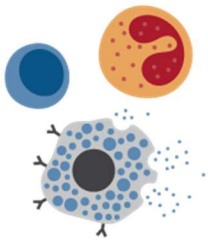

DISCOVERY 2026

New Mechanisms of Type 2 Immunity

Discovery 2026 Abstracts

D01

Germline-encoded recognition of peanut underlies development of convergent antibodies in humans

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Background: The presence of food-specific IgG in response to dietary ingestion has been long-observed. In a subset of individuals, food-specific IgG can class-switch to IgE, potentially causing anaphylaxis. We and others have previously identified highly homologous, or public, peanut-specific antibodies in allergic individuals.

Objective: We hypothesized that we can use the similar, stereotyped pathways of convergent antibodies to peanut to construct germline-encoded structural models of antibody convergence.

Methods: Using a set of epitope-specific antibodies to peanut cloned from peanut-allergic patients, we probed antibody-antigen interactions using mutagenesis, biolayer interferometry and molecular modeling.

Results: Using this set of convergent antibodies with distinct antibody gene rearrangements and similar complementary determining regions (CDRs), we identified had identical epitope-paratope interactions and highly similar energetics in structural models. Peanut specificity of these antibodies is germline-encoded across commonly used gene rearrangements, occurs irrespective of allelic heterogeneity, and has significant junctional malleability. Using molecular modeling, we identified the paratope residues critical for these convergent binding interactions and probed the entire human antibody repertoire to understand the genetic constraints on this

stereotyped recognition of a public epitope on a dominant allergen.

Conclusion: We can utilize molecularly defined germline-encoded recognition of peanut allergen to better understand how the human antibody genome predisposing humans to recognizing peanut proteins.

D02

Recruitment of mast cell progenitors during type 2 inflammation is dependent on the EBI2/Ch25h axis.

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Mast cells (MCs) are tissue-resident granulocytes that expand in the airways during type 2 inflammation (T2I)-associated diseases, where they are thought to play a major role in disease pathobiology. Mast cells arise from rare circulating MC progenitors (MCp), suggesting therapeutic potential in blocking transit of these progenitors to airway tissue, however the signals directing MCp recruitment to airway tissue are unknown. Following dust mite-induced allergic lung inflammation, we find that MCps in WT mice are quickly recruited to the lung parenchyma and retained, where they undergo phenotypic maturation. Single-cell RNA sequencing (scRNA-seq) analysis of flow-sorted murine splenic MCps cross-referenced against human circulating MCps revealed surprisingly few chemotactic receptors shared across species, highlight conserved expression of *Gpr183*, encoding the oxysterol Epstein-Barr virus-induced G-protein coupled receptor 2 (EBI2). EBI2 expression on human MCps was validated by flow cytometry, and the EBI2 ligand 7a,25-dihydroxycholesterol elicited human MCps chemotaxis that was attenuated by a small-molecule inhibitor of EBI2. Endothelial cells taken from control and challenged mice exhibit high transcript expression of the *cholesterol 25-hydroxylase* (*Ch25h*), a necessary enzyme to produce EBI2 ligands. While leukocyte recruitment was broadly unimpaired in mice lacking EBI2 expression on immune cells ($Vav1^{Cre}$; $EBI2^{FL/FL}$) or in Ch25h-deficient mice, MCp recruitment was nearly completely abolished, indicating a central role for the EBI2-Ch25h axis in directing MCp recruitment during T2I. Thus, our findings identify EBI2 as a novel therapeutic target with the potential to block MCp recruitment to airway tissue during T2I.

D03

Flow Cytometric Characterization of Basophil and Eosinophil Metabolism in Th-2 Associated Disorders

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There is growing evidence that basophils play major roles in Th2-mediated immune inflammation independent of IgE-mediated activation, but traditional Basophil Activation Testing only measures proxies of degranulation. Characterization of basophils beyond degranulation could prove valuable insights into Th2-associated disorders (T2AD).

An intracellular metabolic panel of flow cytometric-compatible antibodies was used to evaluate glycolysis (GLUT1), the pentose-phosphate pathway (G6PD), the Krebs cycle (ATP5A and SDHA), and fatty acid metabolism (CPT1A) in 4 healthy donors (HD) and 8 with T2AD. Batch normalized median fluorescence intensities (MFI) of the metabolic markers were collected at baseline and after stimulation with anti-IgE. Mass spectrometry to assess basophil metabolomics is ongoing.

Basophils (CCR3 hi, SSC low, FcER1+) and eosinophils (CCR3 hi, SSC hi) were identified in whole blood and shown to have a transient upregulation of GLUT1 at 10 minutes post-stimulation. The other markers (G6PD, ATP5A, SDA, CPT1A) were maximally upregulated at 60 minutes that largely resolved by 4 hours. Baseline basophil GLUT1 MFI trended higher in T2AD (3.62, 2.98, 2.93, 3.53, 3.49, 3.29, 3.76, 3.47) vs HD (3.53, 2.81, 2.71, 3.01) p=0.17; basophil G6PD MFI similarly trended higher in T2AD (5.14, 4.74, 4.68, 5.37, 6.04, 4.96, 6.24, 6.06) vs HD (6.11, 3.97, 4.24, 4.91) p=0.28. Eosinophils showed similar trends.

Basophil activation is characterized by initial transient increase in glycolysis with subsequent upregulation of fatty acid metabolism, Krebs cycle, and pentose phosphate pathway metabolism. Baseline activity of glycolysis and the pentose phosphate pathway in basophils and eosinophils may be elevated in Th2-associated diseases, warranting further investigation.

D04

Spatially resolved multiomics highlights differential immune responses in atopic dermatitis skin to blocking IL4Rα.

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Background: Blocking IL4Rα reduces pruritis and inflammation in atopic dermatitis (AD). The mechanisms by which blocking IL4Rα resolves inflammation across the epidermal-dermal-neural interface is not well understood.

Objective: This study used spatial transcriptomics and proteomics to study the effect of blocking IL4Rα on gene expression and protein abundance in the epidermis and dermis and in sensory nerves from individuals with moderate to severe AD and to compare these changes to healthy skin.

Methods: Skin biopsies collected from adults with moderate to severe AD (n=62 samples) and healthy adults (n=10 samples) were accessed for GeoMx™ Digital Spatial Profiling of 87-proteins and 1,812 genes. Regions of interest were collected from epidermal, dermal and the dermo-epidermal junction of each skin biopsy. Comparisons were made between AD samples before and after 17 weeks of IL4Rα blockade (dupilumab treatment 300mg every two weeks) and post-treatment with healthy samples.

Results: PCA indicated that IL4Rα blockade shifted epidermal and dermal gene expression and protein abundance in AD skin toward healthy skin profiles. In the dermis, treatment responsive genes were associated with the GOBP pathways ‘neuron projection guidance’ and ‘regulation of neuron differentiation’. Correlations between transcripts and proteins were observed for markers in angiogenesis, cellular responses and cytotoxicity. IL4Rα blockade significantly reduced innate immune gene sets in the epidermis and gene sets associated with adaptive immunity and cell signalling in the dermis.

Conclusions: Blocking IL4Rα and inhibiting IL-4 and IL-13 signalling normalizes inflammatory

pathways in epidermis and dermis toward healthy skin homeostasis, while simultaneously modulating neuronal signalling networks.

D05

Pain in Eosinophilic Esophagitis Exhibits a Distinct Clinical and Molecular Phenotype

Mia Natale

Introduction: Eosinophilic Esophagitis (EoE) is a chronic allergic disease of increasing prevalence. EoE patients report refractory pain despite use of eosinophil-depleting treatment, suggesting a more complex mechanism driving the symptoms of EoE beyond eosinophils in the mucosa. We suggest that EoE patients with and without persistent pain symptoms will exhibit distinct differences in their medical records, comorbid conditions, treatment regimens, biopsy data, and gene expression profiles.
Methods: We conducted a retrospective case-control study comparing pediatric medical records of a "pain positive" group (n=47) (EoE+), and a "pain negative" group (n=47) (EoE-). We analyzed patient demographics, treatment regimens, comorbidities, and biopsy findings. Immunofluorescence microscopy was used to label neurons with bIII-tubulin to compare nerve densities in patient biopsies. Sub-cohorts from EoE+ (n=25) and EoE- (n=21) were chosen for qPCR.
Results: On average, EoE+ patients were evaluated for EoE at an older age (p=0.0122). EoE+ patients exhibited a higher percentage of combined pharmacologic and food interventions (p=0.0069), and altered treatment regimens (p=0.0269). EoE+ patients exhibited increased asthma (p=0.0217) and hypermobility (p=0.0271). Immunofluorescent analysis showed higher sensory nerve density in EoE+ biopsies (p=0.0315). Finally, EoE+ patients showed lower expression of the inflammatory mediator *Alox15* (p=0.002881), even in remission (p=0.0001).
Conclusion: EoE patients with persistent pain are first evaluated at older ages, receive more complex treatment regimens, exhibit higher frequency of comorbid diseases, display an increased density of epithelial nerves, and express lower levels of *Alox15*. Therefore, persistent pain in EoE patients is associated with distinct clinical and molecular features beyond eosinophilic inflammation alone.

D06

Epitope-Specific Neutralizing IgG4 Antibodies Define Natural and Acquired Tolerance in Peanut Allergy

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While early introduction has clinically transformed our care of food allergy patients by inducing long-lived natural tolerance in children, the underlying mechanism of natural tolerance remains unclear, though serum IgG4 levels have been shown to increase in tolerant and peanut consuming adolescents. In oral immunotherapy (OIT), neutralizing IgG4 antibodies have been associated with sustained tolerance. We hypothesize that neutralizing IgG4 antibodies (nAbs) are also induced in peanut-allergic individuals with high thresholds of reactivity. To study this, we developed three biomarker assays: an indirect ELISA which was previous benchmarked in tolerance after OIT, an indirect bead-based assay (iBBNA), and a direct bead-based assay (dBBNA). The ELISA and iBBNA using Ara h 2 specific epitope-specific nAbs to epitopes 1.2 and 3 that block neutralizing epitopes on Ara h 2. The dBBNA uses a mutant Ara h 2 that preserves epitopes 1.2 and 3 and therefore measures nAb levels. Protein G purified serum from peanut-allergic individuals with a low (<144 mg, n=XX) or high reactivity (>144mg, n= XX) peanut were used for profiling. Naturally tolerant individuals exhibited robust neutralizing IgG4 responses, mirrored by OIT subjects achieving sustained unresponsiveness, but absent in transiently desensitized patients. The iBBNA, validated against inhibitory ELISA (R^2 = 0.9, CV = 2.5%), reliably distinguished durable from transient OIT responses. These findings establish neutralizing IgG4 as a shared feature of natural and therapeutic tolerance, supporting their use as predictive biomarkers and guiding hypo-allergen design.

D07

Characterization of the oral antibody response following peanut SLIT in 1-4-year-old peanut-allergic individuals.

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Rationale: A recent peanut sublingual immunotherapy (SLIT) study demonstrated 79% of participants achieved desensitization, with 63% achieving remission. However, the underlying mechanisms of peanut SLIT are poorly understood, therefore, we evaluated the oral mucosal antibody response to SLIT.

Methods: Fifty peanut-allergic children were enrolled in a peanut SLIT trial and were

randomized 1:1 to active peanut or placebo SLIT for 36 months, followed by a three-month avoidance period to evaluate remission. Saliva was collected at baseline, 12, 24, 36, and 39 months, which allowed for evaluation of the longitudinal changes in salivary peanut-specific and peanut component (Ara h 1, 2, and 3)-specific IgA and -IgG4 by ELISA.

Results: Participants who received peanut SLIT, but not placebo SLIT, had significantly increased peanut-specific and peanut component-specific IgA and IgG4 after 12, 24, and 36 months of therapy compared to baseline. This significant difference remained present following the avoidance period at 39 months. When broken down by clinical outcome (remission, desensitization, or failure), peanut- and component-specific IgA and IgG4 was found to be significantly higher throughout therapy in those classified as failures compared to those that achieved remission, challenging the paradigm that IgA and IgG4 are strictly protective antibodies.

Conclusions: Peanut SLIT modified the mucosal immune response by inducing significantly higher peanut-specific and peanut component (Ara h 1, 2, and 3)-specific IgA and -IgG4, compared to those that received placebo SLIT. Interestingly, significantly higher peanut- and component-specific IgA and IgG4 throughout therapy was found in treatment failures, compared to those that achieved remission.

D08

HLA Class I Associations and Multi-Omic Single-Cell/Spatial Skin Profiling Define Cytotoxic T-Cell Pathways in Lamotrigine SJS/TEN

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Introduction:
Lamotrigine, is an anticonvulsant, mood stabilizer, and prevalent cause of Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN). We investigated genetic risk and multiomic responses in lamotrigine SJS/TEN skin and blister fluid using HLA typing, single-cell RNA-TCR-CITE-seq, and spatial transcriptomics.

Methods:
Lamotrigine-induced SJS/TEN cases (n=47) were identified from VUMC, SJS Survivor Study and Boston cohorts. Lamotrigine-tolerant matched controls (n=470) were identified from VUMC BioVU. HLA was typed by high-resolution sequencing or imputation, and association analyses performed at the locus, haplotype, and amino-acid levels with OR and Bonferroni correction (midasHLA). Blister fluid (n=3) was collected from lamotrigine SJS/TEN patients and thermal-burn controls (n=6). For one patient, both affected and unaffected skin and five distinct blister sites were analyzed. Samples were profiled using 5′ scRNA-TCR-CITE-seq with clonotype (ClusTCR), differential (VGAS), TCR-HLA-mapping (DePTH), and proportional (SCANPRO) analyses. Spatial transcriptomics (Visium HD) confirmed cell–cell interactions.

Results:
HLA-C*12:03 (OR 9.2, Pc<0.001) and HLA-B*38:01 (OR 15.7, Pc<0.001) were associated with SJS/TEN, with the HLA-A*26:01/HLA-B*38:01/HLA-C*12:03 haplotype conferring the highest risk (OR 29.0). Threonine at HLA-B position 158 (present in HLA-B*38:01) was significant (Pc<0.001). In one patient a shared TCRαβ clonotype appeared across five blister sites and in affected but not unaffected skin. Across patients, distinct oligoclonal TCRαβ converged on proliferative (*STMN1, TUBA1B, TUBB*) and cytotoxic (*GNLY, PRF1, GZMB*) $CD8^+$ T-cell clusters contacting keratinocytes in spatial analyses.

Conclusions:
HLA-B*38:01 and HLA-C*12:03 define lamotrigine-SJS/TEN risk. Private, drug-expanded cytotoxic $CD8^+$ T-cell clones mediate keratinocyte injury. Integrated genetic, single-cell, and spatial analyses link shared HLA restriction with patient-specific TCR repertoires driving SJS/TEN pathogenesis.

D09

Determinants of mast cell priming potency by peanut allergic patient monoclonal IgE pairs

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The increasing disease burden of peanut allergy worldwide warrants in-depth molecular understanding of factors contributing to severe patient reactions upon peanut exposure. IgE antibodies specific for 2S albumin peanut allergens, Ara h 2 and Ara h 6, are important for pathogenesis, yet some individuals sensitized to these allergens do not show strong clinical reactions to allergen exposure. A potential explanation could be that specific epitope combinations recognized by allergen-specific IgE species lead to optimal patient mast cell priming.

Here, we have tested this idea using biolayer interferometry epitope binning experiments, cryo-electron microscopy structural determination, and beta-hexosaminidase release degranulation experiments in LAD2 mast cell line cells. We find that for monoclonal IgE pairs that bind Ara h 2 or Ara h 6, the inter-Fab angle of allergen binding is correlated with beta-hexosaminidase release from LAD2 mast cell line cells upon Ara h 2 or Ara h 6 addition. IgE pairs with Fabs binding greater than 120 degrees apart on the allergen result in lower beta-hexosaminidase release. Further, we find evidence that inter-Fab binding contacts between members of an IgE monoclonal antibody pair can both improve binding to allergen and increase degranulation of LAD2 cells. Our data support roles for epitope combinations recognized by a patient's IgE clonal species, the angles of allergen binding between IgE species, and potential intermolecular cooperativity of IgE binding as contributors to mast cell priming potency.

D10

CTLA-4 mediates oral immunotolerance against systemic allergic sensitization to food allergens

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Background: Environmental exposure to food antigens through the skin or airways increases the risk of developing food allergy. In contrast, oral antigen exposure early in life reduces this risk.

