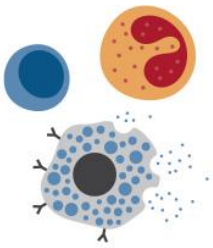

DISCOVERY 2025

Mechanisms of Type 2 Inflammation in Disease and Beyond

2025 Discovery Program Abstract Book

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D01 Flcn regulates Plasma cells, IgE and anaphylaxis

Keyword: Immunoglobulin E

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Plasma cells are terminally differentiated antibody producing cells responsible for proper immune defense but also autoimmunity and allergic reactions. We have found a hypomorphic missense mutation in Flcn named pansy , which was linked to mild (30%) decreased T-dependent and T-independent IgG responses, but a 90% decrease in total IgE response to Ova/Alum. These findings were validated by CRISPR/Cas9 induced knock-in of the Flcn-pansy allele. Biochemically, RNA and protein levels were not affected, which suggests structure is intact, but function is impaired.Folliculin (FLCN) is mutated in Birt-Hogg-Dube' syndrome and regulates mTOR through folliculin-interacting proteins (FNIP)1 and FNIP2. They create a unique activation of mTOR such that the TFE3/TFB transcription factors are activated to regulate metabolism or lysosomes for phagocyte specific deletions, but our hypomorphic mutation has no change in phagocyte numbers and is phenotypically healthy, fertile, and viable. Instead, we found novel changes in plasma cell numbers and function. Study of in vitro plasma cell differentiation showed a developmental block at the plasmablast stage with 50% decreased plasma cells. However, the effects in vivo were more profound with >90% decrease in IgG1 and IgE plasma cell and germinal center cells when immunized with the model allergen, papain. Finally, Flcn deficient mice were re-challenged with papain they were immune to anaphylaxis while age and sex matched wild type controls (n=10) experienced profound hypothermia, shock or death ($p<0.0001$).This study identifies Flcn as a potential target to block plasma cell development to prevent the pathogenic effects of IgE.

D02 High Protein Diet Increases the Risk of Allergic Sensitization But Not Asthma In Mice Through Modulation Of The Cytokine Milieu Toward Th2 Bias

Keyword: Cytokines

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Introduction: The role of different nutrients on allergic sensitization is not clear. In this study we aimed to determine the effect of high protein diet on allergic sensitization, cytokines profile and asthma in mice. Methods: Seven to eight-week old female BALB/c mice were fed either normal (ND) or high protein (HP) diet and were sensitized with ovalbumin intraperitoneally followed by intranasal challenge. Allergic sensitization was tested by measuring anti-OVA IgE, IgG1 and IgG2a antibodies. Cytokines levels were tested by multiplex ELISA in splenocyte supernatants after stimulation. Airway inflammation was tested by measuring total and differential cell counts in bronchoalveolar lavage fluid and by measuring bronchial mucus production, goblet cell hyperplasia and perivascular and peribronchial inflammation severity scores by histologic examination. Results: Mice fed HP diet had a significant increase in weight and higher levels of OVA-specific IgE and IgG1 antibodies compared to the ND group. In addition, they showed a selective Th2 bias in cultured splenocyte supernatants compared to the ND group. However, the level of airway inflammation was comparable between both groups. Conclusions: HP diet increases the risk of allergic sensitization though increase in Th2 cytokines. Efforts should be made to define the upper limit of protein in the diet that does not predispose to allergic sensitization. The effect of diet on health should remain a focus of research for the establishment of optimal health and resilience.

D03 Platelet derived cysteinyl leukotrienes drive IL-33-dependent type 2 lung immunopathology in a sex-biased manner

Keyword: Innate Lymphoid Cell

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Rationale: Cysteinyl leukotrienes (CysLTs), LTC4, D4, and E4, are potent inflammatory mediators that drive type 2 inflammation (T2I). Platelets express LTC4 synthase (LTC4S) and cooperate with 5-lipoxygenase-expressing granulocytes to generate LTC4 and are necessary to drive eosinophil recruitment in models of lung T2I by incompletely understood mechanisms. LTC4 is necessary to expand IL-33-expressing alveolar type 2 (AT2) cells through a CysLT2 receptor-dependent pathway in T2I. IL-33 synergizes with LTC4/LTD4 to drive proliferation and cytokine generation by group 2 innate lymphoid cells (ILC2s). Although sex differences in control of leukotriene production and its consequences potentially contribute to the female predominance of T2I-related diseases, the specific dimorphic features under the control of the CysLTs remain unexplored. Methods: LTC4Sflfl/PF4cre (platelet factor 4 [PF4]-driven LTC4S deletion), IL-33flfl/SPCcre (surfactant protein C [SPC]-dependent IL-33 deletion), and IL-33flfl/VAV1cre (IL-33 deletion in hematopoietic cell) mice were given 12 ug of Alternaria Alternata extract in 20 ul of saline or saline alone by intranasal inhalation on days 0, 3, 6, and 9 and euthanized 24hr after the last dose, monitoring broncho alveolar lavage fluid eosinophilia, ILC2 expansion and activation, and lung levels of IL-33. Results:PF4-dependent deletion of LTC4S from platelets and VAV1-dependent deletion of IL-33 from hematopoietic cells significantly suppressed all features of lung T2I in female but not male mice. SPC-dependent deletion of IL-33 from AT2 cells eliminated the increase in lung IL-33. Conclusions: Platelet-driven transcellular synthesis of LTC4 can contribute substantially to lung T2I features, and may contribute to sex differences in strength of T2I.

D04 Peanut component-specific IgE and IgG4 are modulated by sublingual immunotherapy and are associated with remission in young children

Keyword: Food Allergy

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RationaleOur group recently demonstrated the safety and efficacy of 36-month peanut sublingual immunotherapy (SLIT) in peanut-allergic children aged 1-4. Mechanisms of long-term peanut SLIT and predictors of remission are unknown. MethodsPlasma from 30 participants (17 SLIT, 13 placebo) at 0, 12, 24, 36, and 39 months (3 months off SLIT) was analyzed with ImmunoCAP250 (Phadia, Sweden) to quantify sIgE and sIgG4 to Ara h 1, 2, 3, and 6 (Ah1-6). Clinical outcomes (remission, desensitization, partial desensitization) were previously assessed. Longitudinal changes in component-specific IgE, IgG4, and IgG4/IgE ratios were compared to baseline, and between-group differences were assessed at each time point.ResultsSignificant longitudinal changes in SLIT participants included decreases in median Ah2- and Ah6-sIgE, increases in Ah1-, 2-, and 6-sIgG4, and increases in Ah1-6-sIgG4/sIgE ratios. Ah2 and Ah6 showed the most pronounced changes. No significant changes were observed in the placebo group between baseline and 36 months. Between-group comparisons showed lower median baseline Ah1-6-sIgE and lower Ah2- and Ah6-sIgG4 levels in SLIT participants who achieved remission than in those partially desensitized. These differences persisted throughout the 36-month trial. The most pronounced baseline difference was in Ah2-sIgG4, with remission showing a median of 0.13 mgA/L lower than desensitization ($p=0.0035$) and 0.57 mgA/L lower than partial desensitization ($p=0.0020$).ConclusionsThese findings suggest peanut SLIT primarily modulates Ah2- and Ah6-specific immunoglobulins, with Ah2-sIgG4 levels potentially being the strongest predictor of success. Future studies should focus on developing a predictive tool to inform clinicians on candidate suitability for peanut SLIT.

D05 Intestinal helminth infection induces the dissemination of long-lived Th2 cells with Th1 features to distal organs

Keyword: Helminth Infection

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Intestinal helminth infection can alter the host immune system. Herein, we show that Heligmosomoides polygyrus-specific Th2 cells can be found in organs such as lung, mesenteric lymph nodes, and visceral adipose tissue until at least 6 months after primary infection. Long-lived Th2 cells in distal organs expressed CD69 mRNA and appeared to adopt gene expression features associated with the tissue from which they were isolated. Th2 cells in the lung responded vigorously to the alarmin recombination IL-33 and fungal extracts of Alternaria Alternata. Analysis of T cell receptor clonality demonstrated that distal organs were colonized by a range of Th2 cell clones that were dispersed throughout the mouse. Moreover, single-cell RNA-sequencing pinpointed cells with features of both Th1 and Th2 cells in lung and visceral adipose tissue, suggesting that these cells may be capable of exerting multiple functions. In all, intestinal helminth infection induces the dissemination of T helper cells throughout the organism, which can have important consequences for responses to allergens at distal sites.

