Epithelial science congress highlights: The American Thoracic Society (ATS) International Conference

May 13–18, 2022 | San Francisco

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UNDERSTANDING THE CENTRAL ROLE OF THE EPITHELIUM IN SEVERE ASTHMA AND BEYOND

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Contents

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Aims

- These slides cover **congress highlights** from abstracts that were presented at ATS 2022
- The abstracts were carefully selected to include data that further the understanding of **epithelial science;** this is not an inclusive list of all abstracts

Permissions

- Authors of each abstract were notified that their data will be included in this report
- Please note that **the key takeaways** are not corroborated by these authors, but **were developed** based on the data presented within the abstracts **for the purposes of this report**

Conference details

- Please note this report was **developed specifically for EpiCentral** and independent of the congress
- The ATS international conference was held on May 13–18, 2022 in San Francisco
- Please visit ATS for more information

Report sections

ATS, American Thoracic Society

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Key takeaways

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- Exposure of the epithelium to viral insults can trigger changes in gene expression that may promote asthma exacerbations or asthma development[1,2]
- Differences in clinical biomarkers and gene expression in bronchial epithelial cells, bronchoalveolar lavage, and blood demonstrated the heterogeneity of patients with asthma, and distinguished endotypes that may be associated with disease burden[3,4]
- TSLP may have a role in direct immune responses to viral infection in pediatric asthmatic airways,[5] while nELF cytokines may offer a non-invasive tool to investigate the underlying pathobiology in pediatric asthma[6]
- IL-25 may play a protective role against ASM hypercontractility and bronchoconstriction in asthma;[7] also peripheral blood Eos levels may be a determinant of increased AHR in adult allergic asthma[8]
- Airway remodeling contributes to asthma, with Kp/KISS1R signaling being important in regulating ASM cell migration[9] while bronchoconstriction alone could be sufficient to induce airway remodeling in asthmatic airways[10]
- In murine models, data suggest that frequent exposure to aeroallergens eilicit lung cellular and molecular circuits that trigger neutrophilic asthma,[11] while iNKTs may have a role in T2 inflammation after exposure to fungal allergens.[12] Further research is warranted to understand if these preclinical findings will translate to humans

AHR, airway hyperresponsiveness; ASM, airway smooth muscle; Eos, eosinophils; IL, interleukin; iNKT, invariant natural killer T cell; KISS1R, kisspeptin receptor; Kp, kisspeptin; nELF, nasal epithelial lining fluid; T2, Type 2; TSLP, thymic stromal lymphopoietin

1. Newcomb DC, et al. Poster P973 presented at ATS 2022 (Abstract A3245); 2. Lai Y, et al. Poster P506 presented at ATS 2022 (Abstract A3745); 3. Camiolo M, et al. Poster P547 presented at ATS 2022 (Abstract A3925); 4. Goldfarbmuren KC, et al. Poster P509 presented at ATS 2022 (Abstract A3478); 5. Chorvinsky E, et al. Presentation session B15 presented at ATS 2022 (Abstract A2361); 6. Qiu AY, et al. Presentation session A95 at ATS 2022 (Abstract A2140); 7. Xiong D, et al. Poster P1021 presented at ATS 2022 (Abstract A3281); 8. Imaoka A. Poster P728 presented at ATS 2022 (Abstract A1247); 9. Balraj P, et al. Poster P712 presented at ATS 2022 (Abstract A1229); 10. Mwase C, et al. Poster P980 presented at ATS 2022 (Abstract A3252); 11. Shenoy AT, et al. Poster P512 presented at ATS 2022 (Abstract A3751); 12. Azdale Garcia N, et al. Poster P508 presented at ATS 2022 (Abstract A3747)

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Human and *in vitro* data

Role of the epithelium in asthma

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Early-life RSV infection alters nasal airway epithelial cell development

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- RSV infection during infancy (<1 year) is associated with development of asthma, and early-life RSV infection may lead to metabolic reprogramming and impact development of NAECs
- Single-cell RNA sequencing was performed on differentiated NAECs from healthy 2–3-year-old children with and without early-life (<1 year) RSV infection; data were confirmed by qPCR and metabolic profiling of differentiated NAECs infected with RSV *in vitro* (n=9)

Newcomb D.C.
Med, Vanderbilt Univ, Nashville, TN, USA

- NAECs from children with early-life RSV infection had a slower doubling rate compared with NAECs from controls
- **Compared with NAECs from controls, NAECs from children with early-life RSV infection had:**

↑ Keratin 5-expressing basal cells	↑ Expression of extracellular matrix genes (*TNC*, *VCAN*, *FN1*)	↓ Expression of genes important in tight junctions (*TJP1*)	↓ Goblet cells expressing *MUC5AC* and *MUC5B*	↑ Expression of *ALDOA* and *HK2* markers of metabolism

- *In vitro*, RSV infection of differentiated NAECs confirmed increases in *ALDOA* expression and showed RSV-induced *ALDOA* increases were glucose-dependent

Key takeaway: *RSV infection during infancy may decrease NAEC differentiation and reduces measures of epithelial barrier function, which could increase susceptibility to aeroallergens and environmental factors that lead to asthma*

ALDOA, aldolase gene; *FN1*, fibronectin 1 gene; *HK2*, hexokinase 2 gene; *MUC5AC*, mucin 5AC gene; *MUC5B*, mucin 5B gene; NAEC, nasal airway epithelial cell; qPCR, quantitative polymerase chain reaction; RNA, ribonucleic acid; RSV, respiratory syncytial virus; *TJP1*, tight junction protein 1 (Zo-1) gene; *TNC*, tenascin C gene; *VCAN*, versican gene

Newcomb DC, et al. Poster P973 presented at ATS 2022 (Abstract A3245)

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Primary infection of mast cells with human rhinovirus A16 induces T2 gene expression via an autocrine loop

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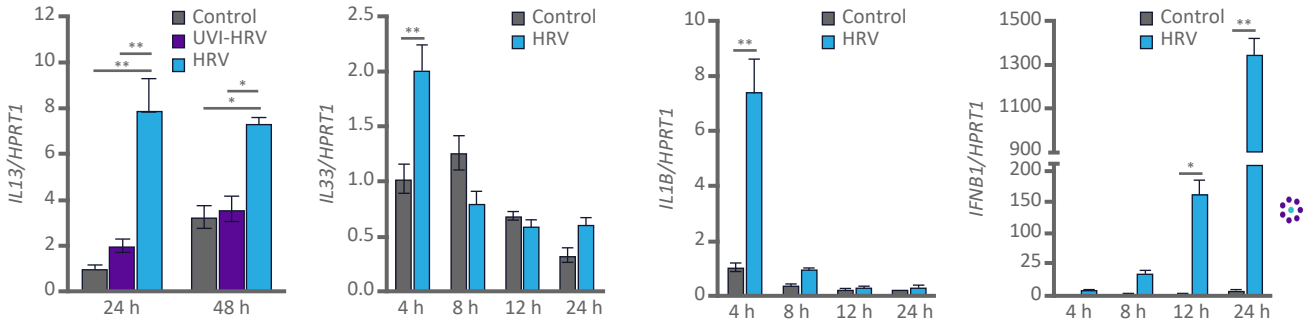
Lai Y.
Department of Medicine, University of Washington, Seattle, WA, USA