Objective: The objective of this study was to investigate the immunologic mechanisms by which oral-route antigen induces systemic immunotolerance and protection from food allergy development in murine models.

Methods: Naive mice were administered ovalbumin (OVA) in drinking water to promote antigen-specific oral immunotolerance. Mice were subsequently exposed intranasally (i.n.) to OVA plus peanut flour as an adjuvant. $CD4^+$ T cells from lung draining lymph nodes (dLNs) were analyzed by single-cell RNA sequencing, MHC-II tetramer staining, and flow cytometry.

Results: Following i.n. exposure to OVA plus peanut flour, mice developed OVA-specific IgE (OVA-IgE) and exhibited symptoms of acute anaphylaxis upon intraperitoneal OVA challenge. Oral OVA feeding prior to i.n. OVA exposure reduced OVA-IgE levels and protected mice from anaphylaxis. Protection was associated with decreased OVA-specific T follicular helper (Tfh) and germinal center B cells in dLNs. Tolerance was reversed by systemic administration of anti-CTLA-4 but was unaffected by genetic deletion of $Foxp3^+Bcl6^+$ T follicular regulatory (Tfr) cells. Accordingly, conventional $Foxp3^+$ T regulatory (Treg) cells were not increased in dLNs of OVA-fed mice. Rather, protection was associated with the emergence of OVA-specific $Foxp3^-CTLA4^+$ cells expressing markers of stemness.

Conclusion: Oral antigen exposure suppresses the development of antigen-specific Tfh cells in remote dLNs, thereby preventing systemic allergic sensitization. Systemic oral tolerance is dependent on CTLA-4 and is unlikely to require conventional $Foxp3^+$ Treg cells.

D11

Characterizing the Local and Systemic Adaptive Immune Responses to Protease Allergens

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Many environmental allergens are known to possess protease activity, but the outcome of the host's adaptive immune response to protease allergens remains incompletely understood. Papain is a cysteine protease found in papaya latex and is commonly used as an experimental allergen. Papain induces potent type 2 inflammation upon primary exposure in the skin and

lungs in mice, leading to the development of type 2 adaptive immunity, including differentiation of papain-specific Th2 cells and anti-papain antibody-producing B cells. However, surprisingly little is known about how papain-specific adaptive immunity modulates host responses to papain upon re-exposure. We found that intraplantar injection of papain in naive mice induced acute edematous inflammation in the footpad that resolved within 24 hours. In contrast, re-exposure of these mice to papain in a different paw led to an attenuation of acute edema but paradoxically prolonged footpad inflammation lasting up to three days at the site of re-exposure. The challenged mice also exhibited hypothermia, indicating a systemic response to a local challenge. Notably, the proteolytic activity of papain is required to drive local edema, whereas papain-specific antibodies are responsible for the modulation of the local response and hypothermia in a mast cell-dependent manner. Inactivated papain was capable of inducing hypothermia without causing local edema in papain-immunized mice. However, when remotely administered, it attenuated acute edema but protracted inflammation in the footpad that was induced by a structurally unrelated protease. These data demonstrate that the pre-existing adaptive immunity to allergens can modulate protease-induced local inflammation in an antigen-nonspecific manner.

D12

Exploring the Role of IgE Autoantibodies and Viral Reactivity in Idiopathic Anaphylaxis and Mast Cell Disorders: A Pathway to Understanding Autoallergy

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Background: Idiopathic anaphylaxis (IA) is a rare, life-threatening mast cell activation disorder characterized by recurrent anaphylactic episodes without identifiable triggers. Emerging evidence suggests that IgE autoantibodies directed against self-antigens (“autoallergy”) may contribute to mast-cell activation in patients with chronic urticaria; however, no evidence exists to date in IA.

Objective: To investigate IgE autoantibody and viral antigen reactivity profiles in patients with IA compared to chronic spontaneous urticaria (CSU) and non-atopic controls.

Methods: Sera from 17 patients—including IA (n = 9), CSU (n = 4), and non-atopic controls (n = 4)—were analyzed using the Infinity Bio antibody-reactome platform, which employs Molecular Indexing of Proteins by Self-Assembly (MIPSA) technology for high-throughput IgE profiling. The

AllerSIGHT™, HuSIGHT™ (full-length and peptide autoantigen libraries), and VirSIGHT™ (viral protein library) assays were used to detect IgE binding to environmental, self, and viral antigens.

Results: Exploratory screening identified IgE reactivity to selected human autoantigens and viral peptides, including *proline-rich protein 34*, *pleckstrin homology domain-containing family B member 2 (evectin-2)*, and Megalocytivirus proteins in patients as well as controls. No statistically significant differences, however, were observed among the groups. These preliminary patterns highlight potential areas for further mechanistic study in larger cohorts.

Conclusion: Autoreactive IgE targeting self-proteins and viruses was detected in some patients with IA and CSU. However, the disease phenotype in IA may be multifactorial, involving additional factors contributing to mast cell hyperreactivity. Ongoing recruitment and expanded sampling are underway to validate these findings and further elucidate IgE-mediated pathways in IA and related mast-cell disorders.

D13

The use of chromosomal microarray analysis to evaluate immune deficiency and hereditary angioedema

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Background:
Hereditary angioedema (HAE) is a rare genetic disorder characterized by recurrent, nonpruritic swelling due to dysregulation of the complement and bradykinin pathways. Common variable immunodeficiency (CVID) is a heterogeneous antibody deficiency disorder affecting humoral immunity. Although uncommon, coexistence of HAE and antibody deficiency has been described in a few case reports, supporting a shared mechanism of immune dysregulation.
Objective:
To test the hypothesis that chromosomal microarray analysis assists with evaluation of clinical presentation in primary immune deficiency and hereditary angioedema.
Methods:
The comparison of chromosomal microarray analysis to evaluate a cohort with primary immune deficiency and hereditary angioedema.
Results:
A proband with HAE and CVID underwent genetic analysis revealing heterozygous variants in

several genes, including C3, ATM, FAT4, EPG5, RNU4ATAC, SLC10A2, and TOP2B. Family history was notable for two daughters and one sister with both CVID and HAE, and multiple extended relatives with HAE. Comparative analysis demonstrated inheritance of shared heterozygous variants across affected family members. The patient and her two daughters shared FAT4, RNU4ATAC, EPG5, and SLC10A2 variants. The patient and her sister shared FAT4 and RNU4ATAC variants, both implicated in antibody deficiency and lymphatic dysfunction.

Conclusion:

This cohort demonstrates multigenerational inheritance of immune dysregulation syndromes involving cumulative heterozygous variants rather than a single monogenic defect. Co-inheritance of variants in complement, lymphatic, and autophagy genes may act synergistically to disrupt immune homeostasis. Recognition of such polygenic inheritance patterns in familial HAE and CVID can improve diagnostic accuracy, guide genetic counseling, and expand understanding of complex immune regulation.

D14

IgE at the Maternal-Fetal Interface: Implications for Immune Interactions in Early Life

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Rationale: Maternal atopy confers higher risk of childhood allergy than paternal atopy. Whether increased placental IgE concentrations contribute to early-life atopic predisposition has not been established.

Methods: The Michigan Sibling Immunity Birth Study is a birth cohort of individuals with atopic siblings, collecting biospecimens and survey data prenatally and throughout the first three years of life. IgE immunofluorescence staining was performed on FFPE placental sections from 8 mothers (4 atopic and 4 non-atopic per ISAAC criteria). IgE was quantified in Fiji by counting positive puncta within defined maternal, fetal, and intervillous regions. Paired maternal and cord blood IgE levels were measured at birth using ImmunoCAP™ assays. Statistical comparisons used Student's *t*-test and the Mann-Whitney U test, as appropriate.

Results: The presence of IgE was significantly higher in atopic compared to non-atopic placentas (214 ± 90.7 vs 132 ± 64.9 puncta/image, $p = 0.05$). Maternal prenatal IgE was significantly higher in atopic vs non-atopic mothers (116.2 ± 52.1 vs 12.5 ± 4.3 kU/L, $p = 0.02$). Cord-blood IgE levels

were not significantly different between groups (1.41 ± 0.81 vs 0.16 ± 0.13 kU/L, p = 0.27).

Conclusions: This pilot study found higher placental IgE in atopic mothers but no corresponding rise in cord-blood IgE, implying minimal transplacental transfer. These findings raise the possibility that increased IgE at the maternal–fetal interface may modulate fetal immune development through local mechanisms. Continued cohort enrollment, combined with longitudinal follow-up, will enable verification of these associations and clarify the mechanisms behind childhood allergy risk.

D15

Molecular characterization of the B cell receptor and serological IgE repertoires in pediatric peanut allergy

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Background: Evaluating food allergy utilizing serologic food-specific IgE assays is standard practice. Available testing has high sensitivity but low specificity and is limited by a high rate of false positives. Additionally, serological levels of food-specific IgE do not correlate with clinical phenotype or severity. Importantly, IgE antibodies responsible for severe allergic reactions remain poorly understood, especially at the molecular level. The advent of BCR-seq—deep sequencing of the B cell receptor (BCR) repertoire—has illuminated molecular features of pathogenic IgE-producing B cells. However, the degree to which the transcribed BCR repertoire represents the circulating secreted immunoglobulin (Ig) repertoire is not known.
Objective: Characterize the circulating IgE proteome in children with peanut allergy, utilizing deep sequenced BCR transcript libraries.
Methods: We used parallel BCR- and Ig-seq methods to describe the circulating IgE, IgG, and IgA repertoires in pediatric donors with peanut allergy. BCR transcript libraries provided personalized proteomic search databases for circulating Ig analyzed using tandem liquid chromatography mass spectrometry (LC-MS/MS).
Results: LC-MS/MS analysis allows for relative quantitation of serological lineages, identification of CDRH3 motifs underlying peanut allergy, description of antibody clone convergence, and analysis of lineage overlap between circulating isotypes.
Conclusion: Distinct food-specific antibody repertoires may underlie food allergy phenotypes. A detailed examination of IgE and IgG profiles will help elucidate clarify the immunologic basis of symptomatic food allergy, asymptomatic desensitization, and resolved food allergy.

Comprehensive characterization of the serological repertoire will elucidate mechanisms underlying B cell and Ig trafficking in food-allergic individuals and inform diagnostic opportunities.

D16

Allergen Independent Activation of IgE-bearing Basophils by A549/Cancer Cell-associated Galectin-3 is Dynamically Modulated by IL-3 Priming

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Background/Rationale: We previously reported a novel mode of basophil activation requiring cell-to-cell interaction between IgE-bearing basophils and A549 lung adenocarcinoma epithelial cells bearing Galectin-3 (Gal-3), referred to as EC-Gal-3 activation. Basophils release histamine and Th2 cytokines (IL-4/IL-13) with this stimulation but also trigger IL-6/VEGF-A secretion from A549 cells in so-called "reciprocal responses". Both are augmented when basophils are first primed by IL-3 and do not require the active secretion of basophil or A549-derived mediators. We propose that IL-3 induces a yet unidentified surface marker on basophils that physically interacts with A549 cells to promote EC-Gal-3-dependent responses.
Methods: EC-Gal-3-dependent responses were measured at various time points following basophil IL-3 washout. IgE-naïve culture-derived basophils (CDBA) basophils with and without microspheres (5 mm) coated with IgE were employed to further elucidate how these components facilitate activation. Cytokine production was determined by ELISA.
Results: The enhancing effect of IL-3 wanes within hours of removing IL-3. We have previously published that neither IgE-coated MS nor IgE-naïve CDBA alone are sufficient to elicit a reciprocal IL-6 response from A549 cells or Th2 cytokines from basophils. However, we show here that combining these components restores IL-6 secretion by A549 cells but not the Th2 cytokines from CDBA.
Conclusions: Basophil/A549 responsiveness to EC-Gal-3 activation requires cell-to-cell interaction and at least two separate signals: one involving IgE and another involving IL-3. Identifying the IL-3-dependent component may lead to new diagnostic and/or therapeutic targets.

D17

Mechanisms Governing IgE Distribution from Production Sites to Effector Tissues

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Rationale: Food protein–specific immunoglobulins can trigger life-threatening anaphylaxis; however, the mechanisms and kinetics by which these antibodies move from their sites of production to effector tissues remain poorly understood.
Methods: An active anaphylaxis mouse model was used to assess antibody distribution through antibody-dependent vascular permeability, quantified by Evans blue dye extravasation. Mice were sensitized subcutaneously with ovalbumin-alum and challenged intravenously at defined time points (days 7–14). Vascular leakage and temperature changes were evaluated in wild-type, AID-deficient, and IgE-deficient mice, as well as in wild-type and IgE-KO mice treated with the FcγRII/III-blocking antibody (2.4G2), to delineate the contributions of IgE and IgG1.
Results: On day 7, Evans blue leakage was localized to the immunization site and accompanied by mild hypothermia. By day 10, vascular leakage became systemic, correlating with sustained temperature reduction and enhanced vascular reactivity. Using AID-deficient and wild-type mice, we confirmed that the vascular response depends on class-switched antibodies. To dissect antibody contributions, we compared IgE-deficient mice with wild-type mice treated with an FcγRII/III-blocking antibody (2.4G2). In IgE-deficient mice, IgG1 mediated both the localized (day 7) and systemic (day 10) vascular leakage. Conversely, FcγR blockade in wild-type mice revealed IgE-driven localized leakage at day 10 that progressed to systemic leakage by day 14.
Conclusions: Antibody-mediated vascular leakage evolves temporally from localized to systemic responses, driven sequentially by IgG1 and IgE. These findings highlight dynamic antibody trafficking during anaphylaxis and identify a need to define mechanisms regulating IgE distribution to effector tissues.

D18

Mechanism of action of ozureprubart, a next generation anti-IgE, for the treatment of allergic diseases

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Immunoglobulin E (IgE) plays a central role in the pathogenesis of allergic inflammatory diseases. Omalizumab, an FDA-approved anti-IgE therapy, is indicated for moderate-to-severe persistent asthma, chronic spontaneous urticaria (CSU), chronic rhinosinusitis with nasal polyps

(CRSwNP), and to reduce allergic reactions to accidental exposure, including anaphylaxis, in individuals with IgE-mediated food allergy. Despite the well-established efficacy and safety of anti-IgE therapy for allergic diseases, significant unmet need remains for treatments that reduce treatment burden and improve patient compliance and outcomes.
Ozureprubart (also known as RPT904 and JYB1904) is a next-generation anti-IgE monoclonal antibody designed to target the same epitope as omalizumab while introducing several improvements. Ozureprubart was engineered to increase affinity to IgE and to significantly increase half-life by increasing affinity for FcRn. Increased binding of ozureprubart to FcRn compared with omalizumab was shown by biolayer interferometry. Ozureprubart shows a 2-to-3-fold increase in ability to block IgE binding to FcεRI compared with omalizumab. Crystal structure analysis verified that ozureprubart maintains the same binding epitope as omalizumab following these modifications. Like omalizumab, ozureprubart dissociates IgE from FcεRI, reduces receptor-bound IgE, and reduces surface FcεRI expression on basophils.
These data show that ozureprubart shares many of the mechanisms of action of omalizumab. Along with its extended half-life and increased ability to block IgE, these properties give ozureprubart the potential to be an improved anti-IgE therapy. Ozureprubart is currently in Phase 2 clinical development for the treatment of food allergy, asthma, and CSU.

D19

Targeted Therapy Partially Normalizes mTOR Dysregulation and CD8+ T Cell Dysfunction in APDS and RALD

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Primary Immune Regulatory Disorders (PIRD) are monogenic diseases characterized by poor infection control, hyperinflammation, autoimmunity, and malignancy. In a subset of PIRD, genetic mutations converge on mechanistic Target of Rapamycin (mTOR), a serine/threonine kinase that regulates immune cell metabolism and function. These “mTOR-opathies”, including Activated PI3K Syndrome (APDS), RAS-associated autoimmune leukoproliferative

disorder (RALD), PTEN haploinsufficiency, and CTLA4 haploinsufficiency, share overlapping clinical manifestations and CD8+ T cell (CTL)-associated pathologies. However, the mechanism by which mTOR hyperactivation contributes to CTL dysregulation is not well understood. We analyzed PBMCs from a cohort of mTOR-opathy patients (APDS, n=18; RALD, n=10; CTLA4, n=10; PTEN, n=10) at baseline and following disease-modifying therapy to identify shared changes in CTL phenotype and function in the context of mTOR hyperactivity. Phenotypically, APDS and RALD had the most abnormal CTL, including increases in terminally differentiated cells, inhibitory receptor expression, and senescence markers. CTLs exhibited functional differences, with decreased IL-2, increased TNF-α and IFN-ɣ production, and impaired PI3K/AKT/mTOR-axis phosphorylation upon stimulation. Paired single-cell CITE-seq and TCR-seq confirmed increases in TNF-α and IFN-ɣ gene signatures in both conditions, while exhaustion was more pronounced in APDS. To determine whether this dysregulation is modifiable with targeted therapy, we studied paired samples from patients pre- and post-treatment with leniolisib (APDS) or sirolimus (RALD). Treatment partially normalized phenotype and IL-2 production, and restored activation-induced PI3K/AKT/mTOR signaling. IFN-ɣ production and gene signature remained significantly different from HC. In conclusion, APDS and RALD share changes in CTL phenotype and function, some of which are modifiable by targeted therapy.