D06 Fibrocytes as Potential Drivers of Type 2 Inflammation in East Asian CRSwNP

Keyword: Cytokines

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RATIONALE: Chronic rhinosinusitis with nasal polyps (CRSwNP) in East Asian populations is more heterogeneous compared to Western populations, with approximately 40-60% demonstrating Type 2 inflammation. The specific roles of individual cell types contributing to this inflammation are yet to be determined.METHODS: Nasal polyps were collected from 10 consented CRSwNP patients undergoing routine endoscopic sinus surgery. Eosinophil counts were assessed on pathology slides per high-power field (HPF). Single-cell suspensions from the nasal polyps were stained with 29 markers. This study focused on six cell types (fibrocytes, ciliated cells, goblet cells, basal cells, neutrophils, and eosinophils) and three Type 2 cytokines (IL-4, IL-5, and IL-13). Analysis was conducted using multiparameter cytometry with the Sony ID7000 system.RESULTS: Fibrocytes showed significantly higher expression of IL-4 and IL-13 compared to other cell types ($p<0.005$ and $p<0.0001$, respectively), with the exception of IL-13 in goblet cells. In contrast, IL-5 expression did not significantly differ among the cell types. No significant differences were found in Type 2 cytokine expression of fibrocytes between eosinophilic (>10 eosinophils/HPF) and non-eosinophilic CRSwNP groups.CONCLUSIONS: Among the cell types analyzed, fibrocytes exhibited the highest Type 2 cytokine expression in this East Asian CRSwNP cohort , independent of eosinophilic status. These findings suggest that fibrocytes may play a significant role in the pathogenesis of CRSwNP in East Asian populations, warranting further investigation.Funding: Grants from the National Science and Technology Council of Taiwan (NSTC 112-2314-B-002-096)

D07 Development of epitope-specific antibody cross-reactivity between peanut allergens Ara h 2 and Ara h 6

Keyword: Food Allergy

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Rationale: Despite new insights into how epitope-specific antibodies mediate efficacy of oral immunotherapy, we have yet to understand how these high-affinity peanut-specific antibodies evolve. Previous literature establishes epitope diversity and polyreactivity as clinically relevant aspects of epitope-specific antibodies. We previously defined the cross-reactivity between immunodominant peanut allergens Ara h 2 and Ara h 6 using a set of monoclonal antibodies from highly sensitive peanut-allergic individuals. We identified three cross-reactive epitopes, including a public epitope identified in multiple cohorts of peanut-allergic patients. We hypothesized that by studying the evolution of antibodies to this epitope, we could better understand epitope diversification and immune progression in peanut allergy. Methods: We utilized Ara h 2-specific monoclonal antibodies previously cloned from the peripheral blood of peanut-allergic patients. We systematically reconstructed antibody evolution using site-directed mutagenesis of the monoclonal antibodies that arose from different IGHV/IGHJ gene rearrangements. Results: Only two of the three distinct IGHV/IGHJ gene rearrangements in the heavy and light chains of monoclonal antibodies are cross-reactive at germline. Systematic addition of mutations in the heavy and light chain complementary determining regions resulted in increased affinity and epitope spread in the third antibody lineage. Conclusion: In summary, allergen-specific antibodies can acquire cross-reactivity and increased affinity through mutation in allergen-binding regions. Our data suggest that a key component of immune progression is the epitope diversification that occurs through affinity maturation. We speculate that these findings may also help explain why early intervention, before epitope diversification progresses, may be more effective in the context of oral immunotherapy.

D08 IL-21 Induced Changes in Monocytes and the Impact of STAT-3 Inhibition

Keyword: Cytokines

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Rationale: Dormant viruses in the body can continuously trigger the immune system, leading to increased production of IL-21 over time. IL-21, a proinflammatory cytokine in the gamma chain family, primarily affects B and T lymphocytes. However, chronically elevated IL-21 levels with aging may also alter the function of other cells, such as monocytes, and influence their response to infections. We investigated how IL-21 affects the production of IL-6 and CXCL-10 by monocytes, given their key role in the cytokine storm associated with COVID-19, which disproportionately affected older adults. Methods: Monocytes isolated from blood were treated with either IL-21 or IL-21 and a STAT3 inhibitor. STAT-3 inhibitor was used because IL-21 signals via the JAK-STAT pathway. Samples were assayed for IL-6 and CXCL-10 levels using ELISA. Results: Monocytes treated with IL-21 displayed a significant increase in the production of IL-6 and CXCL-10 ($p = 0.0122$; $p = 0.0248$). Inhibition of IL-21 signaling via STAT-3 inhibitor resulted in significantly decreased production of IL-6 and CXCL-10 when compared to monocytes treated with IL-21 alone ($p = 0.0283$; $p = 0.0307$). Conclusion: Chronic IL-21 levels enhance the secretion of detrimental mediators from monocytes that are involved in cytokine storms during infections. Further, IL-6 has been postulated to be a master regulator of IL-21. Our results support a more reciprocal understanding of the relationship between IL-6 and IL-21 and call attention to the potential use of STAT3 inhibition in the treatment of diseases with aberrant levels of IL-21.

D09 Nasal commensal bacteria modulate taste receptor mediated sinonasal epithelial innate immune pathways

Keyword: Microbiome

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Bitter taste receptors (T2Rs) are chemosensors that detect microbial products, subsequently inducing rapid and early immune responses against these encountered microbes. Activation of certain T2Rs on ciliated epithelial cells results in an immediate calcium-dependent increase in nitric oxide (NO) production and ciliary beating. Activation of T2Rs on nasal tuft cells results in the release of antimicrobial peptides. However, the effect of commensal nasal bacteria on taste receptors in chronic airway diseases needs further exploration. Hence, we investigated the strain-specific effects of microbial soluble factors produced by commensal nasal bacteria on T2R function. Primary human nasal epithelial air liquid interface cultures from Non-CRS and CRSwNP patients were used to investigate the effect of conditioned media (CM) from commensal airway bacteria on ciliary beating, NO production, and antimicrobial peptide production (e.g. β2-defensin). CM of Staphylococcus aureus USA300 blunted ciliary beating both in CRSwNP and Non-CRS and reduced NO metabolites (NOm). Stimulation with Dolosigranulum pigrum AMBR11 CM increased ciliary beating and NOm in CRSwNP but not in Non-CRS. CM of Lacticaseibacillus casei AMBR2 and Lacticaseibacillus rhamnosus GG did not alter ciliary beating in either group. Evaluation of airway surface liquid β2-defensin concentration, demonstrated elevated levels upon stimulation with L. casei AMBR2 CM in CRSwNP, which was not evident with CM from D. pigrum AMBR11 nor LGG. S. aureus USA300 did increase β2-defensin production in Non-CRS. These findings indicate a strain-specific effect of microbial soluble factors on sinonasal innate immune pathways regulated by taste receptors, that may contribute to chronic respiratory diseases.

D10 Gut Microbiota-Derived Metabolites Regulate Atopic Disease.