- Lower RTI with HRVs are common triggers for asthma exacerbations and have been implicated in asthma development
- Mast cells are a source of T2 cytokines, which can be produced in response to airway epithelial cell-derived cytokines such as IL-33 and IL-1β, and also express the ICAM-1 receptor necessary for HRV binding and cell entry
- qPCR was used to compare gene expression in HRV A16-infected and uninfected mast cells

Mast cell gene expression following HRV infection



- Increased *IL13* expression was induced at 24 h and 48 h after HRV infection compared with that induced by UV-inactivated virus
  - *IL13* expression was significantly attenuated by addition of blocking antibodies against IL-33, IL-1β or IFN-β at 24 hours following HRV infection, and was partially attenuated by coculture with airway epithelial cells
- There was increased expression of *IL33* and *IL1B1* at 4 h post HRV infection, and increased *IFNB1* expression at 12 h and 24 h
  - *IFNB1* expression in mast cells was enhanced by coculture with airway epithelial cells

Key takeaway: *HRV infection of intra-epithelial mast cells may play a key role in viral-induced acute asthma exacerbations and sustained airway inflammation via production of T2 cytokines as well as type 1 interferons*

*$P<0.001$, **$P<0.0001$

h, hours; HRV, human rhinovirus; *HPRT1*, hypoxanthine phosphoribosyltransferase 1 gene; ICAM, intercellular adhesion molecule; *IFNB1*, interferon beta 1; IL, interleukin; qPCR, quantitative polymerase chain reaction; RTI, respiratory tract infection; T2, Type 2; UV, ultraviolet; UVI-HRV, UV-inactivated HRV

Lai Y, et al. Poster P506 presented at ATS 2022 (Abstract A3745)

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Mucin expression in human precision-cut lung slices

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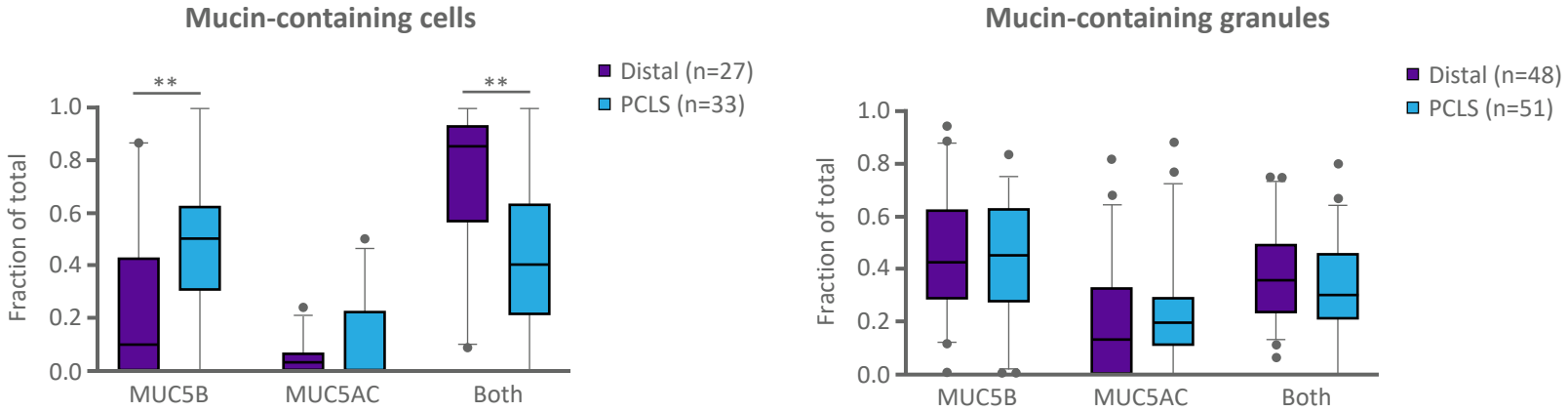
- Small airways (<1 mm diameter) are critical sites of mucus dysfunction in diseases such as asthma
- Precision-cut lung slices (PCLS)* from healthy human lungs were compared with small airways from dissection of fresh tissue to determine if they can be used to study mucin production and secretion in small airways

Hoang O.N.
The University of Texas MD Anderson Cancer Center, Houston, TX, USA

- Immunofluorescence microscopy showed that the mucins *MUC5B* and *MUC5AC* are expressed in PCLS cells
- The proportions of secretory granules containing MUC5B and MUC5AC were comparable between PCLS and freshly isolated distal airways

Mucin localization within cells and granules from PCLS and freshly isolated tissue



Boxes show median and IQR; whiskers show 5th and 9th percentiles; dots show outliers; n represents number of images analysed

Key takeaway: *Mucin expression and packaging within secretory granules of PCLS are similar to those in freshly isolated lung tissue, providing a new platform for studying cellular mechanisms mediating mucin expression and secretion by the human airway epithelium, which could be used to help to understand mucus dysfunction in small airway diseases such as asthma*

*Slices (0.35 mm) from donated lungs inflated with low gelling-temperature agarose were cultured for 2–3 weeks, and then slices containing a small airway (200–1000 µm diameter) with beating cilia were fixed with 4% paraformaldehyde and paraffin embedded; ***P*<0.001
IQR, interquartile range; MUC, mucin; PCLS, precision-cut lung slices
Hoang ON, et al. Poster P982 presented at ATS 2022 (Abstract A5653)

Complexity of severe asthma

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Multi-compartment clustering of asthma patients using network-based transcriptional profiling

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- Integration of molecular phenotype data from multiple compartments offers the opportunity to better understand heterogeneity in severe asthma
- Gene network assembly was performed independently for blood, bronchial epithelial cell, and bronchoalveolar lavage transcriptional data sets from healthy controls and patients with mild-to-moderate and severe asthma, and gene expression data across these compartments were used to cluster patients with asthma using unsupervised machine learning