D20

Focal adhesion kinase as a therapeutic target in mast cell activation and anaphylaxis

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Rationale: As the incidence of severe allergic reactions increases worldwide, therapeutic options for their prevention and treatment remain limited. Preliminary human RNA sequencing has demonstrated that focal adhesion kinase (FAK) signaling is upregulated during early anaphylaxis. Several FAK inhibitors are under clinical development, with first-in-class FDA approval in early 2025. We therefore sought to investigate the effect of FAK targeting in mast cell (MC) activation.

Methods: Cultured murine and human MCs were sensitized by incubation with monoclonal immunoglobulin E (IgE) then stimulated by antigen or ionomycin. MC activation was quantified by beta-hexosaminidase release and CD63/CD107a expression. FAK activity was modulated by

small molecule kinase inhibitors, proteolysis-targeting chimeras (PROTACs), and activators. Balb/c mice were sensitized with intravenous DNP-IgE or intraperitoneal OVA-alum, then challenged with oral DNP-HSA or repeated oral ovalbumin respectively. FAK-targeting agents were administered via two intraperitoneal doses within 24 hours prior to final challenge.

Results: FAK kinase inhibition prevented MC degranulation *in vitro* in a dose-dependent manner without significant cytotoxicity. Kinase inhibition introduced after activation also reduced both IgE- and ionomycin-mediated degranulation in a time-dependent manner. FAK agonism inversely increased degranulation, while degradation exhibited both agonistic and antagonistic effects on degranulation related to PROTAC dose. FAK antagonism in mice significantly reduced several measures of both DNP- and ovalbumin-induced anaphylaxis compared to either vehicle-treated controls or prior ovalbumin challenge.

Conclusion: The suppression of MC mediator release and systemic anaphylaxis by small molecule therapeutics suggests a potential role for FAK targeting in the management of allergic reactions.

D21

Extracellular vesicles produced by activated human mast cells increase the metabolic function of target cells

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Small extracellular vesicles (EVs), or exosomes, are 30-150 nm phospholipid-encased nanoparticles that are particularly difficult to characterize due to their small size and difficulty of isolation using conventional methods.Human mast cell lines (HMC-1 and LAD2) were activated and their production of exosomes was analyzed by proteomic profiling, transmission electron microscopy, nuclear magnetic resonance metabolomic profiling and flow cytometry. Activated mast cells produced at least three distinct subpopulations of EVs and revealed unique structures via EM. Atom scale modeling indicated that each size could potentially carry specific amounts of protein cargo, dependent upon protein size, protein to lipid ratios, and potential limitations on membrane protein function due to physical constrains in the smallest EVs. Proteomic profiling showed that 16 proteins were significantly more abundant and 32 less abundant in EVs isolated from activated mast cells. A STRING analysis of the more abundant proteins revealed a correlation with mitochondrial function (COX6A1), catalytic activity (MAP4K4), calcium

mediated signaling (PPP3CB), as well as regulators of intracellular membrane trafficking (RAB31) and exon junction complex components (CCDC9). STRING analysis of the less abundant proteins revealed strong correlation with microtubule turnover regulation (KIF2C) and chromatin assembly mediator (CHAF1B). The EVs contained 15 mitochondrial-related proteins that are key electron transport chain components such as cytochrome c, complex I and inner mitochondrial membrane ATPAse proteins. This data suggests that activated inflammatory immune cells produce different populations of EVs that differ in size and cargo that activates the metabolomic profile of target cells and thereby increase their metabolic rate.

D22

Serum factor regulation of MC desensitization following combined immunotherapy

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Recently, our laboratory developed cell culture systems allowing selective differentiation of MCs co-expressing tryptase and chymase (MCTC), similar to those residing in peripheral connective tissues, and expressing tryptase alone (MCT), the principal phenotype found in mucosal epithelium. Due to their in vivo location, MCT are likely central drivers of allergic responses in the airways and digestive tissues, but little is known about the differential sensitivity of each subset to both activation and desensitization. To test this, we selectively differentiated MCT and MCTC from peripheral blood-derived CD34+ cells, sensitized them using patient serum samples from a recent study of cat allergic individuals evaluating effects of subcutaneous immunotherapy (SCIT), Tezepelumab (TEZE) treatment, or combined TEZE/SCIT. Our findings indicated that MCT exhibit a striking increase in sensitivity to cat dander compared with MCTC. Both treatments in isolation lead to decreased MC activation, with TEZE limiting MC priming and SCIT limiting MC activation through blocking allergen recognition in an IgG-independent manner. Evaluation of the TEZE/SCIT group indicated that combined therapy leads to robust desensitization of both subsets to a greater degree than either therapy in isolation, highlighting the complementary nature of these two treatments.

D23

Mast cell dedifferentiates through iron-uptake after IgE-mediated activation

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Introduction/Rationale: Mast cells (MCs) are principal effector cell type in the skin in diseases associated with type 2 inflammation (T2I) such as atopic dermatitis. Although MC degranulation exacerbates T2I, the post-activation fate of MCs remains largely unexplored.
Methods: We generated MC_{TC} and MC_T from $CD34^+$ cells sorted from human peripheral blood, and analyzed the cell number up to 7 days after IgE cross-linking. To assess the differentiation state, the expression of integrin β7 (ITGB7), 2D7, and transferrin receptor protein 1 (TFR1) was measured on days 1, 3, and 7 by flow cytometry. Iron contents were determined at 1-, and 24-hours post-activation. Moreover, the expression of 2D7, ITGB7, and TFR1 was analyzed after iron chelation from the culture prior to activation. Morphological assessment was conducted using toluidine blue staining.
Results: While the counts of MC_{TC} and MC_T decreased after IgE cross-linking, MC_{TC} but not MC_T, underwent robust proliferation by day 7. ITGB7 and TFR1 on activated MC_{TC} were detected from day 1 and persisted until day 7, whereas 2D7 expression was upregulated from day 3 to 7. Iron levels in MC_{TC} rapidly decreased 1-hour post-activation, and re-uptake was observed at 24 hours. In contrast, the iron content in MC_T gradually declined from 1 to 24 hours post-activation. Importantly, iron chelation from the culture prior to activation inhibited the expression of ITGB7, 2D7, and TFR1 on MC_{TC}. MC_{TC}, but not MC_T, was regranulated on day 7 post-activation.
Conclusion: Our findings suggest that iron promotes MC_{TC} dedifferentiation, regranulation and expansion following IgE-dependent activation.

D24

Proteomic Profiling Reveals Immune Dysregulation in Hypermobile Ehlers–Danlos Syndrome

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Hypermobile Ehlers-Danlos Syndrome (hEDS) is a heritable connective tissue disorder with unclear etiology. While hEDS has historically been defined based on joint hypermobility and tissue fragility, it has become increasingly recognized as a multisystem disorder that includes gastrointestinal, autonomic, and immune function with patients having comorbid diagnosis of autonomic dysfunction, mast cell activation diseases and gastrointestinal disorders. The goal of this study was to investigate systemic molecular changes in hEDS and explore how immune mechanisms that may be linked to connective tissue pathology. We performed unbiased serum

proteomic profiling in hEDS patients and matched controls, followed by pathway and gene ontology analyses. Differentially abundant proteins were validated by ELISA in an expanded cohort, and cytokine arrays were used to assess inflammatory signaling. Proteomic analysis revealed dysregulated proteins associated with innate immunity, complement activation, kallikrein/coagulation pathways, and oxidative stress. Validation confirmed reduced serum levels of complement proteins in hEDS patients, while cytokine arrays alterations of multiple inflammatory mediators, consistent with immune dysregulation. These findings support an alternative view of hEDS as a systemic disorder involving immune dysregulation, raising the possibility that connective tissue manifestations may be secondary to, or exacerbated by, immune dysfunction. This work provides a foundation for understanding the molecular mechanisms underlying hEDS and highlights potential diagnostic and therapeutic targets.

D25

The role of CD9 in regulating metabolic dysfunction and tissue inflammation during obesity

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Obesity is a global epidemic that is associated with worse outcomes in asthma and allergic disease, in part due to global metabolic dysfunction. Adipose tissue (AT) stores excess energy but can undergo dysfunction, leading to global insulin resistance, ectopic fat deposition, and systemic inflammation. The liver is a critical regulator of AT function during obesity via the secretion of hepatokines, lipoproteins, and extracellular vesicles (EVs). EVs are nanometer-sized particles that deliver proteins and miRNAs to target organs and cells. Despite their relevance to multiple chronic inflammatory diseases, the mechanisms regulating this liver–AT axis, and their influence on inflammatory disease, remain poorly defined.

CD9 is a tetraspanin molecule that plays a role in EV biogenesis, release, and uptake by target cells. We hypothesized that CD9 regulates an EV-mediated liver-AT axis that restrains AT inflammation during obesity. To test this, we generated liver-specific CD9 knock-out (CD9 LKO) mice and placed them on a high fat diet. Compared with obese wild-type littermate controls, obese CD9 LKO mice exhibited marked AT inflammation characterized by macrophage influx and AT fibrosis. These changes were accompanied by systemic metabolic derangements including delayed glucose clearance, ectopic lipid deposition, and hyperlipidemia.

These findings reveal a previously unrecognized role for liver CD9 in suppressing AT inflammation and metabolic dysfunction in obesity, likely through modulation of EV-mediated

inter-organ signaling. Ongoing work aims to define the liver-derived protective signals regulated by CD9 and to determine their broader impact on obesity-associated inflammatory diseases, including asthma and allergy.

D26

Early-Life Vaccine Adjuvant Selection Shapes Durable Immune Polarization to Prevent Allergic Sensitization in Mice

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Background: The immature neonatal immune system is highly malleable, providing a critical window for shaping long-term immune trajectories. Environmental factors, including microbiota and nutrition, alter immune priming to influence responses to subsequent heterologous antigens. Our previous work suggests that vaccine adjuvants may also train the neonatal immune system.
Objective: To determine the influence of early-life immunization with diverse adjuvants on subsequent T cell polarization and susceptibility to allergic sensitization.
Methods: Seven day old neonatal mice were immunized with a diverse panel of adjuvants. Bone marrow–derived DCs were assessed for altered phenotype and function to evaluate the impact of early-life immunization on T cell skewing capacity. Adjuvants were also screened for their ability to prevent allergic sensitization.
Results: Early-life immunization with certain Th1-polarizing adjuvants promoted heterologous Th1 biased immunity, while certain Th2-polarizing adjuvants promoted Th2-biased responses. No heterologous priming effects were observed when mice were immunized at 8 weeks of age. The heterologous immune effects were largely mediated through training of DCs. Bone marrow-derived DCs from adjuvant-primed mice exhibited phenotypic and functional changes that altered their capacity to drive T cell polarization. Importantly, the Th1 bias induced by certain adjuvants prevented allergic sensitization by attenuating the induction of IgE and Th2 cytokines.
Conclusions: These findings demonstrate that early-life adjuvant exposure can imprint long-lasting immune biases that alter the polarization of immune responses to heterologous antigens and modulate susceptibility to allergic sensitization, supporting a strategy of rational adjuvant selection to guide immune development while maintaining critical vaccine-mediated protection.

D27

Toxin and Allergen Co-exposure Drives Persistent Sinonasal Mast Cell Accumulation

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Background: Military Veterans with deployment-related toxin exposures have a higher prevalence of chronic rhinosinusitis (CRS), often without nasal polyps. The mechanisms for this chronic inflammation are unknown. Mast cells (MC), key Type 2 immune cells activated by xenobiotics, are understudied in this CRS phenotype.

Objective: We sought to characterize the immunophenotype in toxin-exposed Veterans and investigate mechanisms of MC persistence using a preclinical model.

Methods: Ethmoid sinus tissue from toxin-exposed Veterans (n=13) and CRS controls (n=10) was analyzed by flow cytometry and bulk RNA-sequencing. In parallel, C57BL/6 mice received 4 weeks of intranasal *Alternaria* (ALT), combustion product constituents (CPC), or ALT+BPC. A subset was monitored for 4 weeks post-exposure to evaluate persistence.

Results: Toxin-exposed Veterans (primarily CRSsNP) showed a >2-fold selective increase in sinus MCs (p=0.0185) versus controls, with no difference in eosinophils or neutrophils. RNA-seq confirmed upregulation of MC-related transcripts (*TPSAB1*, *HDC*) and integrin β7 (*ITGB7*). In mice, ALT+CPC co-exposure synergistically increased sinonasal integrin b7+ MC accumulation. Four weeks post-challenge, MCs declined in ALT-only mice but continued to increase in the ALT+CPC group, demonstrating toxin-driven persistence.

Conclusion: Toxin exposure is associated with a novel, MC-driven CRS endotype. Co-exposure to toxins and aeroallergen establishes persistent sinonasal MC recruitment and accumulation, suggesting a mechanism for disease chronicity and identifying MCs as a therapeutic target for this phenotype.

D28

Intestinal mast cell-derived leukotrienes enhance sensitivity to dietary antigens

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Food allergies are IgE-mediated reactions against protein antigens found in food. Some individuals with food specific IgE can tolerate allergen ingestion, whereas others undergo anaphylaxis. The factors that govern this difference in sensitivity are unclear; however, mouse models suggest intestinal mast cell expansion can heighten responsiveness. We reasoned that by studying intestinal mast cell biology, we may uncover novel mediators that enhance sensitivity to dietary antigens. We focused on leukotrienes as candidate molecules due to their recently identified roles in food allergen avoidance behavior in mice. We utilized flow cytometry, bulk and single-cell RNA sequencing, and *in vitro* models to better understand the development and function of murine intestinal mast cells. Mice genetically or pharmacologically deficient in aLOX5, LTC4S, CysLTR1, or CysLTR2 were tested in oral and systemic anaphylaxis paradigms to elucidate the role of cysteinyl leukotrienes in experimental food allergy. Intestinal mast cells were largely epithelium resident and acquired a unique terminal differentiation state dependent on αvβ6 integrin driven TGF-β release. Intestinal differentiation altered mast cell effector function, with histamine synthesis being strongly suppressed and leukotriene secretion enhanced. Hematopoietic cysteinyl leukotriene synthesis was required for intragastric, but not systemic, allergen challenge. This effect was attributable to non-redundant requirements of CysLTR1 and CysLTR2 in intestinal mast cell expansion and redundant acute requirements of multiple CysLTRs in oral anaphylaxis itself. This data suggests targeting gastrointestinal responses can acutely blunt susceptibility to ingestion-induced anaphylaxis and that leukotrienes may be therapeutic targets in food allergy.

D29

GPR15–GPR15L Axis and Adhesion Molecules Cooperatively Promote Memory CD4$^+$ T-Cell Homing to the Esophagus in Eosinophilic Esophagitis (EoE)

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Background: - Eosinophilic esophagitis (EoE) is a chronic food-allergen driven Th2-skewed inflammatory disease characterized by eosinophilic infiltration of the esophagus. Elevated expression of GPR15, a potential esophagus-homing receptor, has been observed in peripheral pathogenic Th2 (peTH2) clonotypes that are also detected in the esophagus, indicating a role in T cell migration in EoE. This study investigates the contribution of the GPR15–GPR15L axis in T cell recruitment to the esophagus and examines whether adhesion molecules reported to be upregulated in active EoE—PECAM1, MAdCAM1, and ICAM1 facilitate this process.
Methods: We performed in vitro cell migration assays using Transwell filters, coated or uncoated with adhesion molecules. Memory CD4+ T cells from samples were placed in the upper well, while a GPR15L gradient was established in the lower chamber. Migrated cells were quantified and analyzed via flow cytometry.
Results: Flow cytometry revealed a significant enrichment of GPR15-expressing memory CD4+ T cells, particularly GPR15+ regulatory T cells, among migrated cells from EoE patients compared to controls. PECAM1 alone did not significantly impact T cell migration. In contrast, MAdCAM1 and ICAM1 significantly enhanced recruitment of memory $CD4^+$ T cell supporting a cooperative role for adhesion molecules.
Conclusion: These findings demonstrate that GPR15L acts as a chemoattractant for memory $CD4^+$ T cells, and that MAdCAM1 and ICAM1 enhance GPR15-dependent migration, suggesting cooperative chemotactic–adhesive mechanisms. Targeting GPR15 or adhesion pathways may provide therapeutic opportunities to limit pathogenic T-cell homing to the esophagus in EoE.