Keyword: Microbiome

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The incidence of atopic diseases is increasing rapidly in industrialized countries. While diet, pollution, and the microbiota are known to play a role in their development, the mechanisms by which these factors interact with one another and the host, remain to be fully elucidated. We hypothesized that the gut microbiota of individuals with atopic diseases encodes metabolic pathways that can regulate activation of atopic responses in the host. Using cell-free supernatants from gut microbiota strains, we identified the bacteria that produce metabolites that activate primary mast cells. Mast cells were selectively activated by metabolites from specific gut microbiota species in a dose-responsive manner while maintaining cell viability. Biochemical and analytical methods were used to further investigate the bioactive metabolites within the supernatants. And while this analysis is ongoing, results so far have revealed the involvement of small, polar molecules driving mast cell activation and exacerbating mast cell-mediated, IgE-dependent allergic responses. Immunological analysis has ruled out the involvement of common pattern recognition receptors on the mast cells. Ongoing work is focused on further characterization of the bioactive metabolites and the biosynthetic genes involved in their production, and the mechanisms by which these metabolites activate type 2 immune cells and regulate allergic responses in vivo. Identifying novel metabolic pathways encoded by the gut microbiota that activate type 2 immune cells provides potential novel therapeutic targets for small molecule inhibitors to prevent or treat atopic diseases.

D11 CFTR inhibits Th2 cell response in airway allergy

Keyword: TCell/BCells

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RATIONALE: A type 2 (T2) inflammatory endotype has been identified in people with cystic fibrosis (CF). The objective of this study was 1) to determine the role of CFTR in Th2 cell function and Th2 cell-mediated allergic inflammation, 2) elucidate the underlying molecular Th2 cell signaling mechanisms, and 3) to test the therapeutic potential of a clinically approved CFTR potentiator in allergic disease.METHODS: CD4Cre+Cftrfl/fl or CD4Cre-Cftr+/+ mice were challenged with PBS or Alternaria alternata extract (AE). AE-challenged murine Cftr or human CFTR expressing mice and CD4+ T cells were treated with vehicle control or the CFTR potentiator ivacaftor. Human naive CD4+ T cells were isolated from PBMCs, cultured in Th2 polarizing conditions, and treated with either DMSO control or the CFTR potentiator ivacaftor.RESULTS: Cultured murine Cftr-/- CD4+ T cells had increased IL-4, IL-5, and IL-13 production in vitro compared to Cftr+/+ controls and murine CD4+ T cell specific Cftr knock-out resulted in significantly increased allergic inflammation to AE. CFTR expression decreased sensitivity of CD4+ T cells to IL-4 thereby reducing GATA3 expression. CFTR potentiation decreased human CD4+ T cell GATA3 expression and IL-13 secretion in vitro, and significantly T2 inflammation in allergen-challenged hCFTR mice compared to drug vehicle.CONCLUSIONS: CFTR is a negative regulator of Th2 effector function in CD4+ T cells and loss of CFTR results in increased allergic inflammation, an effect reversed by current CFTR modulator therapies. These studies highlight a potential therapeutic use for CFTR modulators in allergic disease.

D12 Dietary stearate alters lung myeloid cell metabolism and causes inflammasome-dependent IL-1β cytokine production

Keyword: Macrophage/Dendritic Cell

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Obesity is a major modulator of immune function and is associated with multiple inflammatory diseases including asthma. Obesity-associated asthma (OAA) is a distinct endotype characterized by type 1 inflammation and neutrophilia. Yet, the underlying mechanisms by which diet-induced obesity alters innate immune cell activation in the lung are unclear. The broad objective of this work is to decipher how a common dietary saturated fatty acid, stearate, alters the transcriptional profiles and metabolic mechanisms of lung myeloid cell populations in the steady state. The central hypothesis is that metabolic processing of stearate induces pro-inflammatory cytokine production in a subset of lung-resident macrophages. To test this hypothesis, we exposed mice to a stearate-enriched or calorie-matched control diet for 5 weeks and examined lung cells by single cell RNA sequencing and flow cytometry. Dietary stearate caused upregulation of genes related to lipid metabolism, cellular stress, and inflammation. Upon flow cytometric analysis, we found that dietary stearate caused intracellular lipid accumulation in lung myeloid cells and induced inflammasome-dependent IL-1β cytokine production. Furthermore, lung neutrophils, monocytes, and a subset of lung-resident macrophages expanded their respective populations in animals fed the stearate-enriched diet. Notably these changes occurred in the absence of weight gain. Together, our data indicate that the saturated fatty acid stearate causes pro-inflammatory shifts in lung innate immune populations. Notably, weight gain is not required for these immune alterations indicating that specific dietary components, rather than obesity per se, are the primary driver of these effects.

D13 QTL mapping with Collaborative Cross mice reveals Ffar3 as a gene that reprograms ILC2 effector function

Keyword: Innate Lymphoid Cell

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RATIONALE: The molecular mechanisms that govern group 2 innate lymphoid cell (ILC2) expansion and effector function are incompletely understood. We hypothesized that there exist genetic regulators of ILC2 that have not yet been described and that could be identified with a quantitative trait locus (QTL) mapping study. METHODS: We examined the number of ILC2 in the lung after 4 consecutive days of Alternaria alternata extract challenge in 48 strains of the Collaborative Cross recombinant inbred mouse panel with known genetic backgrounds. We mapped a 3.43 megabase QTL on mouse chromosome 7 containing 72 protein coding genes that associates with ILC2 number in response to aeroallergen challenge. Through transcriptomic comparison of ILC2s from phenotypically extreme strains, we identified free-fatty acid receptor 3 (Ffar3) as a gene candidate responsible for the QTL effect. We isolated ILC2 from the CC030 recombinant strain (highest ILC2 phenotype), and we cultured them in stimulatory conditions with or without the FFAR3 agonist AR420626. RESULTS: ILC2 from CC030 mice stimulated with the FFAR3 agonist had greater numbers after culture, but they were reprogrammed into a less inflammatory effector state compared to vehicle controls. FFAR3 agonism reduced ILC2 apoptosis, decreased IL-5 and IL-13 expression, and increased IL-10 expression. This reprogramming occurs through FFAR3 signaling upregulating epidermal growth factor receptor (EGFR) expression on ILC2. CONCLUSION: FFAR3 can be expressed on ILC2s and regulates their effector states. These studies highlight FFAR3 as a potential therapeutic target that could be utilized to reprogram inflammatory ILC2s to an anti-inflammatory effector state.

D14 Laboratory Mice Exposed to Pet Store Mice Show Profound Sex- and Tissue-Dependent Immunological Phenotypes

Keyword: Regulation of inflammation

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Rationale: Mice are used for models of human diseases. The immune composition of specific-pathogen-free (SPF) mice most closely resemble that of newborn humans. Adult humans have been exposed to a multitude of pathogens throughout their lives. In this project, we sought to create a mouse model resembling humans by co-housing SPF mice to pet store mice. Methods: Male and Female SPF C57BL/6 mice were co-housed with pet store mice. Multiplexed PCR was used to assess pathogen transmission. After 2 months, lungs, small intestines, and colons were analyzed by high-dimensional mass cytometry and multiplexed cytokine assay.Results: Co-housed male mice were susceptible to infection and frequently succumbed to disease. After two months, co-housed male mice exhibited signs of active infection across all organs examined including, increased CD45+ cell infiltration, and myeloid and T effector cell expansion. Conversely, infection was less fatal for female mice and active sickness was resolved by two months. T cells from female mice exhibited both memory and effector phenotypes. Across tissues, robust increases in monocytes and granulocytes were observed in lungs and colons while increases in CD8+ T cells dominated in the small intestine. Importantly, innate lymphoid cells, such as group 2 innate lymphoid cells, were not displaced but underwent phenotypic changes.Conclusion: Unsynchronized multiple infections in SPF mice co-housed with pet store mice results in dramatic alterations in the immune landscape in a sex- and tissue-dependent manner. When used in disease models, these mice may provide new insight beyond those obtained previously with SPF mice.

