.8% (21.1), FEV_1/FVC was 0.52 (0.14), Low attenuation percentage below -950 HU (LAA%) on CT was 9.7% (11.1). Airway biopsy showed mucosal abnormalities in all measures from this cohort (mean (SD) GCH score 1.4 (0.9), eosinophils 0.5 (0.7), chronic inflammation 0.7 (0.7)) at baseline. Of those 31 patients, seven had FEV_1 greater than 80% at baseline, 4 of which had an FEV_1/FVC > 0.7, with a mean LAA% of 3.1 (2.4). Despite a preserved FEV_1 and LAA, these patients had significant mucosal disease on biopsy (mean (SD) GCH score 1.6 (1.2), eosinophils 0.6 (0.6), chronic inflammation 1.0 (0.9)). An additional 7 patients had FEV_1 between 60% and 80% (GCH score 1.3 (0.9), eosinophils 0.3 (0.9), chronic inflammation 0.25 (0.6), LAA 4.8 (4.8)).
Conclusions: These data suggest that chronic changes to the airway mucosa are present prior to identification of disease using traditional physiologic and imaging measures. These pathophysiologic changes provide evidence that disease processes can be present, independent or preceding traditional metrics of disease, and which may herald development of more progressive disease. Therapies to target mucosal abnormalities could alter symptoms and/or disease progression.

Table 1: Semi-Quantitative Histopathology Scoring Matrix

Goblet Cell Hyperplasia	
0	Normal numbers of goblet cells
1	Moderately increased numbers of goblet cells, but less than a 1:1 ratio of goblet cells to ciliated bronchial epithelial cells
2	Significantly increased goblet cells, with approximately a 1:1 ratio of goblet cells to ciliated bronchial epithelial cells
3	Dramatically increased numbers of goblet cells, with a ratio exceeding 1:1 of goblet cells to ciliated bronchial epithelial cells
Submucosal Lymphocytes and Plasma cells	
0	None/rare lymphocytes
1	Mildly increased
2	Moderately increased
3	Dense submucosal lymphocytic infiltrate
Eosinophils	
0	None/rare
1	Mildly increased
2	Moderately increased
3	Dense submucosal eosinophil infiltrate

Abstract no: 34

Maternal cigarette smoke exposure in mice induces a Th2-biased immune response in offspring

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Introduction: Environmental cigarette smoke (CS) exposure serves as a primary contributor to household air pollution in both developing and developed countries. Direct and indirect CS exposure have adverse health impacts that start during early pregnancy and persist throughout childhood and adolescence. Despite evidence of harmful effects of smoking on the fetus and infants, 20–30% of women who smoke, continue smoking during pregnancy and lactation. Considering the pivotal role of immune development in early life, the present study investigated the effects of maternal CS exposure on allergy development and vaccine responsiveness in the offspring.
Methods: Pregnant dams were exposed to either CS or air during pregnancy and lactation. Two weeks after weaning, the offspring were either intranasally sensitized and challenged with house dust mite extract (HDM) or were twice injected with Influvac vaccine. After the HDM challenges, lung function, T cell subsets in the lungs, bronchoalveolar lavage fluid (BALF) cell counts, and antigen-specific serum immunoglobulins were assessed, while 9 days following the booster vaccination, vaccine-specific delayed-type hypersensitivity (DTH) response, antigen-specific serum immunoglobulins, and splenic T cells were measured following an intradermal antigen challenge.
Results: Lung resistance and antigen-specific serum immunoglobulins (IgE and IgG1) were significantly higher in HDM-sensitized and challenged offspring of CS-exposed dams compared to HDM-challenged offspring born to air-exposed dams. There was no discernible effect of maternal CS exposure on Th1 cell frequency in the lung of HDM-challenged offspring. HDM-sensitized and challenged offspring of air-exposed dams showed increased numbers of eosinophils in BALF, while maternal CS exposure further enhanced this inflammatory response in the offspring.
Conclusions: Vaccinated offspring from both air- or CS-exposed dams exhibited a similar pattern in DTH response and vaccine-specific antibody levels (IgG1 and IgG2a). However, maternal CS exposure resulted in higher splenic Th2 cell activation in vaccinated offspring compared to PBS-treated offspring.
In conclusion, early life exposure to CS affects immune development in offspring. The offspring showed a bias towards Th2 immunity resulting in an exacerbated allergic immune response to allergens.