Camiolo M.
Pulmonary Medicine, UPMC, Pittsburgh, PA, USA

- Patient clustering broadly recapitulated observed clinical inflammatory phenotypes across the T2 immune axis as gauged by FeNO, blood eosinophil count, and bronchial epithelial cell gene expression

Asthma endotype	T2-high (~60% of cohort)		T2-low
Asthma control	Well controlled	High symptom burden and impaired lung function	Not reported
Transcription profile in bronchial epithelial cells, bronchoalveolar lavage, and blood	No hallmarks of intra-epithelial cytotoxic T cells	Hallmarks of intra-epithelial cytotoxic T cells	Systemic inflammatory response featuring prominent neutrophil activation

Key takeaway: *Critical differences in bronchial epithelial cell, bronchoalveolar lavage, and blood gene expression distinguish patients with well-controlled T2-high asthma from those with impaired lung function and high symptom burden, and from patients with T2-low asthma*

FeNO, fractional exhaled nitric oxide; T2, Type 2

Camiolo M, et al. Poster P547 presented at ATS 2022 (Abstract A3925)

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Heterogeneity in clinically and molecularly defined airway type 2 inflammation among children with exacerbation-prone asthma

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- T2 endotype classifications based on common clinical biomarkers (blood eosinophil count, FeNO, and serum IgE) were compared with classifications based on T2 inflammation-related nasal airway gene expression networks,* within a cohort of children without asthma (n=48), with asthma and prone to exacerbations (n=53), and those with exacerbation-resistant asthma (n=25)

Goldfarbmuren K.C.
Genetics, National Jewish Health, Denver, CO, USA

- Children with asthma and prone to exacerbations exhibited elevated IgE, blood eosinophils, and FeNO levels relative to controls and those with exacerbation-resistant asthma
  - Hierarchical clustering of subjects based on any one of these measures revealed subgroups with low, moderate, or high trait levels
  - Children with asthma and prone to exacerbations were more prevalent in 'high' groups for all three measures, but the individuals assigned 'high' for each measure only partially overlapped

	Group	Asthma exacerbation-prone	Asthma exacerbation-resistant	Controls
% children in group based on clustering by blood eosinophil count, FeNO, and serum IgE	High	43	20	15
	Moderate	42	32	48

- Several transcriptional networks whose eigengene expression is significantly correlated with at least one T2 clinical measure, including a classic epithelial T2 response network and independent modules corresponding to eosinophil, mast cell, and mucus production
- Hierarchical clustering of patients by both clinical measures and transcriptional eigengenes revealed asthmatic sub-endotypes with different cellular and molecular aspects of airway T2 inflammation

Key takeaway: *T2 inflammation is complex and heterogeneous across children with asthma who are prone to exacerbations*

*Data from whole transcriptome RNA-sequencing on nasal brushings from a subset of the overall cohort (n=72)
FeNO, fractional exhaled nitric oxide; IgE, immunoglobulin E; RNA, ribonucleic acid; T2, Type 2
Goldfarbmuren KC, et al. Poster P509 presented at ATS 2022 (Abstract A3478)

Epithelial cytokines and the inflammatory cascade

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Nasal epithelial lining fluid (nELF) cytokines as biomarkers to characterize T2-high asthma in children

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- Understanding cytokine profiles in childhood asthma helps to define asthma phenotypes as well as to identify future biomarkers
- Alongside blood Eos count and FeNO, non-invasive measurement of T1 and T2 cytokines* in nasal epithelial lining fluid was used to characterize asthma endotypes in children (n=84; 8–17 years; 45% female, 88% African American)

Qiu A.Y.
Division of Pulmonary and Critical Care Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA

- Children with elevated blood Eos or FeNO had elevations in several T2 cytokines and reduced T1 cytokines
- Nasal epithelial lining fluid cytokine measurements associated with blood Eos, FeNO, and asthma control:

Comparison of asthma phenotypes	**Blood Eos ≥300 cells/µL compared with blood Eos <300 cells/µL**	**FeNO >35 ppb compared with FeNO ≤35 ppb**	**ACT ≤19 (poor control) compared with ACT >19 (good control)**
Associated T2 cytokines†	↑ IL-5, eotaxin-3, MCP-4, and TARC ↓ IL-13	↑ IL-5, eotaxin-3, MCP-4, and TARC ↓ IL-4, IL-13, and MCP-1	↑ IL-5 and eotaxin-3
Associated non-T2 cytokines†	↓ TNF-α, IL-1β, IL-2, and IL-8	↓ IFN-γ, TNF-α, IL-1β, IL-2, IL-6, IL-8, and IL-17	Not significant

Key takeaway: *Nasal epithelial lining fluid may offer a non-invasive tool to expand asthma endotyping and aid understanding of inflammatory phenotypes of childhood asthma*

*Measured via multiplex electrochemiluminescence ELISA; †$p<0.05$

ACT, Asthma Control Test; ELISA, enzyme-linked immunosorbent assay; Eos, eosinophils; FeNO, fractional exhaled nitric oxide; IFN, interferon; IL, interleukin; MCP-4, monocyte chemotactic protein 4; Ppb, parts per billion; TARC, thymus and activation-regulated chemokine; T1, Type 1; T2, Type 2; TNF, tumor necrosis factor

Qiu AY, et al. Presentation session A95 at ATS 2022 (Abstract A2140)

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High TSLP responses in the pediatric airway epithelium are linked to a global pro-inflammatory state instead of type 2 polarization

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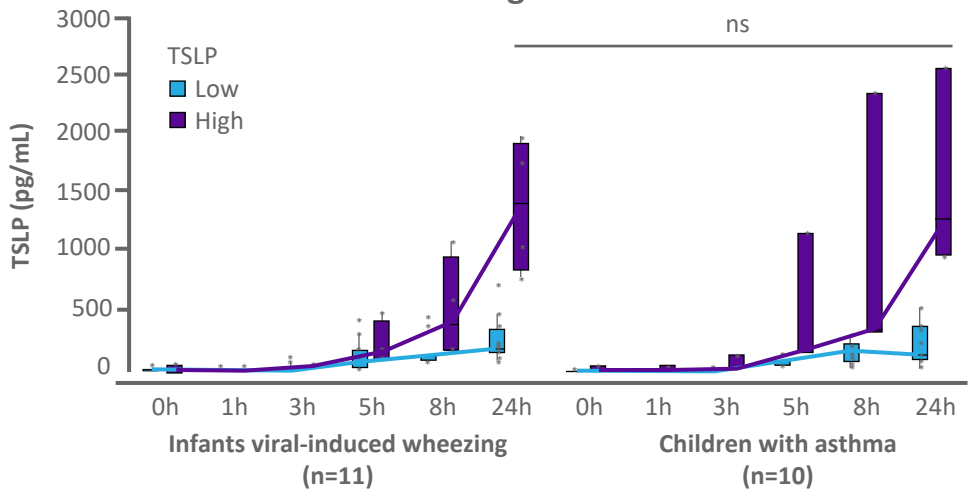
Chorvinsky E.
Center for Genetic Medicine Research, Children's National Hospital, Washington, DC, USA