D15 Early-life immunization with an aluminum adjuvant-based vaccine results in preferential priming of Th2-biased immune responses to heterologous antigens

Keyword: TCell/BCells

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Rationale: While vaccines are commonly administered to infants, a major gap in knowledge exists in understanding their immune effects specific to early life. The immature infant immune system develops dependent upon environmental stimuli. Vaccine adjuvants may play important roles in shaping immune development.Methods: Mice were immunized as neonates or adults with an alum-adjuvanted hepatitis B vaccine (HB), generally administered to humans within days of birth. Mice subsequently received a 2-dose vaccine series of ovalbumin (OVA) and the adjuvant MPLA to determine alterations of heterologous immune responses.Results: Immune responses to HB-alum vaccine were more strongly Th2 polarized when given earlier in life. The effects on Th2 skewing were not limited to HB, as early-life immunization with HB-alum also led to the development of more strongly Th2 polarized immune responses to subsequent immunization with OVA-MPLA. Interestingly, mice that were immunized with HB-alum in adulthood generated Th1-polarized OVA-specific responses more consistent with the responses of naive mice. Early-life immunization with HB-alum also changed the phenotype of dendritic cells in the bone marrow, suggesting a role for trained immunity in the heterologous effects.Conclusions: Immunization with alum in early life induces stronger and more long-lived Th2 immune responses and predisposes mice to develop Th2-biased immunity to subsequent antigen exposure. Because vaccines are crucial elements of health in infancy, furthering our understanding of early life immunization will allow for the development of vaccines that induce beneficial effects on immune development through the choice of adjuvant.

D16 Peanut Allergy-Susceptible CC027 Mice Demonstrate Paneth Cell Dysfunction

Keyword: Food Allergy

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BackgroundSevere peanut allergy (PA) is associated with increased intestinal epithelial cell (IEC) permeability. The CC027 mouse, unlike traditional food allergy models, is susceptible to PA by oral exposure without exogenous immune stimulation. We looked at baseline differences in IEC composition and function in CC027 and PA-resistant (C3H/HeJ) mice to understand how this contributes to increased IEC permeability. Methods We collected jejunal morphometrics on H&E sections, performed quantitative RT-PCR to measure the jejunal expression of IEC subtype markers and antimicrobial peptides (AMPs), and used immunofluorescence (IF) to measure jejunal proliferation (Ki67) and the AMP lysozyme 1 (Lyz1). Results There were no differences between mouse strains in jejunal villi or crypt length. Expression levels of jejunal chromogranin A (enteroendocrine cells), doublecortin-like kinase 1 (tuft cells), guanylate cyclase activator 2a (secretory IECs), mucin 2 (goblet cells), and leucine rich repeat containing G protein coupled receptor 5 (stem cells) were significantly higher in CC027 compared to C3H/HeJ mice, while that of the AMP alpha-defensin 5 (Paneth cells) was significantly lower. Other AMPs (lysozyme 1 (Lyz1) and secreted phospholipase A2) were expressed at low levels in CC027 mouse jejuna, while mucosal pentraxin 2 and regenerating islet-derived 3 γ were highly expressed in CC027 mouse jejuna. IF showed fewer proliferating cells (Ki67) and lower crypt Lyz1 expression in CC027 mice. Conclusions We identified putative altered IEC composition and decreased Paneth cell AMP production in a PA-susceptible mouse, revealing an intriguing link between Paneth cell dysfunction, enteric microbial dysbiosis, and intestinal permeability in PA pathogenesis.

D17 Tissue-Resident CCR8+ST2+ T Regulatory Cells Alleviate Chronic Type 2 Airway Inflammation via IL-10 and Amphiregulin

Keyword: TRegs

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RATIONALE:CD4+ T regulatory (Treg) cells play critical roles in maintaining immune homeostasis. Treg cell heterogeneity has been recognized in different diseases. This project aimed to identify the potent Treg cell population that play a key role in controlling type 2 airway inflammation. METHODS: Naive C57BL/6J mice were intranasally (i.n.) and repeatedly exposed to fungus Alternaria alternata extract. To examine regulation of antigen-specific immune response, Ccr8-/- and Foxp3DTR mice were sensitized to ovalbumin (OVA) i.n. with Alternaria extract as an adjuvant and then challenged with OVA antigen alone. RESULTS: During Alternaria-induced airway inflammation, Treg cells in lung tissues highly expressed transcripts involved in regulatory functions (i.e. Tgfb1, Ctla4, Pdcd1, Il2ra) compared to those in mediastinal lymph nodes. Mass cytometry identified several populations of Foxp3+ Treg cells in the lungs, including those expressing CCR8 and ST2. By Alternaria exposure, CCR8+ST2+ Treg cells expanded within lung tissues in 4 weeks and persisted at least for additional 8 weeks. Blocking immune cell egress from lymphoid organ with FTY720 did not affect the tissue residency of CCR8+ST2+ Treg cells. Treg cells (Foxp3DTR) or CCR8 (Ccr8-/-) deficient mice demonstrated the potent protective role of CCR8+ST2+ Treg cells in antigen-specific airway inflammation. Ex vivo characterization showed superior regulatory functions of CCR8+ST2+ Treg cells as compared to CCR8-ST2+ Treg cells, including increased expression of KLRG1, IL-10 and amphiregulin CONCLUSIONS:Tissue-resident Treg cells, such as the CCR8+ST2+ population, likely serve to counteract Th2-type effector CD4+ T cells in the lungs by suppressing excessive inflammation and promoting tissue repair.

D18 IgE-mediated anaphylaxis increases TEWL and its correlation with severity of reactions in mouse models.

Keyword: Food Allergy

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Background: An increase in transepidermal water loss (TEWL) has been shown to precede food anaphylaxis during clinical oral food challenges. We sought to determine whether TEWL changes during mouse anaphylaxis to model the observed TEWL change in human food anaphylaxis. Methods: Two IgE-based models were utilized: 1) Active systemic anaphylaxis (ASA) with an ovalbumin-alum sensitization followed 2 weeks later by ovalbumin challenges; and 2) Passive systemic anaphylaxis (PSA) with dinitrophenyl (DNP)-IgE given on day 1 followed on day 2 with a DNP-human serum albumin (HSA) challenge. Additionally, histamine IV injections were given in a non-IgE-mediated chemical anaphylaxis model. Diarrhea, reaction score, rectal temperature, and TEWL measurements were recorded pre-challenge and at 15-minute intervals post-challenge. TEWL was obtained by holding the tewameter against the mouse ear. Results: TEWL increased during ASA (3.82 g/m2/h) and PSA (1.54 g/m2/h), whereas histamine challenges (-0.14 g/m2/h) and control mice (-0.36 g/m2/h) had no change in TEWL. Internal temperature drops (ASA: -1.03°C; PSA: -0.96°C; histamine: -4.90°C) and reaction scores (ASA: 3.19; PSA: 1.36; histamine: 3.80) recorded during the trials confirmed that an anaphylactic event occurred. MCPT-1 ELISA results show an increased presence of MCPT-1 in the blood of challenged mice (14.22 ng/mL) as opposed to control mice (5.76 ng/mL), which also supports the transpiration of anaphylaxis. Conclusion: For IgE-mediated anaphylaxis, an increase in TEWL preceded symptoms of allergic reactions, whereas TEWL did not change in control or histamine-treated mice. This aligns with observed TEWL changes in human food anaphylaxis, supporting future mechanistic investigations into this phenomenon.

D19 iNKT Cell Stimulation Induces IgE-Mediated Peanut Allergy Through a Type 2 Conventional Dendritic Cell-Dependent Pathway

Keyword: Food Allergy

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Rationale: Food-specific immunoglobulin E (IgE) is required for food allergies. The environmental factors and mechanisms that lead to food-specific IgE production are not well understood. We aimed to understand the adjuvants and cellular mechanisms that lead to food-specific IgE production. Methods: We screened various adjuvants in mice for the ability to induce food-specific IgE. After identifying a successful adjuvant, we employed various knock out models to understand the cellular mechanism of this adjuvant in peanut-specific IgE production. Results: Most adjuvants we screened did not lead to the production of peanut-specific IgE. We found that oral administration of the iNKT cell-activating lipid KRN7000 with peanut was able to stimulate the production of anaphylactic peanut-specific IgE in mice. This IgE was iNKT cell-dependent and was also CD4+ T cell-dependent and CD40L-dependent. We also determined that only type 2 conventional dendritic cells (cDC2s) were necessary for the production of peanut IgE after treatment with KRN7000. Additionally, we found that the same dendritic cell population carried out both iNKT-activating and antigen-presenting functions. Conclusions: iNKT cell activation by the lipid KRN7000 leads to the production of peanut-specific IgE. This IgE requires cDC2s to both activate iNKT cells and prime CD4+ T cells; CD40L is also required for the production of this peanut-specific IgE. Future work should focus on whether iNKT cell activation could be a relevant pathway in the development of food allergy in people.