D30

Polysorbate 80 Impairs Esophageal Epithelial Barrier Function: Implications for Eosinophilic Esophagitis Pathogenesis

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Introduction/rationale: Eosinophilic esophagitis (EoE) is a chronic, immune-mediated disease characterized by esophageal dysfunction and eosinophilic inflammation. Worldwide EoE incidence and prevalence have increased substantially and coincide with increased global usage of dietary emulsifiers, indicating a possible association between environmental factors and disease pathogenesis. We investigated the effects of polysorbate 80 (P80), a common emulsifier, on esophageal epithelial barrier function and inflammatory responses.

Methods: Immortalized esophageal epithelial cells (EPC2) cultured as monolayers and in air-liquid interface (ALI) were exposed to P80 concentrations ranging from 0.005% to 1%. Transepithelial electrical resistance (TEER), paracellular-flux, and cytotoxicity were assessed. Phase contrast, histology, immunofluorescence, and RT-PCR were performed.
Results: EPC2 cells exposed to concentrations of P80 >0.05% demonstrated decreased TEER, increased paracellular-flux, and cytotoxicity consistent with epithelial barrier disruption. Histology and phase contrast microscopy revealed increased intercellular spaces, enlarged nuclei, cellular detachment, and membrane disruption with administration of P80 concentrations as low as 0.05%. mRNA expression of *TSLP* and *IL33* increased (2.1-fold, $p<0.01$ and 4.2-fold, $p<0.01$, respectively) after 6 hours of exposure. Concentrations <0.05% show no or limited change in barrier impairment or expression in cultures.
Conclusion: P80 at concentrations as low as 0.05% impairs esophageal barrier integrity and induces pro-inflammatory responses. These findings suggest that dietary emulsifiers, like P80, may contribute to EoE pathogenesis by compromising epithelial barrier function, potentially facilitating immune activation.

D31

JAK1 Inhibition with Abrocitinib Modulates the IgE Axis and Basophil Activation in Adults with Peanut Allergy: An Ad Hoc Analysis

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Peanut allergy is a common, persistent, IgE-mediated condition driven by IL-4/IL-13 signaling through the IL4R-JAK1 pathway. Abrocitinib, a selective oral JAK1 inhibitor that is FDA-approved for treatment of atopic dermatitis, may attenuate IgE production and effector cell activation through inhibition of IL4R signaling. We analyzed immunologic changes in 26 peanut-allergic adults randomized to receive abrocitinib 100 or 200mg once daily for four months in the AREA trial (NCT05069831). Blood samples were collected at baseline, months 1 and 4 of treatment, and one month (1M)-post-treatment. Serology included ImmunoCAP for total, peanut-/Ara h 2-specific IgE, and ELISAs for peanut-specific IgG1 and IgG4. Functional IgE activity was tested using RBL-SX38 basophils passively sensitized with participant serum, with activation quantified by ß-hexosaminidase release (OD405nm). On average, abrocitinib treatment significantly

reduced total and peanut-/Ara h 2-specific IgE by month 4, with all levels returning to near baseline by 1M-post-treatment. Peanut-specific IgG1 increased significantly at 1M-post-treatment, reversing its downward trend during treatment. A similar but non-significant pattern was observed for peanut-specific IgG4. Peanut-induced basophil degranulation was significantly decreased by month 4, with inhibition lost by 1M-post-treatment. Despite variability between participants, preliminary analyses suggest greater drug responses among participants with lower baseline peanut-specific IgE. Short-term JAK1 inhibition decreases allergen-specific IgE levels and attenuates Fc??R1-dependent basophil activation in peanut-allergic adults, with reversible effects after withdrawal. These findings support further evaluation of abrocitinib as an adjunct to desensitization and may motivate biomarker-guided selection by baseline allergen-specific IgE level. Further mechanistic and cell-based analyses will be reported following study unblinding.

D32

OIT-mediated desensitization modifies the gut cytokine–APRIL–IgA2 network

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Background: Oral immunotherapy (OIT) is a food allergy treatment modality, facilitating desensitization via ingestion of allergenic food at sub-reactive doses, which gradually increase throughout treatment. The immune-mediated processes occurring in OIT are not completely understood, yet likely involve changes in the gut mucosal milieu. IgA2 antibodies are highly present in gut mucosa, and identified as a putative players in mediating oral tolerance. Patient stool may allow the evaluation of IgA2 and other markers potentially related to OIT-mediated desensitization.
Objective: To gain insight into OIT-mediated desensitization mechanisms involving IgA2.
Methods: Stool samples were collected from patients (n=16) undergoing walnut OIT, before and after successful treatment, and subsequently prepared as filter-sterilized eluents. Cytokines were evaluated by a multiplex array, and walnut-specific IgA2 evaluated by ELISA. Stool eluents were also assessed for their capacity to induce expression of APRIL, an IgA2 inducer, on HT29 cells.
Results: IL-6 was increased in post-OIT compared to pre-OIT samples (p=0.04). Walnut-specific IgA2 levels in post-OIT samples were positively correlated with those of TGF-β1, IL-6 and IL-10 (p=0.04 for each), and with levels of IL-12p70 and IL-17 (p=0.003 for both). The generation of APRIL in stool-treated HT29 cells was positively correlated with the stool levels of IgA2, IL-6, IL-

12p70, IL-17, and CCL20 ($p<0.05$ for all). No correlations in cytokine or walnut-IGA2 levels were observed for pre-OIT stool samples.
Conclusions: Following OIT, walnut-specific IgA2 develops in association with Th1 and Th17-related cytokines, potentially coordinated by IL-6. These findings may highlight the gut mucosal immune mechanisms for promoting allergic desensitization.

D33

Preventive Effects of Lactococcus lactis on Ovalbumin-Induced Food Allergy in Mice

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Rationale

we aimed to investigate the underlying immunological mechanisms of food allergy and evaluate the therapeutic effect of Lactococcus lactis in an ovalbumin-induced food allergy murine model.
Methods
A food allergy model was established in BALB/c mice by sensitization and challenge with ovalbumin (OVA). Mice were randomized into control, OVA, and probiotic-treated groups, with the latter receiving oral *Lactococcus lactis* (strains MG534 or GEN001). Clinical symptoms were monitored. Serum OVA-specific IgE levels was measured by ELISA. Histological analysis and cytokine mRNA expression was assessed in colon. Mesenteric lymph nodes (mLNs) and spleen were analyzed by flow cytometry. Western bloting was performed in colon.
Results
Both anaphylaxis and diarrhea scores were lower in probiotic-treated groups than in the OVA group, with a smaller decrease in rectal temperature. MG534 showed a stronger effect than GEN001 in suppressing food allergy. OVA-specific IgE was reduced in probiotic groups, significantly in MG534. Colon eosinophil infiltration was elevated in the OVA group but minimal in probiotic groups. In colon, IL-4 and IFN-γ showed no group differences, whereas TNF-α was significantly lower in probiotics groups. Regulatory T cells and the proportion of cDC1 among $CD11^+$ dendritic cells were increased in MG534 group compared with the OVA groups. Western blot revealed decreased E-cadherin and increased N-cadherin in the OVA group versus controls.
Conclusions:

Lactococcus lactis is a very effective probiotics for preventing food allergy and the MG534 strain is suggested to be more effective.

D34

Tick bites expand intermediate monocytes and dermal dendritic cells linked to type 2-skewing in alpha-gal syndrome

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Rationale: Whether dendritic cells (DCs), innate antigen presenting cells central to food-protein-immunoglobulin (Ig)E induction and anti-parasite responses, are also required for tick-induced IgE to galactose-α-1,3-galactose (α-Gal) in alpha-gal syndrome (AGS) is unknown. Transcriptomics in human PBMCs revealed increased expression of antigen presentation and cell migration genes in DC-like intermediate monocyte clusters. Thus, we hypothesized that tick bites expanded DC-like monocytes with antigen presenting capacity and conventional (c)DC2s with type 2-skewing capabilities to trigger AGS.

Methods: We infested alpha-gal-deficient (AGKO) mice with adult lone star ticks for $\leq$ 7 days and stained skin leukocytes with antibodies to CD11c and CD301b seven days post infestation initiation (7dpi), and peripheral blood leukocytes with anti-Ly6C, -CD62L, -MHCII, -CD209, and -CD1d, 14, 21, and 28dpi. Human AGS and non-AGS PBMCs were also stained with anti -CD14, -CD209, -CD74, -HLA-DR and -CD11c. Human and mouse cells were analyzed by flow cytometry.

Results: We found increased frequencies of skin cDC2 ($CD301b^+CD11c^+$) 7dpi and higher frequencies of circulating DC-like intermediate monocytes with antigen presenting capacity ($CD209^+CD62L^+MHCII^+$ and $CD209^+CD62L^+CD1d^+$) 14dpi in tick-infested compared to tick-free mice. Human DC-like intermediate monocytes with antigen presenting capacity ($CD14^+CD11c^+CD209^+HLA\text{-}DR^+$ and $CD14^+CD11c^+CD209^+CD74^+$) were increased in AGS donors.

Conclusions: DC-like intermediate monocytes with antigen presenting and migratory capacity are enriched in human AGS donors and in a tick-bite-driven AGS mouse model. Tick bites also increase dermal cDC2s. Thus, tick bites may mobilize DC-like monocytes with migratory and antigen presenting capabilities, and dermal cDC2s to promote allergic sensitization to α-Gal.

D35

Evaluation for changes in SERPINA3 levels in patients post oral food challenges

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Background: Serine proteinase inhibitor A3 (SERPINA3) is an acute-phase reactant that increases in response to inflammatory stimuli. It encodes α1-antichymotrypsin, a major plasma protease inhibitor involved in regulating inflammatory and immune processes.

Objective: To quantify human SERPINA3 in patients who underwent placebo-controlled food challenges to evaluate for changes in SERPINA3 levels post-challenge and to explore its potential relationship to allergic reactions.

Methods: Changes in SERPINA3 levels were measured in blood samples collected from patients who underwent both placebo and allergen oral food challenge (OFC) visits. SERPINA3 concentrations were quantified using an ELISA kit and analyzed in relation to demographic, clinical, and biochemical indicators of allergy. The study included 17 patients between 4 and 30 years of age. Blood samples were collected at least one hour after the OFC for subsequent analysis.

Results: A total of 20 oral food challenges were performed with seven different food allergens. No consistent relationship was observed between SERPINA3 levels, allergen, age, tryptase, reaction severity, or other allergy-related factors. SERPINA3 levels varied in samples collected days after the challenge among the participants.

Conclusion: These findings demonstrate considerable heterogeneity in SERPINA3 levels among patients post oral food challenges, indicating that additional studies are needed to clarify the factors contributing to this variability.

D36

Similarities in Metabolomic Profiles Between Sublingual (SLIT) and Oral Immunotherapy (OIT)

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Background:
Metabolomics has previously been used to demonstrate metabolite differences associated with food allergy and with remission on oral immunotherapy (OIT), though nothing is known about sublingual immunotherapy (SLIT). Here we explore the metabolomic profiles associated with SLIT and compare metabolite profile trends between a placebo controlled SLIT trial and two OIT trials.

Methods:
Untargeted metabolomic profiling was performed on plasma samples collected longitudinally from 42 children (1-4yo) in a placebo-controlled peanut SLIT trial. We compared metabolomic profiles to previously analyzed metabolomic profiles from two cohorts on OIT (PNOIT, ages 7-13yo and DEVIL, ages 1-4yo). Logistic regression models and pathway enrichment analyses were used to compare active and placebo groups.

Results:
We found that in SLIT, the Glycerophospholipid (Glycerophosphocholines ($q=2.6x10^{-17}$), Glycerophosphoethanolamines ($q=1.1x10^{-4}$), Phosphosphingolipid ($q=1.5x10^{-21}$), Fatty Acid ($q=1.1x10^{-25}$) and Amino Acid ($q=1.5x10^{-18}$) subclasses were altered in the active treatment arm compared to placebo. A majority of glycerophospholipids (29/38) were higher in the active treatment group compared to placebo and increased over time while all phosphosphingolipids (13/13) were higher in the active treatment group and decreased over time. Increases in glycerophospholipids over time on OIT were also found in our prior analyses of two separate OIT trials.

Conclusions:
Between multiple age groups and different types of immunotherapy, we found that glycerophospholipids, fatty acids, and amino acids are significantly altered in children who receive SLIT versus placebo. The replication of metabolomic profile enrichment between these independent immunotherapy cohorts could indicate their role in desensitization and potentially

even mechanisms of immune tolerance.

D37

CysLT2 receptor signaling works as a checkpoint to suppress leukotriene-dependent mast cell activation and type 2 lung inflammation through a novel Gβγ-dependent mechanism

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Background: Cysteinyl leukotrienes (CysLTs) are potent lipid mediators in asthma and eosinophilic (type 2) inflammation (T2I). While the types 1 and 3 CysLT-specific G protein-coupled receptors (GPCRs) ($CysLT_1R$ and $CysLT_3R$) promote T2I in animal models, the type 2 cysteinyl leukotriene receptor ($CysLT_2R$) can either protect from or promote T2I in vivo depending on the context.
Objective: To elucidate the mechanisms by which $CysLT_2R$ signaling in mast cells (MCs) negatively controls cysLT-dependent MC activation and T2I.
Methods: We investigated the role of $CysLT_2R$ signaling using in vivo mouse models, in vitro studies with human cord blood-derived MCs, and $CysLT_2R$-transfects. Mechanistic analyses focused on how $CysLT_2R$ signaling enhances adenylate cyclase (AC) activation and downstream cAMP-dependent protein kinase A (PKA) signaling.
Results: In transfected cells, $CysLT_2R$ activates G_q, G_i, and G_{12} proteins. Although it does not directly couple to G_s, prolonged $CysLT_2R$ signaling markedly potentiated AC activation by classical G_s-coupled GPCRs, including prostaglandin E_2 (PGE_2) EP_2 and EP_4 receptors, through a $G_{\beta\gamma}$-dependent mechanism. This $G_{\beta\gamma}$-mediated AC "superactivation" is unique to $CysLT_2R$ among CysLT receptors and leads to cAMP/PKA-dependent inhibition of $CysLT_1R$-driven MC activation. Consistently, MC-specific deletion of $CysLT_2R$ enhanced airway eosinophilia in a dust-mite (Df) inhalation model, and this amplified T2I is blocked by $CysLT_1R$ inhibition.
Conclusions: MC-intrinsic $CysLT_2R$ signaling acts as a negative regulator of allergic inflammation by amplifying cAMP/PKA signaling and suppressing $CysLT_1R$-dependent activation. These findings identify $CysLT_2R$ as a checkpoint receptor and suggest that selective modulation of its $G_{\beta\gamma}$-dependent signaling could offer a new therapeutic approach for allergic asthma.