- High TSLP AEC production is linked to increased asthma severity and has been shown to lead to allergic polarization of the immune response in adults with asthma
- AEC responses to viral mimics (dsRNA) in infants and young children with high TSLP production were evaluated
- Nasal and bronchial AEC lines (n=28) were derived from infants with a history of viral-induced wheezing (n=12, aged 1–12 months) and children with asthma (n=11, aged 2–16 years)
- High TSLP was defined as levels ≥75th percentile

- All AEC lines exhibited prominent dsRNA-induced production on TSLP, which was not significantly different in infants with a history of viral-induced wheezing vs children with asthma
- **Individuals with high TSLP levels had:**
  - ↑ Pro-inflammatory cytokines
  - ↑ T2 chemokines
  - ↑ Antiviral cytokines [IFN type I (β) and IFN type III (λ1)]
- These data suggest that infants and children with a history of severe viral-induced respiratory illnesses have high levels of TSLP in response to viruses

TSLP secretion after exposure to dsRNA in infants with viral-induced wheezing and children with asthma



Key takeaway: *TSLP may have a role in the immune response in pediatric airways to viral infection as well as in allergic responses*

AEC, airway epithelial cell; dsRNA, double-stranded ribonucleic acid; IFN, interferon; T2, type 2; TSLP, thymic stromal lymphopoietin

Chorvinsky E, et al. Presentation session B15 presented at ATS 2022 (Abstract A2361)

Airway hyperresponsiveness

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IL-25 alters human airway smooth muscle responsiveness to isoproterenol

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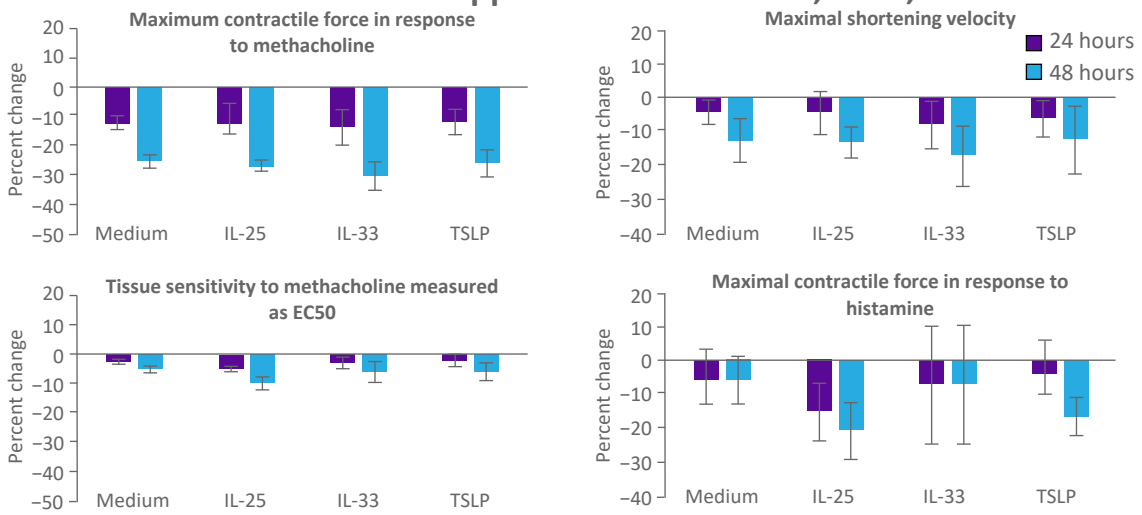
- The alarmins IL-25, IL-33, and TSLP are secreted from the airway epithelium and various immune cells in response to cellular stress
- Alarmins have the potential to activate various inflammatory pathways, leading to the initiation of asthma pathogenesis. Patients with asthma have increased levels of alarmins in bronchoalveolar lavage and blood plasma. The study aimed to evaluate the effects of alarmins on ASM contractility

Xiong D.
Division of Experimental Medicine, McGill University, Montreal, QC, Canada

- In-vitro mechanics of post-mortem asthmatic human lung tissue were measured at 24 and 48 hours after dissection and incubation with individual alarmins:
  - Changes in ASM contractile force, shortening velocity, and response to contractile agonists/stimulants methacholine, histamine, and potassium chloride with isoproterenol were assessed
- Exposure to alarmins did not have any effect on the contractile force or in the velocity of shortening
- Tissue exposed to IL-25 produced a significantly increased relaxation compared with the controls
- A trend of decreased sensitivity to methacholine induced by IL-25 was observed

Change over 24 and 48 hours for ASM tissues incubated in control medium or medium supplemented with Il-25, IL-33, or TSLP



Key takeaway: *IL-25 may play a protective role against ASM hypercontractility and bronchoconstriction in asthma*

ASM, airway smooth muscle; IL, interleukin; TSLP, thymic stromal lymphopoietin

Xiong D, et al. Poster P1021 presented at ATS 2022 (Abstract A3281)

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Eosinophil as a determinant of airway hyperresponsiveness in allergic asthma with elevated blood eosinophils

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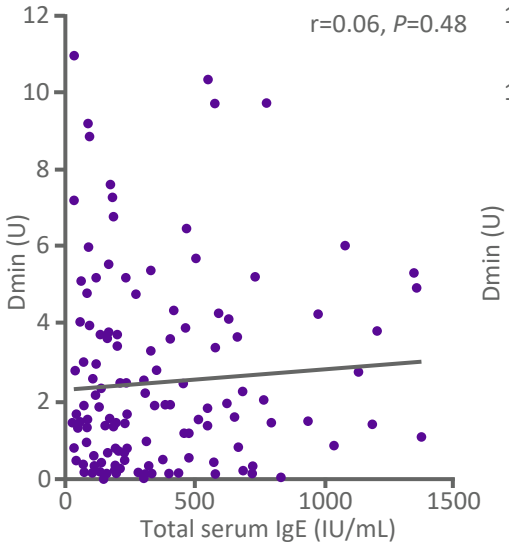
Imaoka A.
Matsuyama Kinen Hospital, Matsuyama, Japan