D20 Early Suppression Of Basophils In Peanut Sublingual Immunotherapy Is Associated With Clinical Remission of Peanut Allergy

Keyword: Food Allergy

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Rationale: Recently, peanut sublingual immunotherapy (SLIT) was shown to safely induce desensitization and remission of peanut allergy in 1-to 4-year-old children. Here, we aimed to evaluate how basophil activation was impacted by peanut 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. Basophil activation was measured by CD63 from blood collected at baseline, 12, 24, 36, and 39 months. Results: Participants receiving peanut SLIT for 12 months demonstrated significantly reduced basophil activation compared to participants receiving placebo SLIT. This difference in basophil activation between those on peanut SLIT and placebo remained present throughout the 36 month protocol. Additionally, participants who received peanut SLIT and achieved remission had both lower CD63 expression at baseline and significant suppression of CD63 expression by 12 months, while treatment failures had higher CD63 expression at baseline and lack of suppression by 12 months. Participants achieving remission had lower basophil activation for up to 36 months while on peanut SLIT compared to those who failed treatment. Conclusions: Following peanut SLIT, participants who achieved remission had significantly suppressed basophils by 12 months, compared to treatment failures. This suggests that early suppression of basophils, based on CD63 expression, may be indicative of peanut SLIT efficacy.

D21 Role for barrier function in the cross-talk between skin and intestine in a model of food allergy

Keyword: Food Allergy

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The incidence of food allergy has increased dramatically over the last several decades, along with several other allergic diseases, including atopic dermatitis. Infants with severe atopic dermatitis are more likely to develop food allergies during childhood. The cross-talk between the skin and intestinal epithelium is currently an area of interest. We have previously found that mice lacking Junctional Adhesion Molecule A (JAM-A) have a leaky intestinal epithelium and a more severe response in a model of food allergy using systemic sensitization, as measured by a drop in body temperature and in increase in systemic mast cell protease. This is primarily due to increased Th2 inflammation and accumulation of mast cells in the intestine. As JAM-A is also expressed in the skin epidermis, we hypothesized that these mice would have a more pronounced response in a skin sensitization model of food allergy. We found that JAM-A-/- mice have increased expression of IL-33 and TSLP in the skin following epidermal disruption by tape-stripping compared to WT C57BL/6 mice. This resulted in an increase in mast cells in the small intestine as determined by histology. Furthermore, allergen sensitization through the skin lead to increased responses to oral challenge as measured by mast cell accumulation and Th2 cytokine production from intestinal tissue and mesenteric lymph nodes. Finally, sensitization through either system or dermal routes lead to increased skin barrier permeability following oral challenge. Together, our results suggest an important role for barrier function in the cross-talk between the skin and gut in allergic disease.

D22 The Role Of Glycerophosphocholines In Outcomes On Peanut OIT Based On Untargeted Metabolic Profiling

Keyword: Food Allergy

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RationaleGlycerophosphocholines have been implicated in cardiovascular disease, bronchiolitis, autoinflammatory disease, and early evidence suggests a role in food allergy. We studied the role of glycerophosphocholines in remission versus non-remission in two independent oral immunotherapy (OIT) trials in children. MethodsTwo independent peanut OIT trails were conducted: PNOIT with older children (ages 7-12, Boston Massachusetts, clinicaltrials.gov#:NCT01324401) and DEVIL with younger children (ages 1-4, Chapel Hill, North Carolina, clinicaltrials.gov#:NCT00932828). In both trials remission status was determined by oral food challenges conducted at the end-of-therapy and the end of a 1-month avoidance period. Untargeted metabolomics was performed longitudinally on plasma samples by LC/MS/MS for PNOIT (n=20), and UHPLC/MS/MS for DEVIL (n=41). Logistic regression models, adjusted for age, were used to detect differences in individual metabolites between remission and non-remission and enrichment analyses were used to assess global trends in chemical subclasses. ResultsWe found that glycerophosphocholines comprised the most significantly enriched subclass associated with remission in both the PNOIT (q=6.8x10-16) and DEVIL trials (q=4.9x10-8). In the DEVIL trial, all (9/9) glycerophosphocholines significantly associated with remission were lower in remission (OR range:0.04-0.3; p range:0.01-0.0499). Approximately half of the glycerophosphocholines (10/18) were lower in remission in PNOIT (OR range:0.04-0.42; p range:0.001-0.045). In both studies,

a majority of glycerophosphocholines increased over time while on OIT (PNOIT-Coefficient range:-0.02-0.04,p range:2x10-4-0.03;DEVIL-Coefficient range:0.0048-0.005,p range:0.0004-0.004).ConclusionsIn two independent studies, glycerophosphocholines increased during OIT and were lower in those who developed remission. Further exploration is required to understand the biological significance glycerophosphocholines have in food allergy and remission during OIT.

D23 Oral administration of antigens prevents allergic sensitization in distant lymph nodes by preventing development of antigen-specific follicular helper T cells

Keyword: Food Allergy

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Rationale: As observed in the 'LEAP' peanut allergy clinical trial, early oral route antigen exposure decreases the incidence of food allergy while environmental exposures through non-oral routes, such as the skin or airways, increase the risk. The goal of this study was to investigate the immunologic mechanisms of how oral allergen consumption protects from sensitization to the same allergens through environmental exposures.Methods: Naive BALB/c mice were orally administered ovalbumin (OVA) in drinking water. Mice were subsequently exposed intranasally (i.n.) or epicutaneously (e.c.) to OVA plus peanut flour (PNF), as an adjuvant. OVA-specific CD4+ T cells from draining lymph nodes (dLNs) were assessed by MHC-II tetramer staining and flow cytometry.Results: Following i.n. or e.c. exposure to OVA plus PNF, mice developed OVA-IgE and OVA-IgG1 and showed symptoms of acute anaphylaxis upon i.p. OVA challenge. Oral OVA feeding prior to i.n. or e.c. antigen exposure did not alter OVA-IgG1 titers but dramatically reduced OVA-IgE titers and protected mice from anaphylaxis. Protection was OVA-specific, as OVA-fed mice still developed peanut-IgE and anaphylaxis upon i.p. peanut challenge. The numbers of total OVA-specific CD4+ T cells and Foxp3+ Treg cells in dLNs were similar between control and OVA-fed mice. However, the majority of OVA-specific CD4+ T cells in control dLNs were CXCR5+PD-1+Tfh cells, which were nearly abolished in OVA-fed mice.Conclusions: Oral route antigen exposure suppresses development of antigen-specific Tfh cells in remote dLNs and prevents systemic allergic sensitization. This distant tolerance may explain immunological mechanisms behind the success of the 'LEAP' study.

D24 Atopic toddlers have increased circulating Th2 populations by 36 months of age

Keyword: TCell/BCells

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Rationale: Th2A cells (CD4+CD45RA-CD49d+CD27-CRTH2+CD161+) are terminally differentiated effector cells that can develop from conventional Th2 cells (convTh2s, CRTH2+CD161-). The natural history of this population is not established. GPR15 is a chemokine receptor associated with eosinophilic esophagitis and unsuccessful food oral immunotherapy (OIT).Methods: 212 PBMC samples from 176 children ages 12, 24, and 36 months with [n=113] or without atopy (history of eczema, allergic proctocolitis, and/or food allergy) were analyzed using flow cytometry and FlowJo. R 4.4.1 was used for statistical analysis (Mann-Whitney, multiple linear regression). Population sizes are described as percentage of memory CD4+ cells.Results: In the atopic group, Th2 populations were larger at 24 and 36 months compared to 12. This pattern was also observed in the subset of children with allergic proctocolitis alone, but not in the non-atopic subjects, The convTh2 population was larger in atopy versus no atopy at 36 months. In regression models, age, but not atopy or any single atopic condition, was associated with increased Th2As and convTh2s. a4b7+Th2As, CLA+Th2As, and GPR15+convTh2s increased over time in atopy. GPR15+convTh2s were higher in atopy versus no atopy at 36 months. In both groups, CLA+convTh2s were increased at 24 and 36 months compared to 12.Discussion: There is an age-related increase in circulating effector Th2 populations, culminating in differences between atopic and non-atopic children by 36 months of age. This observation may suggest a timeline for the establishment of allergic conditions and may have implications for optimal timing of interventions targeting Th2A-mediated disease prevention and/or treatment.