D38

A tuft cell sensory nerve circuit directs allergen and DAMP induced olfactory stem cell proliferation

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Background: How the olfactory neuroepithelium, a sensory organ and integral part of the nasal mucosa is engaged in allergen responses is not clarified. We previously established that olfactory tuft cells, positioned at the luminal surface in direct contact with the inhaled air, are directly engaged by allergens to generate cysteinyl leukotrienes (CysLTs). Tuft cells are required for activation of the quiescent olfactory horizontal stem/basal cells (HBCs), independent of ILC2s or IL-13 receptor signaling.
Objective: To define the discrete steps from allergen sensing by tuft cells to HBC activation.
Methods: Allergen- and ATP inhalation-elicited compositional and transcriptional changes were assessed by immunofluorescence, flow cytometry and bulk-RNAseq in tuft cell-deficient (*Pou2f3*$^{-/-}$), *Chat*^{cre}*Ltc4s*$^{fl/fl}$ (tuft-specific deletion of CysLTs), and mice with deletion of each CysLT-receptor (CysLTR). Nociceptor neuron involvement was investigated in nociceptor-ablated mice (*Nav1.8*^{cre}DTA) and with nociceptor-specific deletion of $CysLT_2R$ (*Nav1.8*^{cre}*Cysltr2*^{flfl}). Innervation was assessed in cleared mouse heads of *Cysltr2*^{cre}-tdTomato, *Nav1.8*^{cre}-tdTomato mice and dual-reporter mice crossed to Chat-eGFP (tuft cells) by light-sheet-microscopy.
Results: Allergen and ATP-elicited olfactory stem cell proliferation was reduced in *Chat*^{cre}*Ltc4s*$^{fl/fl}$ and in *Cysltr2*$^{-/-}$ mice, identifying LTC_4 as an essential tuft cell mediator and $CysLT_2R$ as its receptor. Nerve fibers expressing Nav1.8, CGRP, or $CysLT_2R$ were found near HBCs, some spanning the olfactory epithelium and terminating near tuft cells. Genetic ablation of nociceptor nerve fibers or nociceptor-expressed $CysLT_2R$ significantly reduced *Alternaria* and ATP-elicited HBC proliferation.
Conclusions: Tuft cell-derived CysLTs signal through Nav1.8^{+}/$CysLT_2R^{+}$ sensory neurons to drive HBC-proliferation, linking allergen detection to olfactory stem cell activation via a sensory nerve circuit.

D39

Same slide spatially resolved multi-omic profiling identifies an immunosuppressive immune checkpoint niche in nasal polyposis

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Background: Chronic rhinosinusitis with nasal polyps (CRSwNP) is an inflammatory disease of the nasal mucosa driven by type 2 (Th2) immune responses. Immune checkpoint inhibitors are upregulated in epithelia and immune cells in CRSwNP. The role of immune checkpoint pathways as regulators of type 2 inflammation are poorly understood.
Objectives: We integrated same slide CosMx™ Spatial Molecular Imaging (SMI) RNA data and CellScape™ Spatial Immune Profiling (SIP) protein data on CRSwNP biopsies to map immune checkpoint expression in epithelial, immune and stromal niches within polyps.
Methods: Same slide CosMx SMI (Bruker Spatial Biology, Seattle, USA) with the Human 1,000-plex RNA Discovery Panel and CellScape SIP (Bruker Spatial Biology, Seattle, USA) utilising a 41-plex protein panel was undertaken on sinonasal biopsies. Alignment, visualisation and analyses of RNA and protein data were performed using Weave (Aspect Analytics NV, Genk, Belgium), with Weave's proprietary Multicellular Environment framework utilised to identify recurring cell-cell spatial niches within the tissue and explore relationships between multicellular structures and cell states.
Results: Of 102,540 cells profiled with the CosMx and CellScape platforms, 15 distinct cell types were identified. Dense T-cell and plasma cell niches were co-localised near epithelial cells. Granulocytes could be divided into PD-L1+ and PD-L1- sub-populations. PD-L1+ granulocytes along with PD-L1+ M2 macrophages (CD163+PD-L1+) and monocytes/macrophages (CD68+PD-L1+) were in close proximity to CD4+PD-1+ and CD4+FOXP3+PD-1+ T-cells in polyp stroma.
Conclusions: Integrated RNA-protein profiling identified immunosuppressive immune checkpoint niches in polyp stroma that may contribute to the persistent aberrant inflammation of CRSwNP despite immune activation.

D40

Fecal products from 1 mo-old infants at increased risk of asthma promote lung inflammation through a novel B cell/IL17/neutrophil axis

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Background: The Binational Early Asthma and Microbiome Study (BEAMS; P01-AI148104) investigates why asthma prevalence is four-fold higher in Mexican-American children living in Tucson, AZ, compared to children from Nogales, Sonora, Mexico (PMC5222738). Because marked differences in stool metabolic composition are detected already at age 1 month, we tested the impact of sterile fecal extracts (SFE) on Alternaria-driven experimental asthma.
Methods: SFE from six 1-month old infants were selected based on sufficient volumes and completion of the 6-month symptoms questionnaire. BALB/c mice were sensitized and challenged intranasally with Alternaria with or without SFE. Airway hyperresponsiveness (AHR) and broncho-alveolar lavage (BAL) cellularity measured after challenge were z-scored to the values of Alternaria control mice. Lung RNA-seq was performed on all mice (n=72).
Results: Unsupervised hierarchical clustering of lung transcriptome data identified two distinct groups of SFE-treated mice. Both clusters were protected against Alternaria-induced BAL eosinophilia, type-2 cytokine expression, but AHR,BAL neutrophilia, and *Il17* expression were significantly higher selectively in cluster-2 mice. Genes most strongly correlated with neutrophilia related to gd17 T cells (*Il17a*, *Tcrg-V4*), monocytes (*Cd14*, *Cd33*) and polyclonal B cell activation (*Igha*, *Ighg2b/c, Ighg3*, *Prdm1*). Notably, compared to wildtype controls, airway neutrophils and monocytes were reduced by 65% and 80%, respectively, in B cell-deficient Jh-/- mice treated with a cluster-2 SFE. Strikingly, cluster-2 but not cluster-1 SFEs derived from infants who reported asthma-predictive symptoms at age 6 months.
Conclusions: Fecal products from 1 mo-old infants at increased risk of asthma promote lung inflammation through a novel B cell/IL17/neutrophil axis.

D41

Gut epithelial aryl hydrocarbon receptor deficiency aggravates Candida albicans-mediated allergic airway disease via lung fucosylation regulation

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Fungal and bacterial dysbiosis link to asthma disease severity. A critical barrier to progress in the field of fungi-related allergic airway disease(AAD) has been our incomplete understanding of the associations between fungal dysbiosis, bacterial dysbiosis, and immune cells. Here using germ free (GF) mice and gut epithelial AhR deficient(AhR$^{\Delta IEC}$) mice, we tested effects of *C. albicans* colonization on AAD in a house dust mite (HDM) challenge model. GF mice with *C. albicans* intestinal colonization displayed significantly expanded levels of CD4^{+} Th17 cells in the lung compared with GF mice without colonization. AhR$^{\Delta IEC}$ mice were susceptible to *C. albicans*-mediated AAD in our model and showed the significantly increased IL-17-hyperresponsive neutrophils infiltration and neutrophil-released extracellular traps (NETs). Gene analyses and immunostaining identified the heavily α(1,2) fucosylated lung epithelial cells, which is associated with the decreased abundance of gram-negative fucosidase-producing *Akkermansia muciniphila,* in AhR$^{\Delta IEC}$ mice. Further studies found the administration of *A*. *muciniphila* or deletion of Fut2 reduces lung fucosylation and abrogates the exacerbating effects of *C. albicans* on AAD. Our studies indicate *C. albicans*-induced changes in gut AhR signal, lung fucosylation and neutrophils infiltration can lead to AAD via gut-lung axis.

D42

Progenitor Th2 cells drive chronic pulmonary Type 2 inflammation

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Multipotent progenitor Th2 cells (Th2-MPP) are associated with human type 2 inflammatory disease. These tissue resident cells express transcription factors critical for chronic and memory lymphocyte responses, including TCF1, and can differentiate into effector cells while maintaining self-renewal capacity. To understand the factors that modulate Th2 progenitor differentiation and maintenance, we modeled chronic pulmonary type 2 inflammation in mice with house dust mite and *Alternaria alternata* over months of sensitization. Lung Th2 cells during chronic inflammation were heterogeneous and included a Tcf1+Gata3+St2- progenitor compartment. Progenitor Th2 cells did not require cognate antigen and could self-renew for weeks without contribution from the draining lymph node. Moreover, type 2 inflammation showed minimal features of exhaustion over time and was sustained over months despite chronic stimulation. scRNAseq/scTCRseq analysis identified distinct transcriptional signatures of tissue and lymph

node-resident Th2 progenitors and defined clonal trajectories of the Th2 lineage. Progenitor Th2 cell maintenance was associated with tertiary lymphoid structure formation and partially dependent on local tissue B cells. These results define the function of a progenitor Th2 population with the capacity to sustain chronic type 2 inflammation.

D43

Type 2 cytokines tune regulatory T cell control of allergic inflammation

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Background:
FoxP3^{+} regulatory T cells (Tregs) play an indispensable role in restraining allergic immune responses, but the mechanisms regulating their function in barrier tissues remain unclear. The chemokine receptor CCR4, expressed on both Th2 cells and Tregs, is known to mediate migration to non-lymphoid tissues. However, the lung antigen-presenting cells producing the CCR4 ligands and the role of CCR4 in Treg-mediated suppression during allergic inflammation remain poorly defined.
Methods:
We use a murine model of allergic asthma, RNA-sequencing analysis, and a novel Ccr4 conditional-knockout mouse to define the role of the CCR4 system in controlling Treg suppression of allergic inflammation.
Results:
We demonstrate that a population of activated, type 2 conventional dendritic cells (cDC2s) are the dominant source of the CCR4 ligands during allergic inflammation. We show that the type 2 cytokines IL-4 and IL-13 directly promote cDC2 activation and CCR4 ligand expression. Using a novel, Ccr4 conditional-knockout model, we demonstrate that Tregs require CCR4 to efficiently traffic into the lungs and suppress the expansion of cDC2-CD4^{+} T cell clusters driving allergic inflammation. We show that deletion of CCR4 in Tregs resulted in reduced expression of Asb2, an E3 ubiquitin ligase that regulates actin cytoskeleton dynamics and cell trafficking. We demonstrate that IL-4 directly induces Asb2 in Tregs and promotes Treg trafficking efficiency into the lungs during allergic inflammation.
Conclusions:
Our results suggest that the type 2 cytokines tune Treg trafficking efficiency to control allergic inflammation. Our findings have implications for developing effective Treg-based therapies to durably suppress allergic diseases.

D44

B Cell Profiling Highlights Enrichment of Atypical Memory Subsets and Altered Trafficking in Severe Asthma

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Background: It is established that B cells contribute to the immunopathology of severe asthma; however, features of their dysregulation remain enigmatic.
Objective: To delineate alterations in peripheral B cell maturation, trafficking, and activation profiles in severe asthma.
Methods: Blood B cell profiles unique to severe asthma (n=30 adults) were identified by spectral flow cytometry using a 33-marker panel and by comparing to those in individuals with mild-to-moderate asthma (n=21), and healthy controls (n=29). Expert gating was applied to assess B cell maturation, activation, and trafficking.
Results:T2 transitional B cells ($CD24^+CD38^+CD21^+$) were reduced in mild-to-moderate (p=0.048) and severe asthma (p=0.0013). In severe asthma we found elevated frequencies of $CD62L^+$ class-switched B cells (p=0.0016) characterized by high CCR5 expression, suggesting enhanced lung-homing potential. $CXCR5^+$ B cells were decreased (p=0.0013), particularly within the $CD62L^+$ subsets enriched in severe asthma, consistent with trafficking to lymphoid tissues and lung. Subjects with severe asthma demonstrated expansion of IgG^+/IgA^+ quiescent atypical memory B cells (atBCs) ($FcRL5^+CD11c^-$) expressing CD86 (p=0.012), indicating arming for T cell interaction. AtBCs remained elevated after ≥6 months of biologic therapy (p=0.0015), even as other B cell subsets trended toward control levels. Distinct B cell subsets associated with impaired lung function, particularly linking decreased $CXCR3^+$ cell frequency to worse lung function as measured by FEV1 (Pearson r=0.42, p=0.0226).
Conclusions: Findings indicate broad remodeling of peripheral B cells in severe asthma, with expansion of atBC and $CD62L^+$ subsets and reduction of transitional/IgD-IgM-$CXCR5^+$ cells. $FcRL5^+CD11c^-$ atBC expansion was not ameliorated by biologics inhibiting type-2 inflammation, suggesting new targets for therapy.

D45

Blimp1 is an essential transcriptional regulator for pathogenic CD4 Tissue-Resident Memory T Cells in Allergic airway inflammation.

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Tissue-resident memory T cells (TRM) provide immediate, localized immunity but are also key drivers of chronic inflammatory diseases, including allergic asthma. While CD8 TRM generation is well characterized, the mechanisms governing CD4 TRM development and memory responses are not fully understood. B-lymphocyte-induced maturation protein-1 (Blimp1), a key transcriptional repressor, promotes effector T cell differentiation and is required for CD8 TRM generation. Our study investigates Blimp1's role in the formation and function of CD4 TRM in a model of allergic airway inflammation. Using an adoptive transfer model with Derp-1 (1DER)-specific TCR transgenic cells, we show that a population of CD4 TRM is successfully established in the lungs when mice were exposed to House Dust Mite (HDM). These cells express Blimp1, and its expression is significantly upregulated upon HDM rechallenge. Blimp1+ CD4 TRM displayed higher Th2 cytokine levels. Crucially, adoptive transfer of Blimp1-deficient 1DER CD4 T cells substantially reduced the percentage of CD4 TRMs, indicating Blimp1 is critical for their generation. Our findings reveal an essential, previously unappreciated role for Blimp1 in the development and maintenance of pathogenic CD4 TRM. Future work will employ an inducible Blimp1 deletion model to define the specific time point at which Blimp1 is required during memory formation, and single-cell RNA sequencing to delineate the molecular pathways regulated by Blimp1 that are crucial for CD4 TRM development. This study provides a foundation for exploring Blimp1 as a potential therapeutic target to prevent pathogenic CD4 TRM response that drives allergic asthma.

D46

Prostaglandin E1 (PGE1) suppresses Type 2 allergic airway inflammation by targeting EP2/EP4/IP receptor signaling on ILC2s and mast cells in PKA-EPAC independent manner

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Prostaglandins derived from arachidonic acid are implicated in Type2 airway inflammatory (T2I) disorders like asthma, and aspirin exacerbated respiratory disease (AERD). Compared with the bronchoprotective prostaglandin E2 (PGE2), PGE1 derived from dihomo-γ-linolenic acid (DGLA) is less studied and yet to be explored for its mechanistic role in T2I. We observed that mice lacking microsomal prostaglandin-E synthase-1 (Ptges -/- mice), which are highly susceptible to allergen-driven lung T2I, displayed reduced concentrations of both PGE1 and PGE2 in lung tissues relative to wild-type mice when challenged with Dermatophagoides farinae (Df). Intratracheal PGE1 administration reduced airway T2I in Df-challenged Ptges -/- mice, decreasing both lung group 2 innate lymphoid cells (ILC2s) and mast cells (MCs). PGE1 also reduced proliferation and IL-5 and IL-13 generation by IL-33 stimulated mouse bone marrow-derived ILC2s and bone marrow-derived MCs (BMMCs) in vitro, and potentiated soluble ST2 production by BMMCs, as well as by human cord blood MCs. In vitro studies using selective receptor antagonists demonstrated that EP2, EP4, and IP receptors were involved in mediating anti-inflammatory effect of PGE1. PGE1 mediated its role via cAMP, but not via Protein Kinase-A or EPAC pathways. In vivo studies with EP2Cpa3Cre and EP2KO mice highlighted that PGE1 functions even in absence of EP2 receptors due to its ability to engage EP4 and IP receptors. The broad impact of PGE1 makes it a molecule of immense interest in context of AERD, where ILC2s and MCs display significant activation in a setting of impaired cyclooxygenase/PGE2 synthase function and impaired EP2 receptor signaling.

D47

The asthmatic airway generates a dysregulated immune response to rhinovirus infection in a novel pediatric nasal organoid model

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Pediatric asthma causes millions of hospitalizations every year and is associated with compromised lung function and multiple comorbidities later in life. Rhinovirus infection is the most common trigger of pediatric asthma attacks, and early life exposure to rhinovirus induces airway remodeling that causes asthma later in childhood. We developed a novel airway model by isolating nasal epithelial cells from healthy and asthmatic children and then differentiating nasal epithelial stem cells into human nasal organoids (HNO) ex vivo. HNO are comprised of all major airway cell types, naturally organize to form the compartments of the human airway, and can be cultured in an air-liquid interface indefinitely. We examined differences in the antiviral response of healthy and asthma HNO following rhinovirus infection. Asthma HNOs produced

significantly lower levels of CXCL10 following rhinovirus infection, consistent with reports of defective interferon responses in asthmatic airways. Conversely, asthma HNOs expressed significantly higher levels of proinflammatory cytokines including IL-6 and TNF-α. These altered cytokine responses were associated with significantly elevated levels of LDH, a marker of cellular damage, and significantly increased rhinovirus replication in asthma HNOs. We propose that the epithelial cells from asthmatic airways in children generate a dysregulated response to rhinovirus infection, marked by defective interferon production and an exaggerated proinflammatory response, ultimately leading to increased viral replication and excessive cell damage. This study highlights how epithelial cells regulate the immune response, identifies dysregulated pathways and novel therapeutic targets in pediatric asthma, and is proof-of-concept for a new model of rhinovirus-induced asthma.