- Both IgE and eosinophils play important roles in the pathogenesis of asthma; however, it remains unclear which of the two biomarkers is the main driver of allergic asthma when elevated blood Eos levels are present
- A total of 131 steroid-naïve Japanese adults (59 male and 72 female, aged 20–92 years) with allergic asthma and peripheral blood Eos ≥150 cells/µL were recruited

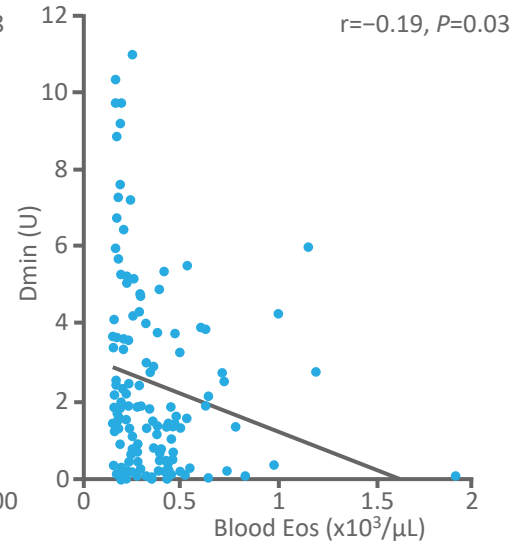
- AHR was measured using continuous methacholine inhalation method (Astrograph). The cumulative dose of inhaled methacholine measured at the inflection point at which respiratory conductance starts to decrease (Dmin) was used as an index of AHR
- Dmin was calculated so that 1 U of Dmin equalled 1 minute of inhalation of aerosol solution at 1.0 mg/mL during quiet breathing
- Dmin values were correlated retrospectively with total serum IgE levels and peripheral blood Eos counts

- Dmin values were not significantly correlated with total serum IgE levels (r=0.06, *P*=0.48)
- Dmin values were weakly negatively correlated with peripheral blood Eos counts (r=−0.19, *P*=0.03)

Relationship between total serum IgE and Dmin



Relationship between peripheral blood Eos and Dmin



Key takeaway: *The results suggest that peripheral blood Eos, and not serum IgE, is a determinant of increased AHR in adults with allergic asthma and elevated blood Eos levels. Further mechanistic studies are warranted to explore the correlation between blood Eos levels and induction of AHR*

AHR, airway hyperresponsiveness; Dmin, the cumulative dose of inhaled methacholine measured at the inflection point at which respiratory conductance starts to decrease; Eos, eosinophils; IgE, immunoglobulin E

Imaoka A. Poster P728 presented at ATS 2022 (Abstract A1247)

Airway remodeling

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Kisspeptin attenuates airway smooth muscle cell migration by regulating Rho GTPase signaling pathway

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Balraj P.
Pharmaceutical Sciences, North Dakota State University, Fargo, ND, USA

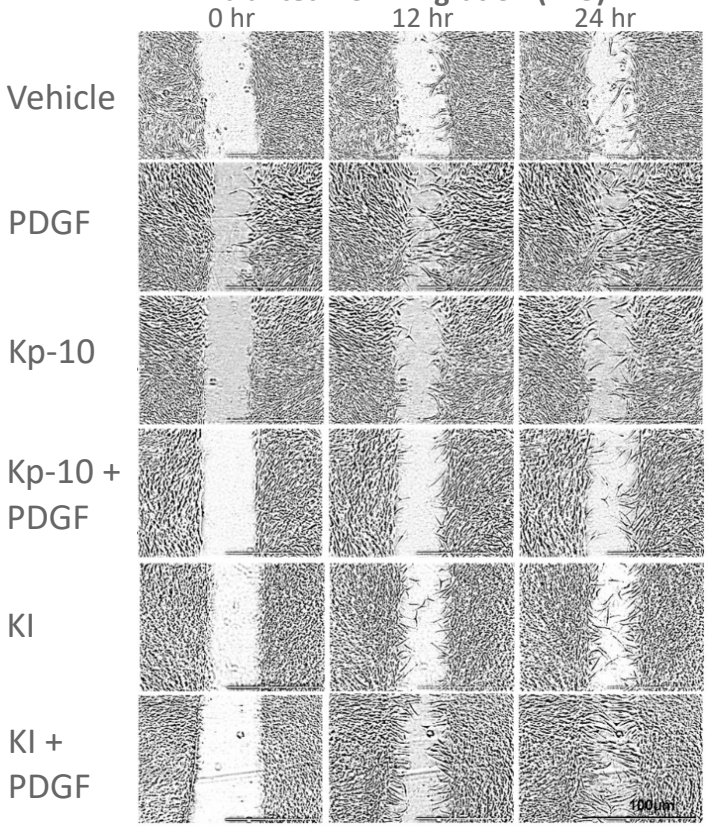
- Studies have shown that alongside hypertrophy and hyperplasia, ASM cell migration plays a key role in irreversible airway remodeling (involving structural changes in the airway wall and increased ASM cell mass) and increased AHR
- Kp is expressed in human ASM and inhibits mitogen PDGF induced ASM cell proliferation; however, the role of Kp/KISS1R in ASM cell migration via the Rho GTPase signaling pathway is unexplored

- Human ASM cells were isolated and treated with Kp-10 (KISS1R agonist), KI (KISS1R antagonist) with/without PDGF for 24 hours
- The role of Kp/KISS1R signaling on regulating actin polymerization was determined by measuring the F/G-actin ratio and KISS1R-shRNA transduced ASM cells

- Kp-10 treatment demonstrated an inhibition of actin polymerization by decreasing F/G-actin ratio (n=4)
- The inhibitory effect of Kp-10 was further confirmed by knockdown of KISS1R with lentiviral KISS1R shRNA
- Kp-10 exposure showed a significant reduction in PDGF-induced mRNA and protein expression of cell adhesion proteins like paxillin and talin compared with PDGF treated group
- Kp-10 treatment downregulates ASM RhoA-GTP and is possibly implicated in the mechanistic basis of Kp's inhibitory role in ASM migration (n=3)

Key takeaway: *Kp/KISS1R signaling is important in regulating ASM cell migration and therefore airway remodeling in asthma*

Exposure to Kp-10 for 24 hours significantly blunted ASM migration (n=5)



AHR, airway hyperresponsiveness; ASM, airway smooth muscle; KISS1R, kisspeptin receptor; Kp, kisspeptin; mRNA, messenger ribonucleic acid; PDGF, platelet-derived growth factor; RhoA-GTP, active Rho A; shRNA, short-hairpin ribonucleic acid

Balraj P, et al. Poster P712 presented at ATS 2022 (Abstract A1229)

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In human airway epithelial cells, mechanical compression induces release of extracellular vesicles containing tenascin c