D25 Regulatory T cell and T follicular regulatory cell populations are disrupted in mice with UTX-deficient T cells

Keyword: Regulation of inflammation

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Rationale: T cell-specific histone H3 lysine 27 (H3K27) demethylase UTX deficiency hinders T-follicular (Tfh) cell function and germinal center B cell responses to T-helper (Th)1-skewing chronic viral infection. T cell-specific UTX deficiency also impairs allergic sensitization to peanut, blunting anaphylaxis after intraperitoneal peanut challenge, despite type 2-skewed, polyclonal CD4+ T cell and antibody responses. Defects in CD4+ Foxp3+ regulatory T (Treg) cells are linked to elevated IL-4+ Th2 cells, IgE, and IgG1. Whether UTX in T cells regulates Foxp3+CD4+ Tregs and T-follicular regulatory (Tfr) cells is unknown. Methods: Splenocytes from mice with T cell-specific UTX deficiency ('UTX-TCD') and littermate controls ('UTXfl/fl') were stained with fluorophore-conjugated antibodies labeling Tregs (CD3+CD4+Foxp3+), Tfr (CD3+CD4+CXCR5+PD-1+Foxp3+), and IL-10+ cells, and acquired with flow cytometry. Suppressive function of CD4+CD25+ T cells from UTX-TCD mice and controls was assessed with in vitro Treg suppression assays. Results: UTX-TCD mice had reduced frequencies and cell numbers IL-10+ Foxp3+ Tregs and Tfr cells compared to UTXfl/fl controls. UTX-deficient CD4+CD25+ T cell suppression of naive CD4+ and CD8+ T cell proliferation was significantly impaired in the absence of T cell activation. Activating T cells with anti-CD3, anti-CD28, and IL-2 improved suppressive capacity of UTX-deficient CD4+CD25+ T cells and enhanced IL-10 concentrations in culture supernatants. Conclusions: T cell-specific deficiency of H3K27 demethylase UTX alters IL-10+Foxp3+ Treg and Tfr populations and blunts suppressive function of CD4+CD25+ Tregs. Thus, epigenetic regulation by histone demethylases may modulate Treg and Tfr compartments, promoting dysregulated, type 2-skewed, immune profiles, and defective antigen-specific antibody production.

D26 Lactobacillus Rhamnosus and Microbial Metabolism Protect Against Airway Mucus Hypersecretion in Allergic Asthma through Gut-Lung-Axis

Keyword: Microbiome

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Rationale: This study aimed to investigate the ameliorative effects of probiotic Lactobacillus Rhamnosus 76 (LR76) on ovalbumin (OVA)-allergic mice and the mechanism of LR76 affecting mucus secretion in asthma. Methods: OVA-allergic mice were supplemented with LR76 and the inflammation and mucous secretion were exhibited. Intestinal microbiome was detected by 16S rRNA gene sequencing. Serum metabolites of mice were detected by high-performance liquid chromatography. OVA-allergic mice and 16HBE cells were supplemented with microbial metabolism 4-hydroxybenzoic acid (4-HB). Expression of AHR, NRF2, SOD1, ROS, p-STAT6 and MUC5AC were examined. Results: LR76 alleviated airway inflammation, mucus secretion and decreased the expression of MUC5AC in OVA sensitized mice. Compared with the OVA group, the homeostasis of intestinal microbiome in the LR76 group was recovered; enriched metabolite 4-HB was significantly correlated with the microbiota marker. 4-HB increased the expression of AHR/NRF2/SOD1, decreased the levels of ROS, p-STAT6, and MUC5AC in 16HBE cells and OVA-allergic mice. Computer modeling results showed that the optimal bonding energy of 4-HB and AHR is -6.0 kcal/mol, and the specific bonding modes were hydrogen bond and hydrophobic interaction. AHR inhibitor CH223191 and silent expression of AHR decreased the expression of NRF2/SOD1, increased the levels of ROS, p-STAT6 and MUC5AC. Conclusions: Probiotics LR76 alleviated airway inflammation and mucus hypersecretion, restored intestinal microecology and host metabolism in OVA-sensitized mice. The metabolite 4-HB may alleviate allergic airway inflammation and mucus hypersecretion through the gut-lung axis, and reduces the expression of MUC5AC in airway epithelial cells through the AHR/NRF2/ROS/STAT6 pathway.

D27 Sympathetic regulation of B cell IgE production during type 2 lung inflammation

Keyword: Immunoglobulin E

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Sensory neurons significantly contribute to immune regulation in the lung. The hypersensitization of sensory neurons by allergens leads to the immediate local release of neuropeptides which heighten the allergic immune response. Sensory neurons also stimulate classic autonomic reflexes which stimulate efferent neurons. Recent evidence shows that efferent sympathetic neurons form a part of the classic autonomic reflex to mediate asthmatic inflammation. However, B cells express adrenergic receptors and may be stimulated by sympathetic neurotransmitters. Therefore, we hypothesized that efferent sympathetic neurons contribute to B cell IgE production during fungal allergen-induced type 2 lung inflammation. Using our established asthma model with Alternaria alternata exposure, we measured B cells, immunoglobulins, and lung resistance in vehicle control and chemically sympathectomized mice. Resident memory B cells and IgE levels were reduced in sympathectomized mice but IgG1, IgG2a, IgG2b and IgG3 remained similar to vehicle mice. Further, lung resistance was suppressed in sympathectomized mice in response to doubling doses of methacholine. Notably, sympathetic neurotransmitter norepinephrine was reduced in chemically sympathectomized mice in response to A. alternata compared to vehicle controls. Our results show that norepinephrine released from efferent sympathetic nerves contributes to IgE production from resident memory B cells.

D28 Bidirectional crosstalk between sensory neurons and γδ T cells regulates allergic responses

Keyword: TCell/BCells

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Sensory neurons densely innervate the skin where they directly detect allergens, leading to itching sensations and the release of neuropeptides that regulate the downstream allergic immune response. We recently identified a poorly characterized subset of epidermal γ δ T cells, termed GD3 cells, whose production of IL-3 fine-tunes the responsiveness of sensory neurons to allergens upon first exposure, thereby governing allergic itch and allergen sensitization. However, the upstream factors that regulate GD3 cells, thereby underlying allergen sensitivity, are obscure. Though multiple factors are required for GD3 cell development, including the thymic environment, MHC expression, and the microbiota, we found an unexpected role of sensory neurons in the homeostatic maintenance of GD3 cells in the skin. Confocal microscopy revealed that GD3 cells are closely associated with sensory neurons, forming direct contacts with free nerve endings in the skin. Using genetic mouse models, we found that inducible deletion of sensory neurons in vivo led to a significant reduction in GD3 cells. To further investigate the interaction between GD3 cells and sensory neurons, we utilized an in vitro co-culture system and discovered that sensory neurons directly support the survival, proliferation, and functionality of GD3 cells, but not other γ δ T cell populations. Thus, sensory neurons promote the homeostatic maintenance of GD3 cells in the skin by enhancing their survival and expansion. These findings suggest that GD3 cells and sensory neurons engage in dynamic bidirectional crosstalk and that dysfunction of this neuroimmune axis may underly differences in allergic susceptibility.