Abstract no: 36

Interleukin-13 represses epithelial cell differentiation in primary bronchial epithelial cells from patients with **asthma**

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Introduction: Asthma is a heterogeneous lung disease, characterized by remodeled airways and mucus hypersecretion. Previously, we showed that the airway composition is changed in patients with asthma, showing more mucus-producing cells such as goblet and mucous ciliated cells compared to healthy controls. Within these cells we observed an enrichment for IL-4/IL-13 signaling in asthma. Here, we aim to characterize the short- and long term effects of IL-13 stimulation in primary bronchial epithelial cells (PBECs) from controls and patients with asthma.
Methods: PBECs were isolated during a bronchoscopy from healthy controls and patients with asthma and fully differentiated over the course of 28 days in air-liquid interface. Upon fully differentiated, 5 day chronic stimulation with IL-13 or vehicle was started. After either 3 (early) or 17 (late) days after the last stimulation cells were isolated for scRNAseq, which was subsequently analysed for differential abundance and differential gene expression.
Results: At the early timepoint, IL-13 stimulation results in fewer ciliated, deuterosomal and goblet cells. In healthy PBECs, this coincides with an increase of basal resting cells, whereas in asthma an increase in suprabasal cells is observed. At the late timepoint, IL-13 stimulation induced an increase in goblet cells and a decrease in club cells, effects that were both larger in asthma. Differential gene expression analysis reveals repressed cilia development and chemokine production, which persist on both asthma and healthy PBECs at the late timepoint.
Conclusion: Five-day IL-13 stimulation of differentiated ALIs results in epithelial cell dedifferentiation after three days in both asthma and control PBECs. The larger increase in goblet cell proportions two weeks after stimulation suggests prolonged effects of IL-13 in asthma PBECs compared to controls. Thus, these results will help to explain the altered airway epithelial composition in patients with asthma.

Abstract no: 37

Increased presence of ferroptosis features in lungs of patients with Chronic Obstructive Pulmonary Disease (COPD)

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Background: Chronic obstructive pulmonary disease (COPD) is a chronic, degenerative lung disease, mainly caused by cigarette smoke (CS) and characterized by obstructive bronchiolitis and the development of pulmonary emphysema. Excessive cell death may be responsible for the loss of alveolar walls and the inflammation observed in emphysema. Ferroptosis, an iron-dependent form of regulated necrosis, is characterized by lipid peroxidation that leads to oxidative stress and cell death. The aim of this study was to assess the presence of ferroptosis markers in lungs of patients with COPD. Glutathione peroxidase 4 (GPX4) is a regulatory molecule able to convert toxic lipid peroxides into non-toxic lipid alcohols, thereby limiting the overwhelming iron-dependent production of reactive oxygen species (ROS). E06 binds oxidized phosphatidylcholine, reflecting the extent of lipid peroxidation during ferroptosis.
Materials & methods: Markers of ferroptosis were quantified in lung tissue samples from never-smokers, smokers without airway obstruction and patients with COPD GOLD stage II and GOLD stage IV. mRNA expression levels of GPX4 were quantified by RT-qPCR in whole lung tissue. Protein levels of GPX4 were quantified in airway epithelium by immunohistochemistry (IHC). Lipid peroxidation was quantified in macrophages of human lung tissue by E06 staining. In addition, lipid peroxidation was measured by C11-BODIPY *in vitro* cultured primary human bronchial epithelial cells (pHBECs), stimulated with increasing percentages of cigarette smoke extract (CSE) or the ferroptosis inducer RSL3.
Results: mRNA expression levels of GPX4 were significantly increased in lung tissue of smokers without COPD and patients with COPD compared to never smokers. GPX4 protein levels significantly increased in airway epithelium of COPD patients compared to never-smokers and smokers without COPD. Moreover, both mRNA and protein levels of GPX4 were significantly higher in patients with COPD GOLD stage IV, compared to patients with GOLD stage II. E06 staining revealed a significant higher expression in lung macrophages of current smokers without COPD and current smoking patients with COPD compared to never-smokers. In the *in vitro* study, CSE and RSL3 significantly induced lipid peroxidation in human bronchial epithelial cells
Conclusion: These data are indicative of an increased presence of ferroptosis in lungs of patients with COPD. More research is needed to demonstrate the role of ferroptosis in the pathogenesis of COPD.