D48

Single Cell Characterization of IL-13 and Rhinovirus-Stimulated Bronchial Epithelial Cells in Pediatric Asthma

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Background/Objective:
This study provides single-cell characterization of bronchial epithelial cells (BECs) from pediatric patients with asthma. We hypothesized that T2 inflammation affects pulmonary epithelial cell composition and gene responses to rhinovirus infection, the most common trigger of viral-induced pediatric asthma exacerbations.

Methods:
BECs were collected from pediatric patients with (n=15) and without (n=5) asthma. They were

differentiated to an organotypic state at an air-liquid interface (ALI) for 21 days, then pretreated with IL-13 (10ng/mL) for 7 days. Differentiated cultures were infected with RV-A16 (MOI 0.5). BECs were analyzed via single-cell RNAseq prior to infection and 2 days-post infection. Leiden clustering identified cell clusters that were annotated based on established gene signatures.

Results:
We identified 11 distinct cell clusters. Further subclustering characterized rare populations including Hillock, ionocytes, pulmonary neuroendocrine, and tuft cells. Comparing healthy and asthma, preliminary analyses showed no differences in cell composition but identified transcriptomic differences, ranging from 50-2825 DEGs among the distinct cell populations (FDR<0.01), both pre- and post-rhinovirus infection. In rhinovirus-infected cells, pretreatment with IL-13 to model T2 inflammation decreased club and deuterosomal cells and increased goblet and mucous secretory cells.

Conclusions:
Preliminary results suggested there were transcriptomic differences in specific bronchial epithelial cell populations at baseline and post-rhinovirus infection when comparing pediatric patients with asthma to healthy controls. T2 inflammation impacted both epithelial cell composition and gene expression in rhinovirus infection. This study provides a novel dataset, characterizing the largest pediatric asthma single-cell dataset of primary bronchial epithelial cells differentiated at ALI to an organotypic state.

D49

Long Acting Bispecific Antibody Co-blocking OX40L and TNFα for Hidradenitis Suppurativa

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Rationale: Hidradenitis suppurativa (HS) is a chronic, immune-mediated skin disease. HS affects many millions of patients, yet current therapies such as anti-TNFα antibodies, anti-IL17 biologics, and surgery are suboptimal. Here we aim to develop an OX40L/TNFα targeting bispecific antibody, LQ095, for the great unmet needs in HS.
Methods: The in vitro activity of each part was assessed by TNFα blockade in L929 cell proliferation assay, as well as suppression of OX40L induced IL-2 release in human T cells. To evaluate the synergistic effect of LQ095, an in vitro MLR assay of DC/T cell coculture was performed with PBMCs from different donors. Additionally, we established mouse models of

human PBMCs induced GvHD to evaluate the in vivo activity of LQ095. PK profile was evaluated in cynomolgus monkeys via single-dose SC at 10 mg/kg.
Results: LQ095 binds to OX40L and TNFα simultaneously with high affinity. LQ095 strongly inhibited OX40L and TNFα signaling in vitro better than its corresponding benchmarks. In human DC/T cell coculture assay, LQ095 synergistically suppressed GM-CSF production, better than adalimumab, amlitelimab and oxelumab analogue. Furthermore, LQ095 demonstrated significantly extended average time of survival in mice compared to adalimumab, amiltelimab and oxelumab analogue. In cynomolgus monkeys, LQ095 exhibited favorable PK profile with half-life extended to 2-3 times compared to traditional monoclonal antibodies.
Conclusions: The impressive in vitro and in vivo activity data of LQ095 make it a very promising therapy for HS treatment.

D50

Post-Transcriptional Control of Th2 Immunity by HuR Inhibition Destabilizes GATA3 mRNA and Suppresses Th2 Cytokine Networks in Human Lung CD4⁺ T Cells

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Background: Persistent Th2 inflammation in allergic asthma is maintained by post-transcriptional stabilization of mRNAs encoding GATA3 and downstream cytokines. Our prior work showed T cell-specific HuR conditional knockout inhibited allergic airway inflammation, highlighting its essential role in regulating CD4⁺ T cell differentiation and Th2 cytokine expression. The RNA-binding protein HuR (*ELAVL1*) regulates these transcripts, suggesting its inhibition may attenuate Th2 responses.
Objective: We hypothesized that KH-3, a small-molecule HuR inhibitor, would reduce GATA3 mRNA stability and thereby Th2 cytokine expression in human lung CD4⁺ T cells.
Methods: Human lung tissue (n=4 donors) was digested with collagenase to generate single-cell suspensions, and CD4⁺ T cells were isolated using anti-CD4 magnetic beads and column separation. Cells were pretreated for 2 hours with KH-3 or inactive analog KH-3B, then activated with plate-bound anti-CD3/CD28 antibodies for 4 days. Cytokine secretion was quantified by Luminex assay. mRNA decay (actinomycin-D) and steady-state transcript levels were evaluated by RT-qPCR.
Results: KH-3 significantly reduced secretion of IL-4, IL-5, and IL-13 versus KH-3B ($p<0.05$), while

IL-10, IL-2, and IL-1β were unchanged. Actinomycin-D data showed accelerated decay and lower steady-state levels of GATA3 and Th2 cytokine mRNAs in KH-3–treated cells, indicating reduced mRNA stability from HuR inhibition. mRNA stability of RORγt and T-bet wasn't changed. **Conclusion:** HuR inhibition by KH-3 disrupts post-transcriptional maintenance of GATA3 and Th2 cytokine mRNAs in human lung $CD4^+$ T cells. These findings identify HuR as a central regulator of Th2 effector responses and support its targeting as a potential therapeutic strategy for allergic asthma.

D51

Selective STAT6 Disruptors Block Type 2 Inflammation and Improve Atopic Dermatitis in Mice

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STAT6 is the central transcription factor mediating IL-4/IL-13 signaling and plays a pivotal role in Type 2 inflammation. Human STAT6 gain- or loss-of-function variants have been shown to drive or protect against allergic diseases such as atopic dermatitis (AD) and asthma. Although monoclonal antibodies targeting IL-4Rα or IL-13 and JAK inhibitors have proven effective, whether direct STAT6 inhibition can translate to symptomatic relief in allergic diseases has remained unclear, partly due to the challenges of drugging this transcription factor. Using an AI-assisted, structure- and knowledge-based drug design platform, we developed small molecules that bind the Src Homology 2 (SH2) domain of STAT6 and disrupt its interaction with IL-4Rα. These compounds potently inhibited STAT6 reporter activity with >300-fold selectivity over other STAT family members. In primary human peripheral blood mononuclear cells and mouse splenocytes, the molecules suppressed IL-4–induced CCL17 release with IC_{50} values below 30 nM. In the MC903-induced mouse model of AD, oral administration of STAT6 disruptors significantly reduced serum IgE—reflecting inhibition of STAT6-dependent B-cell class switching—and attenuated ear thickening and back-skin irritation. Histological analysis confirmed decreased epidermal thickness and lower pathological scores, accompanied by reduced pruritus as measured by scratching frequency. Together, these results demonstrate that selective small-molecule STAT6 inhibitors can effectively block Type 2 inflammation and ameliorate AD in vivo. This work established the first proof-of-concept for pharmacologic disruption of the IL-4Rα–STAT6 interface and suggests an orally available alternative to current anti-IL-4Rα/IL-13 biologics for allergic diseases.

D52

Serum Proteome of Severe Atopic Dermatitis Is Enriched With Epithelial-Derived Proteins Correlating With LDH and Th2/IL22-Markers

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Background: Disease severity of atopic dermatitis (AD) varies widely, with little known about the key drivers. We identified 469 differentially-expressed proteins (DEPs, FDR ≤0.05) in the serum of severe compared to mild AD. Tissue enrichment analysis highlighted that many DEPs were epithelial-derived.

Objective: We evaluated whether epithelial-derived DEPs correlate with adaptive pathways (Th1, Th2, Th17, Th22) and/or LDH (a marker of tissue damage).

Methods: 469 DEPs (Olink-Explore3072) from AD with mild (n=33;EASI≤7) or severe (n=34;EASI≥20) disease (ADRN02;NCT01494142) were analyzed for enrichment in human tissues (HPAStainR), and evaluated for correlation (Pearson) with LDH, Th1 (CXCL9/10/11), Th2 (CCL17/18/26), Th17 (IL17A, CXCL8) or Th22 (IL-22) biomarkers.

Results: DEPs in severe AD were enriched in skin epidermal (233/469; FDR≤0.01), tonsillar squamous (243/469; FDR≤0.02), and esophageal squamous (231/469; FDR≤0.03), with 193 overlapping across these three. Among the 233 epidermal DEPs, 68 correlated with LDH (r≥0.6, FDR<0.05). 27/233 correlated with Th2, including CCL27 (r=0.86), and CDH3 with IL-22 (r=0.79). 26 showed dual Th2 & IL22 correlation including LGALS7/LGALS7B (galectin-7; [Th2]r=0.84, [IL22]r=0.77), LY6D ([Th2]r=0.82, [IL22]r=0.78), EPPK1 ([Th2]r=0.73, [IL22]r=0.77), NECTIN4 ([Th2]r=0.72, [IL22]r=0.75) and PI3/elafin ([Th2]r=0.65, [IL22]r=0.78), all correlations demonstrated FDR<0.05. No correlations (r≥0.6) were observed with Th1 or Th17 biomarkers.

Conclusion: Severe AD serum is enriched with epithelial-derived biomarkers, that likely reflect tissue damage at barrier sites, and whose expression may be driven by Th2 and Th22 pathways. Our findings highlight the potential of serum proteins as biomarkers for disease severity, immune endotype classification, and tissue injury—offering promising tools for

precision medicine and therapeutic monitoring of AD.

D53

Biparatopic TSLP and IL-13 VHHs Based Bispecific Antibody with Extended Half-life for Atopic Dermatitis

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Rationale: Although biologics for atopic dermatitis have demonstrated efficacy to some extent, there remain substantial unmet needs in this condition. To address this, we developed LQ085, a bispecific antibody targeting both TSLP and IL-13, aiming to enhance therapeutic efficacy and improve administration convenience.
Methods: TSLP neutralization in BaF3/TSLPR-IL7R cell proliferation assay, and IL-13 blockade in reporter assay were used for in vitro evaluation of each part. Inhibition of TARC release from PBMCs was used to evaluate the in vitro synergistic activity. OVA induced mice model was used for in vivo efficacy assessment of LQ085. Non-human primates were used for pharmacokinetics study of LQ085.
Results: The TSLP part, consisting of two VHHs with different epitopes, was developed under the guidance of artificial intelligence, exhibiting superior in vitro activity approximately 200-fold better than tezepelumab. LQ085 showed consistent TSLP activity with the biparatopic VHH and better IL-13 neutralizing activity than lebrikizumab and lunsekimig. It exhibited synergistic inhibition of TARC induced by combined treatment with TSLP and IL-13 in primary PBMCs and demonstrated superior anti-inflammatory effects in an OVA-induced asthma mouse model compared to tezepelumab and lebrikizumab. LQ085 also showed excellent PK profiles in NHPs, suggesting a clinical dosing regimen of approximately once every 2–3 months.
Conclusions: LQ085 demonstrated exceptional efficacy in both cellular and animal models, establishing it as a frontrunner bispecific antibody targeting TSLP and IL-13 with the potential to become the best-in-class treatment for atopic dermatitis.

D54

Mechanisms of dietary fatty acid-induced pulmonary eosinophil recruitment

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Mixed granulocytic asthma (MGA) is a severe, treatment-refractory endotype characterized by airway eosinophil and neutrophil accumulation. Obesity is a major risk factor for MGA, yet the mechanisms linking obesity to mixed granulocytic lung inflammation remain unclear. We previously found that mice fed a high fat diet (HFD) developed exaggerated pulmonary inflammation, with significantly increased eosinophils, neutrophils, and mononuclear phagocytes (MNP) compared to normal chow (NC)-fed controls. Untargeted lipidomics revealed enrichment of the long chain fatty acid stearate (SA) within pulmonary MNPs of HFD-fed mice. To test the direct effects of SA, we fed mice a SA-enriched diet and observed that it was sufficient to induce mixed granulocytic lung inflammation. We next investigated the mechanism of SA-driven lung eosinophil recruitment. In monocyte-deficient *Ccr2* knockout mice, pulmonary eosinophil accumulation to the SA-fed lung was markedly reduced, demonstrating a monocyte-dependent pathway for eosinophil recruitment. Using single-cell RNA-sequencing, we identified MNP derived mediators potentially driving eosinophil recruitment in the SA-fed lung. MNPs upregulated *Il33*, *Cxcl3*, *Ccl17*, *Ccl22*, and *Tgfb2*, suggesting potential therapeutic targets that may mediate SA-induced eosinophil recruitment and activation. These findings establish dietary SA as a driver of mixed granulocytic airway inflammation and reveal a previously unrecognized monocyte-dependent pathway for eosinophil recruitment. Ongoing studies aim to define the molecular mediators of this axis and expand our understanding of the interplay between dietary fatty acids and mixed granulocytic lung inflammation.

D55

Interferon Signaling Pathways Are Activated in Ozone Induced Airway Eosinophilia in Asthmatic Rhesus Macaques

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Background
Eosinophils are traditionally linked with type 2 (T2) inflammation; however, recent evidence suggests that eosinophils also respond to type 1 (T1) cytokines, including interferon (IFN) signaling activated during viral infections.

Objective
The interplay between IFN-driven T1 pathways and T2-mediated eosinophilic inflammation remains poorly understood, particularly in the context of oxidative stress–induced asthma exacerbations.
Methods
Rhesus macaques (aged 1.5–5.25 years) from the California National Primate Research Center were screened for methacholine hyperreactivity (EC150 <4 mg/ml). Asthmatic animals were evaluated at baseline and after 6-hour exposure to 0.3 ppm ozone (O_3) or filtered air (n=6 per group). Twelve hours post-exposure, bronchoalveolar lavage (BAL) was analyzed by cytospin, flow cytometry, and poly-A bulk RNA sequencing with differential expression adjusted for cohort, sex, age, and within-animal correlations.
Results
O_3 induced eosinophilic airway inflammation, enhanced methacholine responsiveness ($p<0.05$), and upregulated ccr3, il5ra, epx, il6r, tlr7, and st2 (logFC=6.72-0.81; $p<0.001$). T-cell IFNγ expression increased alongside cd8a, nkg7, and gzmb (logFC=2.36-0.83, $p\leq0.03$). epx correlated with ifngr2 ($r=0.997$, $p=0.0003$), while ifng inversely correlated with stat6 in both the filtered air ($r=-0.934$, $p=0.02$) and O_3 exposed groups ($r=-0.995$, $p=0.005$).
Conclusions
In response to O_3-induced oxidative stress in asthmatic rhesus macaques, T-cell derived interferon pathways were activated and correlated with the extent of airway eosinophilia. While IFNγ -mediated activation of airway eosinophilia has been shown in viral-induced asthma exacerbations, our study indicates the significance of this mechanism in mediating the effects of air pollutants.
Funding: T32 HL007013 (GKS); R42AI132012 (AH); 1R21AI185883(AH); P51OD011107(AH); TRDRP 556126/2018A(AH)

D56

IL-13 alters calcium and ciliary dynamics in nasal epithelial cells through IL-13Rα2 activation.