D29 Targeting the IL-17RB Subunit to Unravel Structural and Molecular Mechanisms of IL-25 Signaling Inhibition

Keyword: Cytokines

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IL-17A and IL-25, members of IL-17 family cytokines, are important regulators of immune responses. While IL-17A engages IL-17RA and IL-17RC, IL-25 relies on IL-17RB and IL-17RA, with IL-17RA serving as a shared essential subunit for their activity. IL-25 play a key role in driving type-2 immune responses, such as allergic asthma. Our recent studies elucidated the structural complexes of IL-25 receptor signaling and revealed a unique assembly where IL-25 interacts solely with the IL17RB subunit to allosterically promote the formation of IL-25-IL17RB-IL17RA ternary complexes via an IL-17RB-IL17RA tip-to-tip interface. In this work, we explored how this unique architecture of IL-25 receptor complex signaling influences pharmacological mechanisms of inhibition with biological therapies. We utilized an anti-IL17RA antibody, Brodalumab, an approved treatment for psoriasis, and AP684, an anti-IL17RB antibody and elucidated the structural basis of their therapeutic binding mechanisms using cryo-EM. Our results show that Brodalumab binds to the D1 domain of IL-17RA without interfering with the critical IL-17RA-IL-17RB interaction necessary for IL-25 signaling. Conversely, AP684 binds to the tip of IL-17RB, completely preventing the binding of IL-25-IL-17RB complex to IL-17RA. Consistent with these structural findings, PBMC assays reveal that AP684 effectively inhibits IL-25 induced type2 cytokine production, whereas Brodalumab only partially blocks it. Furthermore, in vivo experiments using a human IL-25/IL-17RB/IL-17RA KI mouse model showed a markedly superior effect of AP684 antibody treatment over Brodalumab in mitigating IL-25-induced type 2 inflammation. Our integrated structural and functional data provide crucial insights into therapeutic inhibition of IL-25-IL17RB-IL17RA -signaling complexes.

D30 CD301b+ DC-derived IL-2 dictates effector CD4T cell fate

Keyword: Macrophage/Dendritic Cell

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Differentiation of effector CD4T helper (Th) cells is critical for functional adaptive immunity. Different subsets of dendritic cells (DCs) preferentially induce different types of Th cells, but the underlying mechanism for the Th type 2 (Th2) cell differentiation remains elusive, as the critical DC-derived cue has not been clearly identified. CD301b+ DCs, a major subset of migratory type 2 conventional DCs, are specifically required for the Th2 differentiation of antigen-specific CD4T cells induced by allergens, adjuvants and helminth parasites, but the mechanism for their requirement remains unclear. Here, we show that CD301b+ DCs instruct the Th2 cell fate through cognate interaction by 'kick-starting' IL-2 receptor signaling in antigen specific CD4T cells. Mechanistically, upon cognate interaction, CD40 engagement induces selective production of IL-2 from CD301b+ DCs to maximize the expression of CD25, the high affinity subunit of IL-2 receptor, in antigen-specific CD4T cells. This maximal CD25 upregulation in antigen-specific CD4T cells is specifically required for their differentiation into Th2 cells but not Th1 cells. In addition, the full activation of STAT5, a major downstream of IL-2 receptor signaling, in antigen-specific CD4T cells requires CD301b+ DC-intrinsic CD25 expression, suggesting that CD301b+ DCs utilize their own CD25 to facilitate the directed action of IL-2 toward the cognate CD4T cells presumably by transpresenting IL-2. Furthermore, the CD301b+ DC-intrinsic CD40-IL-2 axis skews CD4T cells away from the T follicular helper fate. These results highlight the critical role of DC-derived IL-2 in dictating effector CD4T cell fate at the time of priming.

D31 Single-Cell RNA Sequencing to Understand Type 2 Inflammation in Chronic Rhinosinusitis with Nasal Polyps

Keyword: Single Cell Sequencing

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RATIONALE: Chronic rhinosinusitis with nasal polyps (CRSwNP) is recognized as a type 2 (T2) inflammatory disease. While gene expression studies provide insights into its development, the cellular complexities remain underexplored. Single-cell RNA sequencing (scRNA-seq) offers a powerful tool to investigate the transcriptome of biopsied tissues, revealing mechanisms driving CRSwNP and responses to treatment.METHODS: Nasal polyp biopsies were obtained from 11 patients pre- and post-treatment with dupilumab (N=6) or placebo (N=5). Samples underwent Chromium scRNA-seq, yielding individual transcriptome for a total number of cells of over 59,400, with an average of 23,071 genes detected per cell.RESULTS: Our analysis revealed a complex cellular environment within nasal polyps, highlighting multiple epithelial clusters that reflect the previously unappreciated richness of the nasal mucosa. We observed significant changes in lymphocyte subpopulations and rare cell types, including ionocytes and tuft cells. Notably, gene modulation in olfactory glia and microvillar cells-supportive cells of olfaction-suggests candidates for rapid improvement in sense of smell post-dupilumab. Additionally, we identified diminished expression of T2 markers, including CST1 and POSTN, in multiple epithelial cell clusters, underscoring alterations in the local immune landscape.CONCLUSION: These findings underscore the value of scRNA-seq in understanding CRSwNP within the T2 inflammatory context. The diverse cellular landscape of nasal polyps suggests a complexity that goes beyond epithelial composition, advancing precision medicine by providing a roadmap for targeted therapies and enhancing our understanding of treatment responses in CRSwNP.This study was supported by Sanofi-Regeneron (NCT04596189).

D32 CRISPR/Cas9 in vivo screens for new regulators of B cell activation and plasma cell differentiation

Keyword: TCell/BCells

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The complex cellular and microenvironmental signals that occur in vivo affect immune cell activation and differentiation, and cannot be easily modelled in vitro. CRISPR-based genetic screens in in vivo mouse models are emerging to unravel regulatory mechanisms of immune cell fate and function. We established an in vivo model for pooled sgRNA CRISPR/Cas9 screens to identify new regulators of B-cell response development. The model is based on ex vivo transduction of Cas9-expressing naive B cells with ecotropic lentiviral (LV) particles carrying gene-specific sgRNAs, subsequent transfer of transduced naive B cells to recipient mice, immunization of these mice to induce B cell differentiation into plasma cells, and evaluation of sgRNA abundance or loss in the generated plasma cell population. Because naive B cells are poorly transduced, we developed a mouse carrying the receptor of the ecotropic LV (solute carrier Slc7a1) in the Rosa26 locus. Conditional overexpression of the ecotropic receptor in B cells improves LV transduction without activation. We performed successful screening experiments in which approximately 380 genes were simultaneously studied. As a result of these screenings, 25 potential positive or negative regulators were identified, and 70% of these genes were validated. Many of the validated genes have not been previously described as regulators of B-cell immune responses. These novel regulators include genes involved in adhesion, enzymes, transporters, and signaling molecules. We would like to expand our studies by using the in vivo CRISPR/Cas9 screening model to investigate other B cell-related developmental pathways, such as differentiation to IgE-secreting plasma cells.

D33 BCG Induced Innate Immune Response Heterogeneity and Susceptibility to Pediatric Tuberculosis

Keyword: Genomics/Transcriptomics

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Background: Although immune responses to BCG and susceptibility to pediatric tuberculosis (TB) vary across individuals, the underlying cellular mechanism regulating this heterogeneity is poorly understood.Method: We used a nested case-control study with a 2-year prospective observation period to examine whether genetic variation is associated with BCG-induced innate immune responses and susceptibility to pediatric TB (N= 134 cases, 516 controls) in BCG-vaccinated infants. Whole blood collected at 10 weeks of age was stimulated with BCG or media and examined with flow cytometry to measure BCG-induced PDL1, CD40, and cytokine expression in myeloid (mDC) and plasmacytoid (pDC) dendritic cells, monocytes, and neutrophils in a subset of control infants. We used a cellular and clinical GWAS to assess for associations between genetic variants, BCG-induced innate immune responses, and susceptibility to TB.Results: We identified 11 lead genetic variants at genome-wide level significance associated with BCG-induced cytokine and surface expression markers including PDL1 (5 pDCs, 3 mDCs, 1 monocytes), CD40 (1 mDCs), and IL-6 (1 monocytes). An IGLL1 variant (rs2096522) was associated with CD40 expression on mDCs (p=1.6e-08) and was also discovered as a significant variant using a gene-based method. We identified 39 lead variants mapping to 74 genes suggestive of an association with susceptibility to pediatric TB (p<1e-05), but no variant reached genome-wide significance. A PDE8A region variant (rs1023844, p=9.6e-07) was an expression quantitative trait loci and associated with BCG-induced monocyte PDL1 expression.Summary: We identified genetic variants associated with heterogeneity in infant innate immune responses to BCG with potential immunoregulatory mechanisms.