Abstract no: 39

Serum levels and genetic variants of CC16: Associations with asthma and lung function development in childhood and adolescence

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Rationale: Club cell secretory protein 16 (CC16) is a uteroglobin secreted by non-ciliated Club cells in the small airways, which may function to mitigate airway inflammation. Serum CC16 levels were shown to associate with asthma and lung function levels during development. However, it is uncertain if the association between CC16 and lung function development differs in subjects with and without asthma.
Measurements: Serum CC16 testing was performed using ELISA testing in the PIAMA cohort (NL) at ages 4, 8, 12 and 16. We tested lung function by interrupter resistance (RINT) at age 4 and spirometry at ages 8, 12 and 16.
Methods: We investigated the cross-sectional association between ln-transformed serum CC16 and asthma during childhood (ages 4-8) and adolescence (ages 12-16). We also investigated the association between serum CC16 and lung function levels at each time-point in addition to lung function change between the ages of 8, 12 and 16 with stratification for asthma status. Subsequently, we performed a GWAS analysis to identify SNPs associated with serum CC16 which were investigated for differential association with lung function based on asthma status.
Main Results: Higher serum CC16 was associated with a lower OR for asthma during adolescence (OR 0.52 (0.30, 0.89) p=0.017). Furthermore, higher serum CC16 levels were associated with lower airway resistance at age 4 in subjects with asthma (β -0.18, 95% CI (-0.32, -0.04)) and higher resistance in subjects without asthma (β 0.08, 95% CI (0.01, 0.15), p-interaction: 0.002). Serum CC16 levels at ages 12 and 16 were positively associated with annual change in FEV1 % pred. and FEV1/FVC % pred. between the ages of 8 and 16, with similar estimates in subjects with and without asthma. We identified 2 SNPs (rs10836314, allele A rs502857 allele C) which associated with serum CC16 levels and also associated with greater airway resistance at age 4 in subjects with asthma. The risk allele for rs3741240 and rs2509956 were also associated with a lower annual increase in FEV1/FVC % pred (p=0.002) between the ages of 8 and 16.
Conclusion: Serum levels and genetic variance of CC16 levels are associated with airway resistance during childhood and a lower lung function development during childhood adolescence. The association between CC16 and lung function is differs depending on asthma status.