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Background: Interleukin-13 (Th2 inflammation cytokine), contributes to the pathophysiology of airway diseases like asthma and chronic rhinosinusitis (CRS) by inducing airway hyperresponsiveness, goblet cell hyperplasia, and increased MUC5AC mucus secretion. Since

these effects have been attributed to its canonical heterodimeric receptor IL-13Rα1/IL-4Rα, therapeutic development has primarily focused on blocking this receptor with monoclonal antibodies (e.g, anti-IL-4Rα dupilumab).
Objective: Here we further investigated IL-13 effects on nasal epithelium cell composition, intracellular calcium dynamics, and cilia function. We initially hypothesized that Th2 airway hyperresponsiveness and mucus hypersecretion might be driven by increased calcium signaling.
Methods: Primary nasal epithelial cells were treated with IL-13 for 14 days during air-liquid interface culture. IL-13 effects on nasal epithelium were assessed *in vitro* by immunostaining, quantitative PCR, live cell imaging and ciliary beat frequency measurements.
Results: As expected, IL-13 increased goblet cell density and *MUC5AC* gene expression. Surprisingly, cytosolic calcium responses after treatment with various stimuli, were consistent with a decrease in ER calcium store. Additionally, the cilia lost their sensitivity to stimulation by calcium elevation. Selective ERK inhibition restored healthy calcium and ciliary dynamics. The non-canonical IL-13 receptor IL-13Rα2, once thought a decoy receptor, has been linked to ERK. Gene expression of IL-13Rα2 was increased with IL-13 stimulation, supporting its involvement in IL-13 signal transduction.
Conclusions: Our results suggest that chronic IL-13 signaling involves IL-13Rα2 in the upper airways, with downstream activation of ERK. Thus, reducing the detrimental effects of IL-13 in diseases like CRS may require blocking IL-13Rα2 signaling in addition to IL-13Rα1/IL-4Rα.

D57

ALOX15 promotes CST1 expression through ERK and SPDEF in goblet cells in eosinophilic chronic rhinosinusitis with nasal polyps

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Backgroud
Upregulation of cystatin SN (CST1) is a hallmark of eosinophilic chronic rhinosinusitis with nasal polyps (ECRSwNP). CST1 promotes eosinophil recruitment and activation, amplifies type 2 inflammation, and is associated with disease severity. This study aims to elucidate the regulatory mechanisms governing CST1 expression in ECRSwNP and to explore potential therapeutic interventions.
Methods
We characterized the expression and localization of CST1 in ECRSwNP using scRNA-seq, immunofluorescence, and quantitative RT-PCR. Mechanistic studies on CST1 expression were

conducted using an air-liquid interface culture model of human nasal epithelial cells, employing IL-13 stimulation, pharmacological inhibitors targeting ALOX15 and the ERK pathway, and siRNA-mediated knockdown. To assess the impact of epithelial lineage switching on CST1 expression, Notch signaling was inhibited using the inhibitor DAPT.

Results

CST1 was highly expressed in goblet cells of ECRSwNP. In tissue samples, CST1 and ALOX15 levels correlated positively with goblet cell hyperplasia and eosinophil infiltration; ALOX15 correlated with SPDEF and CST1, and SPDEF correlated with CST1. In HNECs, ALOX15 knockdown or enzymatic inhibition attenuated IL-13–induced upregulation of CST1 and SPDEF. Similarly, SPDEF knockdown or ERK inhibition reduced IL-13–induced CST1 expression. Furthermore, inhibition of Notch signaling not only suppressed IL-13-induced CST1 expression but also accelerated the clearance of pre-synthesized intracellular CST1 by promoting epithelial lineage reprogramming from goblet to ciliated cells.

Conclusion

ALOX15 activates ERK and induces SPDEF, cooperatively driving CST1 upregulation in goblet cells. Inhibiting Notch signaling reprograms epithelial lineage from goblet to ciliated cells and sharply lowers CST1 levels, supporting lineage-remodeling strategies as a therapeutic approach for ECRSwNP.

D58

Single-Cell Mapping of the Gut Epithelium Reveals Paneth Cell Lysozyme Deficiency in Peanut Allergy

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Background: A growing body of evidence links small intestinal epithelial cell (IEC) barrier dysfunction to the development of food allergies. A compromise in this barrier results in dietary antigens circumventing the normal process of immune tolerance. In a murine model of peanut allergy (the genetically susceptible CC027/GeniUnc strain, "CC027"), we previously demonstrated increased IEC barrier permeability following peanut sensitization and challenge, which correlated with allergen absorption and reaction severity.
Objective: We use the allergy-susceptible CC027 mouse in its peanut unexposed, baseline state, and the allergy prone C3H/HeJ mouse, which does not develop peanut allergy in this model, to generate a high-resolution single-cell atlas of the intestinal epithelium.
Methods: Single-cell RNA sequencing (scRNA-Seq), and immunofluorescence and electron microscopy were used to compare jejunal IECs between unexposed, peanut allergy-susceptible CC027 mice and unexposed, allergy-prone C3H/HeJ mice. Our findings were verified in small intestinal biopsies from pediatric patients with and without peanut allergy.
Results: Paneth cells from CC027 mice showed enrichment of genes associated with the unfolded protein response and mitochondrial dysfunction, findings corroborated by electron microscopy revealing marked mitochondrial stress. Notably, transcriptomic profiling demonstrated an absence of lysozyme-1 (Lyz1) expression in CC027 mice, corresponding to a genomic deletion of the Lyz1 locus. Consistent with these findings, small intestinal crypts from patients with peanut allergy showed decreased epithelial LYZ expression.
Conclusion: Together, these results provide new insight into epithelial-intrinsic pathways contributing to peanut allergy susceptibility and highlight the potential role of Paneth cell dysfunction in maintaining immune tolerance to dietary antigens.

D59

Dupilumab treatment restores basal cell hyperproliferation in chronic rhinosinusitis with nasal polyps (CRSwNP)

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Background:

Airway basal cells (BCs) are progenitor cells that maintain the epithelial integrity through different functions. In allergic rhinitis and chronic rhinosinusitis with nasal polyps (CRSwNP), BC dysfunction has been suggested to contribute to tissue remodeling. Although the IL-4Rα antagonist dupilumab reduces the disease burden and nasal polyp size, the effects of dupilumab on BC functions remain unknown.

Objective:

To investigate BC abundance, migration and proliferation in CRSwNP, identify mediators driving BC hyperproliferation, and evaluate the impact of dupilumab treatment on epithelial BC abundance.

Methods:

Nasal epithelial cells were isolated from inferior turbinates of controls and CRSwNP tissue. Double-positive BCs ($CD142^+CD151^+$) were sorted, cultured, and analyzed for their migratory capacity and proliferation with/without mediator stimulation (IL-4 and/or IL-13, EGF and TGF-ß). In a double-blind, placebo-controlled trial, 24 CRSwNP patients received placebo (n = 6) or dupilumab (n = 18) for 6 months. Nasal biopsies were collected at baseline, 3 months and 6 months and stained for BCs ($KRT5^+$).

Results:

CRSwNP patients showed increased abundance, hypermigration and hyperproliferation of BCs. Moreover, stimulation of healthy BCs with IL-4 and IL-13 significantly increased proliferation, whereas EGF and TGF-ß did not. CRSwNP BCs were unresponsive to IL-4/IL-13. However, dupilumab significantly decreased epithelial BC abundance at 3 and 6 months to levels similar of healthy controls.

Conclusions:

CRSwNP is associated with BC hypermigration and hyperproliferation. Hyperproliferation can be induced by type 2 cytokines IL-4 and IL-13. Interestingly, dupilumab treatment restores basal cell homeostasis by decreasing their abundance and proliferation in CRSwNP, demonstrating disease-modifying effects.

D60

MS4A8B as an integral membrane protein for airway motile cilia function.

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Background: MS4A8B is a membrane protein strongly enriched to motile cilia of airway epithelial cells from the nose to the lungs. MS4A8B is a member of the membrane-spanning 4-domain subfamily A (MS4A) group of genes most well characterized in leukocytes, making MS4A8B's airway localization unique. MS4A homolog's molecular mechanisms are unclear but have putative functions in Ca^{2+} signaling. Our prior experiments using heterologous expression systems have characterized a role for MS4A8B in regulation of plasma membrane calcium

channels.
Objective: Specific functions for MS4A8B in the airway epithelium, especially motile cilia, have not been tested. We aim to determine the molecular function of MS4A8B as a marker of motile cilia.
Methods: Experimental approaches include culture of differentiated primary human nasal epithelial cells (HNECs) for ciliary beat frequency measurements and immunofluorescence. MS4A8B gene knockdowns were generated by anti-sense oligonucleotide (ASO) transfection.
Results: ATP treatment of HNECs increased ciliary beat frequency of intact cilia as expected, but ATP treatment failed to increase the population of beating cilia in cells treated with ASOs targeting MS4A8B compared to control scramble ASOs. Immunofluorescence labeling of ß-Tubulin IV in ASO-treated HNECs revealed marked cilia loss in cultures with MS4A8B knocked-down.
Conclusions: Knockdown of MS4A8B in differentiated HNECs induced ciliary dysfunction. Future studies will address MS4A8B-regulated pathways in motile cilia homeostasis. Because cilia loss and acquired cilia dysfunction is common in type 2 inflammatory airway diseases like asthma and chronic rhinosinusitis with nasal polyps, we hypothesize that this work will reveal therapeutic targets to enhance cilia function.

D61

Transcriptomic comparison of airway epithelial cells from Collaborative Cross mice reveals possible mechanisms of TSLP secretion

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Thymic stromal lymphopoietin (TSLP) is a pro-inflammatory cytokine released from airway epithelial cells (AECs) after exposure to protease-containing aeroallergens and represents an essential part of the Type 2 inflammatory response. Understanding the mechanisms regulating TSLP expression and secretion could uncover novel therapeutic targets for inflammatory airway diseases such as asthma. To identify genetic factors regulating TSLP production, we leveraged the extensive genotypic and phenotypic diversity of the Collaborative Cross (CC) recombinant inbred mouse panel. Mice from 47 strains of the CC were challenged with *Alternaria alternata* extract and TSLP was quantified in the bronchoalveolar lavage (BAL) fluid 6 hours post-challenge. Strains differed significantly in TSLP secretion, and we hypothesized

that comparing the transcriptomes of AECs from high-TSLP and low-TSLP strains at the peak of TSLP secretion would reveal mechanisms that regulate this process. We isolated EPCAM+ AECs from 3 high-TSLP and 3 low-TSLP strains 6 hours post-challenge and performed bulk RNA sequencing. Differential gene expression and gene set enrichment analyses revealed that multiple olfactory receptors and their downstream signaling partners were altered between high and low TSLP-producing strains. Our results confirm the expression of olfactory receptors on the airway epithelium and suggest a role for these receptors in regulating TSLP secretion following aeroallergen exposure. This study highlights the utility of the CC for identifying new avenues of mechanistic investigation and identifies olfactory receptor signaling as an unexpected pathway that may modulate alarmin release in Type 2 inflammation.

D62

mTORC1 Signaling Mediates Epithelial Dysfunction in Eosinophilic Chronic Rhinosinusitis with Nasal Polyp (eCRSwNP).

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Background: eCRSwNP is a type 2 (T2) upper airway disease featuring basal cell (BC) hyperplasia and epithelial barrier disruption. The lab has previously identified increased mTORC1 signaling in epithelial cells from eCRSwNP surgical specimens compared to non-T2 controls.

Objective: To determine whether increased epithelial mTORC1 signaling mediates these features of epithelial dysfunction.

Methods: BC mTORC1 signaling was assessed in a published single cell RNA-sequencing dataset featuring ethmoid sinus surgical samples from 21 adults (5 healthy, 5 CRSsNP, 5 neCRSwNP, 6 eCRSwNP). Gene sets correlating with BC mTORC1 signaling were identified from over 9,000 candidate gene sets using Spearman tests.

Epithelial cells from eCRSwNP patient surgical samples (n=6) were grown in air-liquid interface (ALI) culture for 21 days, then treated with TGF-β ± the mTORC1/C2 inhibitor Torin-1 for 3 or 7 days. Expression of various protein markers was measured through immunofluorescence, and statistical analyses were performed using Friedman tests.

Results: BC mTORC1 signaling was elevated in eCRSwNP compared to healthy controls (*p =

0.004), and significantly correlated with TGF-β signaling and epithelial-mesenchymal transition (EMT) gene sets (*p = 0.010, *p = 0.010) after Benjamini-Hochberg p-value correction.

At 3 days, ALIs treated with TGF-β had elevated numbers of BCs compared to control, which was abrogated with Torin-1. At 7 days, ALIs treated with TGF-β had reduced expression of the barrier-related proteins Claudin 1 and E-cadherin, compared to controls, which was rescued by Torin-1.

Conclusion: mTORC1 signaling mediates BC hyperplasia and barrier disruption in TGF-β treated ALI cultures from eCRSwNP patients.

D63

Epac as a novel target for regulation of airway motile cilia beating

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Rationale: Ciliary beating (CB) is a defense mechanism that removes debris from the airway through mucociliary clearance (MCC). Acquired defects in CB and MCC likely contribute to the pathology of chronic rhinosinusitis (CRS). Enhancing MCC may have therapeutic benefits for CRS. Calcium and cAMP are 2 major regulators of CB in nasal epithelial cells. cAMP enhances CB through protein kinase A, but we explored a different, understudied downstream effector, Epac.
Methods: Primary nasal epithelial cells (HNECs) and nasal tissue were collected from CRS (with polyps, CRSwNP; without (sans) polyps, CRSsNP) and non-CRS patients undergoing planned procedures and cultured at air-liquid interface. Epac isoform expression was assessed by qPCR in middle turbinate, ethmoid, and polyp tissues. Epac stimulation with selective agonist 8-pCPT-2'-O-Me-cAMP-AM (8-CPT) and inhibition with pan-Epac inhibitor ESI-09 were investigated with calcium imaging and ciliary beat frequency (CBF) measurements. Epac inhibition on CBF stimulation with b-adrenergic receptor agonist isoproterenol was also studied.
Results: Epac1 was the predominant isoform expressed in sinus tissues from non-CRS, CRSwNP, and CRSsNP patients; there was no difference in expression across samples. Epac stimulation with 8-CPT acutely enhanced CB by ³50%. Long-term exposure (>1 hr) to ESI-09 resulted in a significant, sustained decrease in CB. CBF stimulation with isoproterenol was not sustained in HNECs pre-treated with ESI-09.
Conclusions: Epac may contribute to baseline CBF and may be activated by cAMP signaling

downstream of b-adrenergic receptors in nasal epithelial cells. Ubiquitous expression of Epac1 suggests it might be a novel target for CB across CRS endotypes.

D64

STING agonist MSA-2 directly induces ILC2 apoptosis independent of type 1 IFN receptor

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Cytosolic DNA sensing is a central mechanism of host defense against intracellular pathogens and oncogenic stress. Upon recognizing cytosolic double-stranded DNA, cGAS synthesizes the second messenger 2',3'-cGAMP, which activates STING to induce type I interferon (IFN-α/β) production via the TBK1-IRF3 and NF-κB pathways. Although STING agonism is known to suppress ILC2s indirectly through alveolar macrophage activity and IFNAR1 signaling, the intrinsic role of STING within ILC2s remains unclear. Here, we delineate a cell-intrinsic metabolic-apoptotic pathway linking STING activation to ILC2 dysfunction, independent of IFNAR1 signaling. To examine direct effects on ILC2s, sorted lung ILCs were stimulated with IL-33 and two STING agonists: cGAMP (natural) and MSA-2 (membrane-permeable). MSA-2 caused a more pronounced reduction in proliferation, Ki-67 expression, and IL-5/IL-13 levels in culture supernatants. Transcriptomic profiling revealed no significant decrease in Il5 or Il13 expression, implicating reduced cell viability rather than transcriptional repression. Despite similar suppression of proliferation, MSA-2 elicited weaker type I IFN gene induction than cGAMP, suggesting functional differences between the two agonists. STING activation upregulated apoptotic genes, including Bcl2l11 and Pmaip1, and increased Annexin V binding in ILC2s, with a stronger effect in MSA-2-treated cells, whereas IFNAR1 blockade failed to prevent apoptosis, indicating a type I IFN-independent mechanism. Notably, STING agonists induced Hif1a and a mitochondrial dysfunction-associated transcriptional signature, suggesting impaired metabolic adaptation. Together, these findings demonstrate that STING activation directly drives ILC2 apoptosis through metabolic failure, uncoupled from canonical type I interferon signaling.