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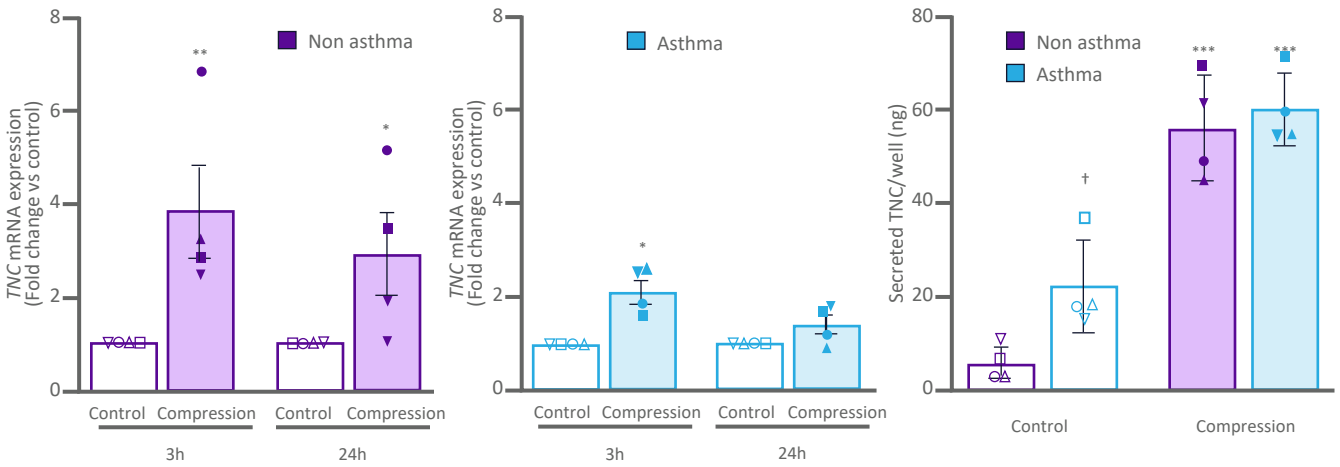
Mwase C.
Environmental Health, Harvard TH Chan School of Public Health, Boston, MA, USA

- Airway remodeling may be caused by dysregulated AECs excessively producing pathologic mediators during bronchospasm when they are compressed in the narrowed airway
- TNC is an ECM protein that remodels tissues and is highly expressed in asthmatic airways; it is differentially overexpressed in mechanically compressed airway epithelial cells
- Primary HBE cells from non-asthmatic (n=4) and asthmatic donors (n=4) were treated with either an ERK inhibitor (U0126) or a TGF-β receptor 1 inhibitor (SB431542) to determine intracellular signaling pathways of TNC production, and explore if TNC secretion can be mediated by EVs

- TNC expression was not significantly different between asthmatic and non-asthmatic cells
- The concentration of secreted TNC protein at baseline was significantly higher in asthmatic than in non-asthmatic cells
- Compression-induced TNC secretion was attenuated by inhibition of both the ERK and TGF-β pathway
- TNC was detected in EVs that were isolated from basolateral conditioned media from compressed HBE cells

Mechanical compression induces TNC mRNA expression and secretion in HBE cells



Key takeaways: *Bronchoconstriction alone may induce remodeling of the asthmatic airway. Compression of AECs induced basolateral release of EVs that contain high concentrations of TNC (an extracellular matrix protein)*

*$P<0.01$; **$P<0.001$, ***$P<0.0001$, significantly different from no compression control; †$P<0.05$ significantly different between non-asthma and asthma

AEC, airway epithelial cell; ECM, extracellular matrix; ERK, extracellular signal-regulated kinase; EV, extracellular vesicle; h, hour; HBE, human bronchial epithelial; mRNA, messenger ribonucleic acid; TGF, transforming growth factor; TNC, tenascin C

Mwase C, et al. Poster P980 presented at ATS 2022 (Abstract A3252)

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Asthmatic lung fibroblasts produce increased levels of TSLP upon TNFα stimulation via the ER stress response pathway

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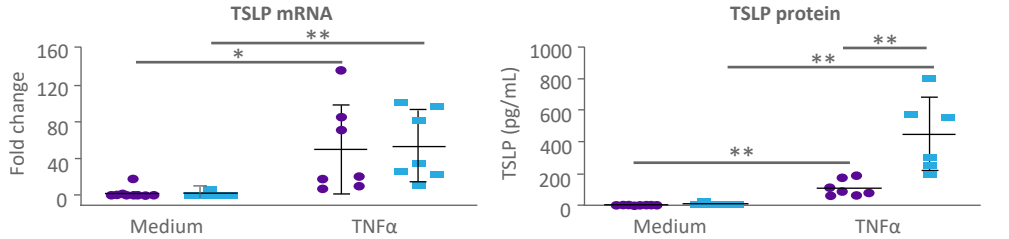
Drake LY.
Department of Anesthesiology and Perioperative Medicine, Mayo Clinic, Rochester, MN, USA

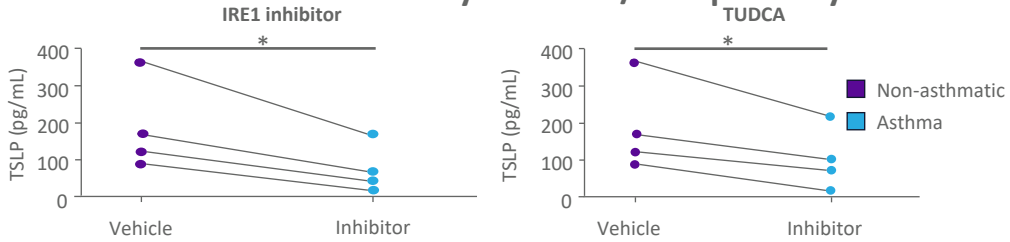
- Lung fibroblasts are an important cellular source of TSLP production in inflammatory conditions
- The study aimed to investigate if human asthmatic fibroblasts produce more TSLP than non-asthmatic fibroblasts, and the molecular mechanism underlying TSLP production in fibroblasts
- Lung fibroblasts were isolated from non-asthmatic and asthmatic human tissue, and stimulated with TNFα (a cytokine known to be upregulated in asthmatic lungs)
- The expression of TSLP and ER stress response-associated genes were examined by qRT-PCR