Abstract no: 40

Respiratory viral infections cause lipid peroxidation in lung epithelial cells

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Introduction: Influenza A virus (IAV) and human adenovirus type C5 (HAdV-C5) are two common causes of respiratory infections that can lead to severe illness in high-risk groups. Both viruses have been linked to exacerbation in patients with underlying chronic respiratory diseases, such as COPD. IAV are enveloped, single-stranded RNA viruses that cause seasonal influenza. Contrastingly, HAdV-C5 are non-enveloped, contain double-stranded DNA genome and have been known to cause acute respiratory illness. The infection mechanism of these two viruses differs greatly, but both viruses can infect the epithelial cells of the upper and lower respiratory tract and cause cell death, leading to loss of lung tissue. Recent studies show that swine influenza A virus drives the cells to a ferroptosis-like cell death to enhance its replication[1]. The aim of this study is to investigate the involvement of ferroptosis during two different respiratory viral infection in bronchial and alveolar epithelial cells.
Material and methods: Bronchial (BEAS-2B) and alveolar (A549) epithelial cells were infected with IAV A/PR8/(H1N1) or HAdV-C5 at a multiplicity of infection (MOI) of 1 and treated with ferroptosis inducer, RSL-3 (200 nM). Ferrostatin (Fer-1, 5 μM) pre-treatment was added 1h before infection. Infected and control cells were collected 24 hours post-infection (h.p.i.). Malondialdehyde (MDA), a marker of lipid peroxidation, was quantified following the thiobarbituric acid reactive substances assay (TBARS)[2].
Results and discussion: IAV and HAdV-C5 infections cause a significant increase in lipid peroxidation in both bronchial and alveolar epithelial cells. Pre-treatment with Fer-1, a ferroptosis inhibitor, during 1 hour prior to the infection reduces the lipid peroxidation in the infected alveolar epithelial cells, but no significant reduction was observed in the bronchial epithelial cells pre-treated with Fer-1. This might indicate that alveolar epithelial cells are more susceptible to lipid peroxidation caused by viral infection.
Conclusion: Influenza A virus and adenovirus type C5 infections cause an increase in lipid peroxidation in bronchial and alveolar epithelial cells. Lipid peroxidation is a marker of ferroptotic cell death, therefore these results suggest ferroptosis is involved during respiratory viral infections and it contributes to cell death of bronchial and alveolar epithelial cells.

Abstract no: 41

TNF signaling plays a role in lipopolysaccharide (LPS) induced lung epithelial repair response

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Introduction: Respiratory infections, particularly those caused by bacterial pathogens, pose a significant global health threat and are a leading cause of mortality. Patients with chronic obstructive pulmonary disease (COPD) and asthma are particularly vulnerable to exacerbations of their condition due to bacterial infections. However, the mechanisms underlying bacterial-induced inflammation and tissue repair in the lungs remain poorly understood. In this study, we aimed to investigate the impact of lipopolysaccharide (LPS), a component of Gram-negative bacteria, on murine lung organoids to better understand these mechanisms.
Material and method: Lung epithelial cells (CD31-CD45-Epcam+) were isolated from either wild-type mice or TNF receptor 1/2 knock-out mice and co-cultured with wild-type mouse CCL206 fibroblasts. Following 14 days of exposure to 100 ng/ml LPS, organoid numbers and size were measured. Immunofluorescence analysis was performed to evaluate the differentiation of organoids, using pro-surfactant protein C (SPC) and acetylated α tubulin (ACT) as markers for type 2 alveolar cells and ciliated cells, respectively. After 3 days of exposure to 100 ng/ml LPS, organoids were resorted into Epcam+ cells and fibroblasts for bulk RNA sequencing analysis. Organoids treated with LPS for 24 hours on day 14 had their medium collected for measuring mouse KC secretion (the homologue of human interleukin-8) using ELISA.
Results and discussion: LPS treatment increased both the number and size of organoids, with a higher abundance of SPC+ organoids observed, indicative of alveolar-type organoids. This suggests that LPS affects the activation of epithelial progenitor cells and the proliferation and differentiation of lung organoids. Furthermore, LPS stimulation led to elevated secretion of the pro-inflammatory cytokine KC. Bulk RNA sequencing analysis showed that LPS upregulated several inflammatory and fibrosis-related markers in both Epcam+ cells and fibroblasts, including Cxcl3, Cxcl5, Ccl20, Mmp13, and Il33. Gene enrichment analysis revealed that LPS enriched several pathways, including TNF-α signaling via NF-κB and epithelial-mesenchymal transition pathways. TNF receptor 1, but not TNF receptor 2, knock-out mice derived organoids showed no difference in organoid characteristics compared to controls, indicating an inhibitory effect of LPS on epithelial progenitor cell activation and organoid proliferation and differentiation, particularly when TNF receptor 1 is deficient.
Conclusion: Overall, these findings suggest that LPS induces lung epithelial repair by stimulating epithelial progenitor cell activation, organoid proliferation, and inflammatory cytokine secretion. The TNF signaling pathway appears to play a role in this process, highlighting its importance in LPS-triggered lung epithelial repair.

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