D65

Helminth-induced migratory ILC2s confer innate tolerance against allergic inflammation

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Hygiene hypothesis proposes that the microbial partners participate in human immune system development and in predisposition or protection from immune-related diseases, and the improvement of hygiene conditions contributes to a steady increase in the prevalence of allergy and inflammatory disorders such as asthma. Epidemiologically, the rise in asthma incidents strongly correlates with reduced exposure to environmental microbes, particularly parasitic helminths. However, the immunological mechanisms by which intestinal helminth infection modulates immune response in the lung remain incompletely understood. We observed that chronic helminth exposure of *Heligmosomoides polygyrus* provides protection against house dust mite (HDM)-induced airway inflammation in mice. This effect was absent during early infection and emerged only after the establishment of host resistance, coinciding with the attenuation of systemic type 2 immune responses. By employing various genetic mouse lines, we demonstrated that such protection is largely mediated by *H. polygyrus*-induced inflammatory ILC2s (iILC2s), which migrate from the gut to the lung during helminth infection. Using a parabiosis model, we found that these gut-originated iILC2s persist in the lung and replenish local natural ILC2s (nILC2s) over the time of chronic infection. ATAC-seq analysis revealed that those re-adapted iILC2s exhibit a dramatic decrease in chromatin accessibility at Th2 locus, resulting in significantly reduced production of IL-13 and IL-5. Thus, we propose that helminth exposure-elicited iILC2s confer innate tolerance in the lung, providing protection against allergen-induced asthmatic inflammation.

D66

CFTR Deficiency Increases ILC2 IL-4 Responsiveness Independent of IL-33 Release

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Background & Objective: Loss of the cystic fibrosis transmembrane conductance regulator (CFTR) gene is associated with type 2 inflammatory diseases. We have previously shown increased IL-33 release from airway epithelia following allergen challenge in $Cftr^{-/-}$ compared to $Cftr^{+/+}$ mice. Whether increased type 2 inflammation requires loss of CFTR regulation of IL-33 release or may result from other contributing CFTR mediated effects, including cell regulation of group 2 innate lymphoid cells (ILC2s) is unknown.
Methods: Pulmonary ILC2s from $Cftr^{-/-}$, $Cftr^{+/+}$, $Cftr^{-/-}Il33^{-/-}$, and $Cftr^{+/+}Il33^{-/-}$ underwent flow cytometric analysis for expression of activating markers including CD124, ST2, KLRG1, GATA3, and ID2. A portion of $Cftr^{-/-}$ and $Cftr^{+/+}$ ILC2s were also cultured ex vivo with and without IL-4 stimulation.

Results: ILC2s from $Cftr^{-/-}$ and $Cftr^{-/-}Il33^{-/-}$ mice exhibited increased expression of CD124, KLRG1, and ID2 compared to $Cftr^{+/+}Il33^{-/-}$ and $Cftr^{+/+}$ controls. IL-4 stimulation significantly increased proliferation in $Cftr^{-/-}$ compared to $Cftr^{+/+}$ ILC2s.
Conclusions: These findings demonstrate that $Cftr^{-/-}$ ILC2 have increased IL-4 responsiveness compared to $Cftr^{+/+}$ controls, and this effect is not explained by the increased IL-33 release seen in CFTR deficiency. Further studies are needed to study the role of CFTR and IL-4 signaling in ILC2 biology.

D67

Cutaneous Type-2 Innate Lymphoid Cells Amplify Neuronal CGRPa

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The skin is a densely innervated and immunologically active organ - the first line of defense against a wide variety of microbial pathogens. Recently, it is appreciated that sensory neurons and the neuropeptide Calcitonin gene-related peptide alpha (CGRPa) play an important role in physiologic and pathologic cutaneous inflammation. A growing body of literature describes immune sources of the neuropeptide CGRPa in organs such as the lung, gut and bladder. However, the cellular source/s of CGRPa in the skin remain poorly characterized. **Based on preliminary data, I hypothesized that CGRPa released by neurons in the skin is amplified by Type-2 Innate Lymphoid Cells (ILC2).** Our data support this hypothesis and demonstrate the following. (1) Optogenetic and chemical activation of cutaneous TrpV1+ neurons are both sufficient to drive local induction of CGRPa-coding *Calca* in the skin. (2) Using flow cytometry we observe that *Calca*-GFP is specifically induced/reported by ILC2 following TrpV1+ neuron activation. (3) Whole tissue and ILC2-produced *Calca,* down-stream of TrpV1+ neuron activation, are significantly attenuated by pharmacologic and genetic perterbation of CGRPa:Ramp1 signaling. Together these findings reveal a mechanism by which neuronal CGRPa is sensed and amplified by cutaneous ILC2. To understand the significance of this pathway, my work now investigates the disease context/s in which cutaneous ILC2 amplify CGRPa. Based on published science in the lung and gut, outlining similar pathways, **I hypothesize that CGRPa from ILC2 is a potent suppressor of cutaneous Type-2 inflammation.**

D68

Cysteinyl Leukotrienes are Potent Chemoattractants for Platelets in Type 2 Lung Inflammation

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Platelets accumulate in the airways in asthma and in nasal polyps in aspirin-exacerbated respiratory disease (AERD), and this infiltration correlates with disease severity. Although platelets are known to drive Type 2 inflammation (T2I), the mechanisms for their recruitment to the airways are unknown.

Cysteinyl leukotrienes (CysLTs) are potent lipid inflammatory mediators derived from arachidonic acid by the 5-lipoxygenase (5-LO)/leukotriene C_4 synthase (LTC_4S) metabolic pathway in mast cells (MCs) and other cell types. Previously, we demonstrated that platelets engage MCs in a bilateral, cysLT-driven feed-forward loop. Platelets express both $CysLT_1R$ and $CysLT_2R$. We hypothesized that MC-derived cysLTs might be important in platelet recruitment to the lung during experimentally induced lung T2I.

Our results indicate that IL-33-stimulated mast cells, as well as exogenous LTC_4 and LTD_4, strongly induce mouse and human platelet chemotaxis in 2D assays. This attraction was reduced by $CysLT_1R$ (montelukast) and $CysLT_2R$ (HAMI-3379) antagonists and in platelets from *Cysltr1*$^{-/-}$ or *Cysltr2*$^{-/-}$ mice. In an AERD-like mouse model (*Ptges*$^{-/-}$ mice challenged with aspirin), both airway resistance and platelet recruitment were increased. Deleting *LTC4S* lessened these changes, which were restored by intranasal LTC4. Furthermore, Cryo-EM showed LTC4/LTD4 stimulates platelets to form filamentous extensions necessary for movement.

Conclusion: These studies suggest CysLTs are key chemotactic factors for platelets in T2I, acting through the combined actions of $CysLT_1R$ and $CysLT_2R$. Simultaneous blockade of these receptors may be needed to suppress T2I by blocking platelet recruitment.

D69

Gut microbial SCFA and bile acid metabolism associate with farming lifestyle and atopic disease development

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Background: Farming lifestyle and gut microbiome composition during infancy have been associated with allergic disease risk; however, how farming lifestyle modulates the microbiome to protect against allergies remains unclear.
Objective: To identify early life gut metabolomic biomarkers associated with farming versus urban lifestyle and development of atopic disease.
Methods: We performed untargeted and targeted metabolomics via UHPLC-MS in 6 wk, 6 mo, and 12 mo stool samples (n=130-144/timepoint) from infants in the Old Order Mennonite (OOM) traditional farming community and urban/suburban Rochester (ROC) high risk families and analyzed their association to lifestyle and atopic dermatitis and/or food allergy development by 2 years.
Results: Metabolomic pathway enrichment analysis showed strong shifts in bile acid (BA) and short-chain fatty acid (SCFA) metabolism over time between lifestyles and ROC atopic and non-atopic children. At 6 wk, OOM infants demonstrated more BA deconjugation and increased production of lactate, while ROC infants harbored elevated conjugated primary and secondary BA and higher propionate and butyrate, the latter of which were negatively associated with fecal IgA1/IgA2 at 6 mo. By 12 mo, OOM samples were enriched with butyrate and conjugated secondary BA relative to ROC. A collection of secondary BA were among metabolites most significantly associated with development of atopic disease in ROC. Further, ROC atopic infants demonstrated elevated propionate at 6 wk and lower butyrate by 12 mo relative to ROC non-atopic infants.
Conclusions: Given the immunomodulatory potential of SCFA and BA, these may be key early host/microbial pathways related to protection against allergic disease.

D70

Environmental exposure to livestock functionally shapes the human nasal microbiome

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In recent decades, it has become appreciated that agricultural exposures can impact the microbiome and modulate health. Recent work has focused on how early life microbial exposures from farms can protect against atopic disease. However, many have studied primarily the gut microbiome, where the environmental exposures to the farm are less direct. Here, we examine how dairy cow exposure alters the composition and functions of the human nasal microbiome. We analyzed >2,200 nasal swabs collected from dairy farmers, their cows, and non-farming controls using 16S rRNA gene sequencing. We also used selective media to isolate and whole genome sequence over 500 *Staphylococci* and other genera, including a subset of bacteria capable of degrading mucin as a primary carbon source. In summary, we found that the nasal microbiomes of dairy farmers and cows are more compositionally similar than non-farmers and cows. Cultured isolates from farmers and cows exhibited greater taxonomic diversity and enriched carbohydrate-active enzymes (CAZymes), including mucin-degrading CAZymes. Furthermore, we were able to isolate more mucin-degrading bacteria from farmer and cow nasal swabs than non-farmers. Our findings suggest that livestock exposure not only shapes the composition of the nasal microbiome, but the functional diversity as well. Mucin is a primary defense of the human innate immune system, and its degradation may influence the likelihood of infection and antigenic exposure. These results underscore the ecological complexity of nasal microbial communities shaped by environmental exposures and raise new questions about how such interactions may influence susceptibility to colonization, infection, and atopic disease.

D71

Lung-infiltrating Tfh Cells Differentiate Locally to Promote Pathogenic Antibody Responses During Allergic Airway Disease

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T follicular helper (Tfh) cells orchestrate germinal center responses and promote high-affinity antibody production. A specialized subset of IL-13–expressing Tfh cells (Tfh13) drives IgE production in draining lymph nodes. Increasing evidence suggests that IgE responses are compartmentalized, with locally produced IgE contributing to allergic inflammation in the lung. We hypothesized that pathogenic IgE production in lung tissue requires coordinated activity between local Tfh and Tfh13 cells. Using genetically engineered mice and a house dust mite (HDM) sensitization and challenge model of allergic airway disease, we examined the

development and function of lung-resident Tfh populations. Both Tfh and Tfh13 cells were abundant in allergic lung tissue, with Tfh13 cells comprising 5–25% of $CD4^+CXCR5^+PD\text{-}1^+$ T cells and exhibiting robust type 2 cytokine production. Single-cell RNA sequencing revealed that lung-resident Tfh13 cells possess a distinct transcriptional program relative to other lung Tfh subsets, enriched for *Il1rl1* (IL-33 receptor), *Gata3*, *Pdcd1*, and *Pparg* expression. Adoptive transfer experiments demonstrated that lung Tfh13 cells arise from lymph node–derived Tfh progenitors that migrate to the lung and undergo stepwise differentiation through an effector Tfh intermediate. Conditional ablation of effector Tfh cells abrogated lung Tfh13 differentiation, diminished germinal center B cell responses, reduced HDM-specific IgG and IgE in bronchoalveolar fluid, and attenuated eosinophilic inflammation. Together, these findings demonstrate that *in situ* differentiation and cooperation between effector Tfh and Tfh13 cells sustains local pathogenic antibody responses and potentiates allergic lung inflammation.

D72

Asymmetric recall responses to local and distal boosts govern B-cell fate

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The development of broadly neutralizing antibodies (bnAbs) against HIV requires sustained germinal center (GC) activity and efficient recall of memory B cells (Bmem) into secondary responses. Using mice with the *DH270* antibody lineage unmutated common ancestor (UCA) knock-in and a GC fate-mapping system, we investigated how GC persistence and local antigen recall shape the maturation of bnAb precursors following immunization with CH848 10.17DT-NP, a ferritin nanoparticle–displayed HIV Env trimer.

Immunization induced steady increases in SOSIP-specific IgG and robust, long-lived GC (LLGC) responses in draining lymph nodes, where early-entering founder clones persisted, underwent progressive somatic hypermutation, and acquired key mutations linked to neutralization breadth. Ipsilateral boosting efficiently recalled these founder-derived cells, expanded GC size, and promoted further affinity maturation, whereas contralateral boosts preferentially drove plasma cell differentiation and recruited naïve or atypical memory populations. This atypical memory subset was somatically mutated but lacked measurable affinity for the immunizing antigen and was enriched for IgM, IgD, CD11b, and CD11c expression. T follicular helper (Tfh) analysis revealed that ipsilateral nodes contained more pre-existing and early-expanding Tfh compared to contralateral sites, indicating that both intrinsic properties of Bmem and local Tfh

composition shape asymmetric recall fates.

These findings demonstrate that LLGCs can sustain bnAb-lineage maturation after a single immunization and that local boosting facilitates founder recall—principles that collectively inform rational lineage-based vaccine design.

D73

Integrated Immune Transcriptomic Module Predicts Radiographic Severity in Chronic Rhinosinusitis with Nasal Polyps

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Background:
Chronic rhinosinusitis with nasal polyps (CRSwNP) is a heterogeneous inflammatory disease of the upper airway, yet the molecular pathways underpinning nasal polyps (NP) immunopathogenesis and endotypic diversity remain incompletely understood.
Methods:
We profiled tissue bulk RNA-seq from 88 NP and 317 ethmoids from CRS. Weighted gene co-expression network analysis (WGCNA) identified NP-associated modules, followed by differential expression and functional enrichment.
Results:
WGCNA revealed six NP-associated modules compared to ethmoids, and the blue module was most NP-associated. Pathway analyses for the blue module highlighted Th1/Th2/Th17 differentiation, cytokine–receptor and chemokine signaling, and hematopoietic regulation, indicating robust immune activation consistent with T2 and mixed T2/T3 endotypes. GSEA further highlighted cell adhesion/activation, leukocyte and mononuclear cell migration, actin filament organization, and GPCR signaling, supporting coordinated immune trafficking and epithelial–immune crosstalk in nasal polyp progression. Protein-protein Interaction Network (PPI) from the blue module centers on SYK/BTK (B cell/FcR signaling), integrin/ICAM1 mediated cell adhesion and migration, and epithelial cell remodeling (ACTB). In addition, this module showed the strongest link to radiographic severity, correlating with Lund-Mackay ($r=0.60$, $p<0.001$), indicating that the coordinated immune gene expression best predicts disease

burden.

Conclusion:

NP immunopathogenesis is dominated by a blue immune-activation module that aligns with T2/T3-mixed programs and most strongly predicts radiographic severity. This network-level signal outperforms single biomarkers, supporting comprehensive, module-based profiling over cytokine-centric approaches. Convergent GSEA and PPI mapping implicate SYK/BTK signaling and integrin/ICAM1-mediated adhesion alongside epithelial remodeling, highlighting coordinated epithelial–immune crosstalk as a driver of polyp progression and pointing to tractable therapeutic targets.

D74

Targeting pathogenic Th2 TRMs formed after early life respiratory viral infection mitigates asthma pathology.

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Background: Viral lower respiratory tract infections, which are remarkably common in the first 3 years of life, confer an increased risk of asthma development by age 7. Asthma is the most common chronic disease of childhood and is often driven by Th2 immunity characterized by eosinophil recruitment, mucus production, and airway hyperresponsiveness.

Objective: To model asthma development following early life viral infection and to disrupt the immunologic connection linking these two pathologies.

Methods: Neonatal mice were infected with human metapneumovirus (HMPV) on day of life 4-6, then reinfected as adults. Tissue resident memory cells (TRMs) were identified via exclusion of intravascular cells followed by purification for single cell RNA sequencing. TRMs were depleted with local administration of an anti-CD4 antibody. Th2 TRMs were specifically targeted with fedratinib, a selective JAK2 inhibitor.

Results: Mice infected with HMPV as neonates and reinfected as adults demonstrated asthma pathology, while mice infected initially as adults did not. Following neonatal infection, a clonally expanded TRM population demonstrated Th2 effector functions. Local depletion of TRMs reduced eosinophil recruitment and mucus production. The Th2 TRMs had several identified differentially expressed genes, including Jak2. Treatment of HMPV-reinfected mice with

fedratinib, a selective Jak2 inhibitor, reduced eosinophil recruitment and mucus production.

Conclusion: Targeting Th2 TRMs formed after early life viral infections mitigates asthma pathology in the setting of virus-induced wheeze. Future studies could further explore targeting these cells in a translational or clinical approach in children with recurrent wheeze and asthma.