- TNFα induced upregulation of TSLP mRNA expression and TSLP protein secretion by lung fibroblasts
- There was no significant difference in TSLP mRNA expression in asthmatic and non-asthmatic fibroblasts; however, **asthmatic lung fibroblasts** stimulated with TNFα **had increased secretion of TSLP compared with non-asthmatic lungs**
- Lung fibroblasts stimulated with TNFα exhibited increased ER stress response-associated genes (*ATF6*, *PERK*, and *ERN1*); ***ATF6* mRNA expression was increased in asthmatic vs non-asthmatic fibroblasts**
- ER stress protein expression was dysregulated in asthmatic lung fibroblasts
- ER stress inhibitors decreased TSLP protein secretion by asthmatic lung fibroblasts

TSLP secretion is increased in asthmatic lung fibroblasts



TSLP secretion is inhibited by ER stress/UPR pathway inhibitors



Key takeaway: *TNFα activates lung fibroblasts to secrete TSLP via the ER stress response pathway. Fibroblasts from asthmatic lung tissue have an increased ER stress response to TNFα, which may contribute to higher levels of TSLP secretion by these cells*

ATF, activating transcription factor; ER, endoplasmic reticulum; *ERN1*, endoplasmic reticulum to nucleus signaling 1; mRNA, messenger ribonucleic acid; *PERK*, PKR-like ER kinase; qRT-PCR, real-time quantitative reverse transcription polymerase chain reaction; TNF, tumor necrosis factor; TSLP, thymic stromal lymphopoietin; TUDCA, sodium tauroursodeoxycholate; UPR, unfolded protein responses

Drake LY, et al. Poster P1028 presented at ATS 2022 (Abstract A3288)

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Preclinical data

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Fungal allergen induces expansion of invariant natural killer T cell in an acute innate type 2 lung inflammation

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- Skin-test positivity to *Alternaria*, a ubiquitous fungus that is abundant in indoor and outdoor environments, is linked to asthma severity
- Alternaria induces T2 inflammation in murine models, in part, via IL-33 dependent ILC2 activation; however, the role of CD1d-restricted iNKTs has not been characterized, and the natural ligands or cytokines promoting iNKT activation is unknown

Azdale Garcia N.
Laboratories at Research Institute of McGill University, Montreal, QC, Canada

- Mice were exposed to PBS or *A. alternata* extract intranasally
- Lungs were collected for flow cytometry to quantify iNKT, ILC2, total eosinophils, activated eosinophils, and neutrophils. Lung cells were restimulated with IL-7, α-GalCer, lipid antagonist DPPE-PEG350, and IL-33; the released cytokines and chemokines were then quantified by multiplex ELISA

- Lung cells cultured with α-GalCer produced large amounts of IL-4, IL-13, and IL-17, which are cytokines characteristically produced by iNKT phenotypes
- **Exposure to *A. alternata* resulted in**:
  - ↑ Eosinophil influx and activation
  - ↑ CD4+ and CD8-CD4- (double negative) iNKTs, including those producing IL-4 and IL-13
  - ↑ ILC2 producing IL-5 and/or IL-13
- iNKT inhibited the production of IL-5
- Inhibition of IL-4 production by DPPE-PEG350 demonstrated that ILC2 may promote iNKT activation in a CD1d dependent mechanism
- ILC2 may also present endogenous lipids to increase iNKT activation as observed in co-cultures without α-GalCer

Key takeaways: *A single exposure to Alternaria results in activation of ILC2 and iNKTs, and increases eosinophilic lung inflammation. Enhanced expansion of functional CD1d+ILC2 and iNKTs suggests that these cells may interact to promote a T2 inflammatory environment in the lung in response to fungal allergens*

α-GalCer, α-galactosylceramide; CD, cluster of differentiation; DPPE, dipalmitoylphosphatidylethanolamine; ELISA, enzyme-linked immunosorbent assay; IL, interleukin; ILC2, type 2 innate lymphoid cells; iNKT, invariant natural killer T cell; PBS, phosphate-buffered saline; PEG, polyethylene glycol; T2, type 2; TCR, T cell receptor

Azdale Garcia N, et al. Poster P508 presented at ATS 2022 (Abstract A3747)

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Characterization of neutrophilic responses in a pollutant-aggravated asthma mouse model

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- DEP are the main component of traffic-related pollutants, and are implicated in both the development of asthma and in asthma exacerbations
- Neutrophils may contribute to inflammation by releasing NETs (networks of DNA and granule proteins such as NE and MPO); however, it is unknown how neutrophils modulate the DEP-aggravated airway inflammation
- Murine models were exposed to DEP, HDM, and DEP+HDM
- The exposed lung tissues were evaluated to quantify neutrophils and detection of NETs; HBECs were also exposed *in vitro* to detect expression of chemokines

De Volder J.
Ghent University, Ghent, Belgium

- **Neutrophil numbers were significantly higher** in BALF and lung tissue **in combined HDM+DEP-exposed mice** compared with the control groups, and were positively correlated with eosinophils
- **Neutrophil attracting chemokines CXCL1, CXCL2 and CXCL5 were significantly increased after HDM+DEP exposure** in both BALF and mRNA in the lungs
- dsDNA and NE were significantly elevated in BALF of HDM+DEP mice; NETs were also detectable in lung tissue
- After HDM+DEP exposures HBECs showed an increased mRNA expression of CXCL1 and CXCL8 compared with the controls

Key takeaway: *Exposure to HDM+DEP induced neutrophilic responses in both murine models and HBECs. The neutrophilic responses correlate with eosinophilic inflammation which suggests an interaction between neutrophils and eosinophils in pollutant-aggravated allergic asthma*

BALF, bronchoalveolar lavage fluid; CXCL, chemokine (C-X-C motif) ligand; DEP, diesel exhaust particles; HBEC, human bronchial epithelial cell; HDM, house dust mite; MPO, myeloperoxidase; mRNA, messenger ribonucleic acid; NE, neutrophil elastase; NET, neutrophil extracellular trap

De Volder J, et al. Poster P211 presented at ATS 2022 (Abstract A3572)

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Mouse model of late-onset neutrophilic asthma reveals novel cellular and molecular circuits underlying destructive airway neutrophilia

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- Late-onset asthma often presents as severe, steroid-resistant, lung-damaging neutrophilic disease with poor outcomes
- Conventional mouse models of allergic asthma were used to study neutrophilic allergic disease

Shenoy AT.
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- Conventional mouse models were exposed to ovalbumin; extensively exposed mice exhibited neutrophilic inflammation
- Intracellular cytokine and TF staining demonstrated that mice with neutrophilic allergic airways disease expressed diverse clusters of lung-resident CD4+ TRM cells including a novel RORγt-negative IL-17A+ Th17 subset
- The RORγt-negative Th17 cells rapidly secreted IL-17A on antigenic reencounter, which in turn caused lung epithelial and stromal cells to express CXCL5 and instigate neutrophil recruitment and subsequent airway inflammation

Investigation performed in mice models	Cellular and molecular outcomes
Ovalbumin exposure	
Naïve mice acutely exposed	Phenotypes of childhood-onset eosinophilic asthma
Naïve mice underwent recurrent exposure over an extended duration	Increased susceptibility to allergic airway neutrophilia
Memory recall exposure of aeroallergen experienced mice	Induced features of neutrophilic asthmatic exacerbations including rapid peribronchial neutrophilic inflammation, worsened vascular leakage, and severe lung disease
Flow- and spectral-cytometry Lungs of aeroallergen experienced mice	Extensive remodeling of the tissue-resident and recruited-myeloid and lymphocytic landscape suggesting a new altered state of tissue homeostasis in asthmatic lungs
Intracellular staining Mice with neutrophilic allergic airway disease	Increased lung-resident CD4+ TRM cells including RORγt-negative IL-17A+ Th17 subset
MHC-II ablation in aeroallergen experienced lung epithelial cells	**Epithelial antigen presentation** to CD4+ T cells **regulated severity of neutrophilic airway disease** by skewing CD4+ TRM phenotypes

Key takeaway: *Transient and frequent exposure to aeroallergens may reprogram lung cellular and molecular circuits to trigger neutrophilic asthma. A subset of pathogenic Th17 TRM cells may be a critical regulator of neutrophilic asthma*

CD, cluster of differentiation; CXCL, chemokine (C-X-C motif) ligand; IL, interleukin; MHC, major histocompatibility complex; RORγt, RAR-related orphan receptor gamma; TF, transcription factor; Th, T helper cell; TRM, tissue-resident memory T cell

Shenoy AT, et al. Poster P512 presented at ATS 2022 (Abstract A3751)

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Remodilins – a new class of small molecules that blunt human airway smooth muscle contractile protein accumulation and allergen-induced airway hyperresponsiveness in mice

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- ASM hypertrophy contributes to airway constrictor hyperresponsiveness and occurs in many patients with asthma

- A novel class of small molecules, **remodilins**, not previously known to relax ASM, was identified from a high content screen of 10000 small molecules for compounds that reduce traction force exerted by cultured human airway myocytes grown on a flexible substrate

- Force reduction may blunt transcriptional activation of SRF, required for ASM contractile protein expression. Therefore, tests were conducted on the remodilins to confirm:
  - Their ability to inhibit TGF-β-stimulated SRF activation
  - Their ability to blunt TGF-β-stimulated MYH11 and ACTA2 protein accumulation in cultured human ASM cells in a dose-dependent fashion

- One remodilin, R187, partially blocked airway constrictor hyperresponsiveness in mice with experimental airway inflammation induced by HDM administration

Key takeaway: *In mice models, remodilins are a novel class of small molecule compounds that can blunt ASM hypertrophy in vitro and AHR in vivo*

ACTA2, smooth muscle alpha-actin-2; AHR, airway hyperresponsiveness; ASM, airway smooth muscle; HDM, house dust mite; MYH11, smooth muscle myosin heavy chain 11; TGF-β, transforming growth factor-beta; SRF, serum response factor

Chen B, et al. Poster P1036 presented at ATS 2022 (Abstract A3296)

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Leptin augments human airway fibroblast invasion and IL-13-induced eotaxin production in a murine model of allergic airway disease

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- Allergic asthma is characterized by chronic airway eosinophilia and is often associated with comorbidities such as obesity
- Elevated airway levels of leptin and IL-13 in allergic asthma with comorbid obesity may stimulate profibrotic airway fibroblast functions and increase eotaxin secretion

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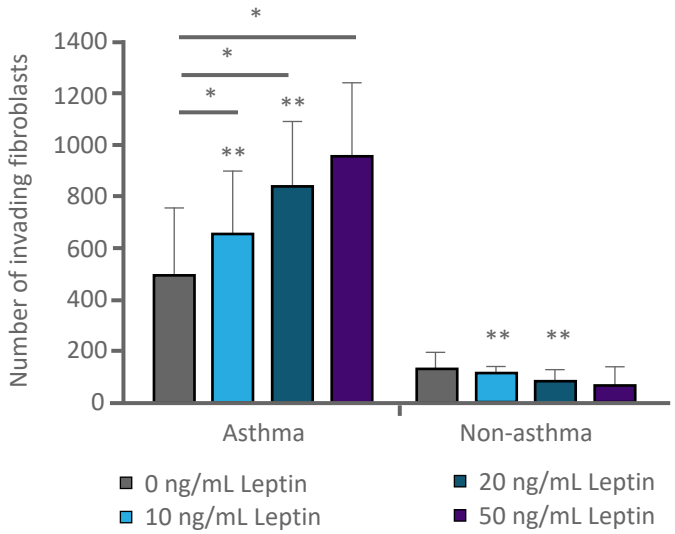
- **Eotaxin** is an eosinophil-specific chemokine that contributes to allergic asthma by attracting eosinophils to sites of inflammation; it is also a secretory product of adipose tissue and its expression is increased in obesity
- **Leptin** is a pro-inflammatory, pro-fibrotic adipokine that amplifies the chemotactic response of eosinophils to eotaxin
- **IL-13** is a T2 cytokine involved with allergic airway responses, which stimulates eotaxin production in mouse and human lung fibroblasts

- The invasiveness of human airway fibroblasts from patients with asthma was significantly increased in response to increasing doses of leptin ($P<0.05$)
- Eotaxin secretion was significantly elevated in MLFs isolated from HFD-fed mice compared with normal chow-fed mice ($P=0.004$; n=2 and 3, respectively)
- The effect of IL-13 on MLF eotaxin secretion was significantly greater in cells isolated from HDM-challenged mice compared with saline-challenged mice, regardless of diet ($P<0.05$; n=2–3 for each group)
- Combined IL-13 and leptin significantly augmented eotaxin secretion in lean, saline-challenged mice dosed with exogenous leptin compared with untreated mice ($P=0.01$) and compared with IL-13 alone in obese, HDM-challenged mice ($P=0.02$)

The effect of leptin on human airway fibroblast invasiveness



Key takeaway: *Leptin promotes airway fibroblast invasiveness and works with IL-13 to enhance eotaxin secretion by lung fibroblasts in a mouse model of obese allergic asthma*

*$P<0.05$; **$P<0.05$ asthma vs non asthma
HDM, house dust mite; HFD, high-fat diet; IL, interleukin; MLF, mouse lung fibroblast; T2, type 2
Mcquade V, et al. Poster P501 presented at ATS 2022 (Abstract A3740)