D34 Engrafted NSG-SGM3 humanized mice produce abundant functional human IgE without sensitization

Keyword: Immunoglobulin E

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NSG-SGM3 (NOD scid-IL2Rgnull-3/GM/SF) humanized mouse models are well-suited for studying human immune physiology. We previously characterized a new and simpler NSG-SGM3 mouse engrafted with human donor umbilical cord-derived CD34+ hematopoietic stem cells without prior bone marrow ablation or human secondary lymphoid tissue implantation that still retains human mast cell- and basophil-dependent passive anaphylaxis responses. Its capacities for human IgE antibody production and human B cell maturation, however, remain unknown. Here, we show that NSG-SGM3 mice engrafted without prior marrow ablation spontaneously produce abundant human antibodies, including IgE, without prior sensitization. These human IgE are polyclonal with specificities to a diverse array of allergens, such as millet, egg, and wasp venom, that are otherwise absent from mouse diet or housing environments. Furthermore, naive engrafted NSG-SGM3 splenocytes expand human CD20+ CD27+ plasma cell and CD138+ CD27+ memory B cell populations in response to stimulation with human CD40L and IL-4 ex vivo. Notably, engrafted NSG-SGM3 mice, but not non-engrafted controls, also exhibit dose-dependent passive systemic anaphylaxis responses to intraperitoneal goat anti-human IgE challenge as compared with isotype control. Together, our results demonstrate that naive engrafted NSG-SGM3 mice without prior ablation can spontaneously produce abundant and functional human antibodies, including polyclonal IgE that can directly facilitate anaphylaxis, and possess the upstream capacity to support human B cell maturation into antibody-producing and memory cells. We therefore present engrafted NSG-SGM3 mice without prior ablation as a simpler humanized model for studying broader anaphylaxis and IgE biology.

D35 Bruton’s tyrosine kinase (BTK) deficiency alters regulation of commensal microbe Akkermansia muciniphila

Keyword: Primary Immunodeficiency

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Bruton's tyrosine kinase (BTK) is a signaling protein present in B cells. Patients lacking BTK have few B cells, and they are prone to both enterocolitis and chronic infection with commensal non-H. pylori helicobacter. We recently reported that BTK supports mucosal homeostasis via regulation of the microbiota in a mouse model of rheumatoid arthritis. Here, we present further evidence of the importance of BTK in the gut in the non-obese diabetic (NOD) model of type 1 diabetes (T1D). We previously found that Btk-/-NOD mice had increased levels of Akkermansia muciniphila (A.M.), a commensal microbe associated with protection against T1D, compared to WT NOD controls. Thus, we monocolonized germ-free WT and Btk-/-NOD mice with A.M. to test whether loss of BTK facilitated increased colonization of A.M. and whether this microbe alone could confer protection against T1D. Consistent with our previous findings, Btk-/-NOD mice had reduced Peyer's patch size, germinal center area, and number of B cells. These Btk-/-NOD mice also made less small intestinal IgA, and their A.M. was less coated with IgA. Btk-/-NOD mice had more A.M. colonization compared to WT NOD mice and greater intestinal mucus production. Only the monocolonized Btk-/-NOD mice were protected against T1D. They had reduced CD4+ T cells in pancreatic islets, and a greater proportion of them were FOXP3+, suggesting these T regulatory cells may contribute to protection against T1D. Overall, we conclude that BTK plays a previously unrecognized role in gut homeostasis, with Btk-deficiency driving microbiome alterations important in T1D pathogenesis.

D36 The role of mTORC1-mediated neuroimmune training in the initiation of allergic immunity

Keyword: Macrophage/Dendritic Cell

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Environmental allergens in both physiological and experimental settings are generally encountered via recurrent, low-dose exposures. In such cases, any single exposure to an allergen may not be sufficient, but repetitive exposure somehow could promote the initiation of allergic immunity. Our laboratory previously showed that allergens are directly detected by sensory neurons, which induce the sensation of itch and release neuropeptides that promote the migration of Th2-skewing CD301b+ dendritic cells (DCs) into the draining lymph node (dLN) to initiate Th2 cell differentiation. We hypothesized that allergens induce a state of 'neuroimmune training' in which previous allergen exposure leads to boosted neuronal responses upon re-exposure. We developed a model of recurrent allergen exposure in which re-exposure to an allergen augmented the sensory response as well as the migration of CD301b+ DCs into the dLN. This augmented response was independent of adaptive immune cells but was lost if neurons were transiently silenced during initial allergen exposure, indicating the allergic neuroimmune training was at least partially intrinsic to sensory neurons. Transcriptional analysis of sensory ganglia revealed the activation of mTORC1 pathways after allergen exposure. Pharmacologic or genetic blockade of mTORC1 function in sensory neurons did not impact initial allergen-induced responses but did lead to a loss of this trained response upon re-exposure. Mechanistically, mTORC1 signaling augmented neuronal responses to allergens by regulating the metabolic status. Finally, antigenically unrelated allergens with shared functional characteristics could prime the sensory neuronal and immune responses to each other, providing a potential mechanism to explain the polysensitization of allergy.

D37 Blimp-1 maintains the transcriptional identity of ILC2 cells to promote allergen-driven airway inflammation and pro-tumor immunity.

Keyword: Innate Lymphoid Cell

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Type 2 innate lymphoid cells (ILC2s) respond to alarmins triggered by tissue damage and play key roles in anti-helminth immunity, allergy, and cancer. How ILC2s maintain type 2 transcriptional identity during tissue inflammation is not known. We explored the expression and function of Blimp-1 in ILC2 cells in settings of lung inflammation. Blimp-1 was rapidly expressed in lung ILC2s responding to the alarmins IL-33 and IL-25, house dust mite, papain, and worm infection. Blimp-1 was also upregulated in skin or gut ILC2s upon inflammatory challenge, suggesting Blimp-1 upregulation is a global feature of ILC2 activation. Both IL-33 and IL-25 could induce Blimp-1 expression in ILC2s in vitro through indirect regulation of the cytokine IL-9. IL-33 rapidly induced IL-9, leading to activation of Blimp-1 that was eliminated via IL-9 blockade. Deletion of Blimp-1 in ILC2s drove increases in type I cytokines including TNF and IFNg, while suppressing the type 2 cytokines IL-5 and IL-13. Paradoxically, Blimp-1 deletion increased IL-9. Increases in chromatin accessibility in the absence of Blimp-1 confirm its role as a repressor. The shift in cytokine balance in the absence of Blimp-1 led to increased lung inflammation including recruitment of mast cells in settings of allergic inflammation, but also potently suppressed metastatic melanoma by shifting the type I/type 2 balance in the lung. Collectively, our study suggests Blimp-1 is a key regulator of type 2 transcriptional identity that acts to promote ILC2-mediated allergic lung disease while suppressing anti-tumor immunity.

D38 How format impacts activity: Structure-activity relationship for optimal FcRn engagement of Fc-based therapeutics.

Keyword: Autoimmunity

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Background: Increasing the affinity of the IgG Fc to the neonatal Fc receptor (FcRn) is a versatile tool to extend serum half-life, increase cellular uptake or introduce FcRn antagonizing capacity to the Fc of therapeutic antibodies. Moreover, antagonizing this interaction is a promising therapeutic approach in IgG-mediated autoimmune diseases. Recently, it was found that the IgG Fabs can sterically impair efficient engagement of the IgG Fc with FcRn, likely due to the membrane proximity of Fabs when IgG is bound to FcRn. Aims: Here, we further dive into these structure activity relationships with a focus on how format impacts serum half-life, clearance and FcRn blocking capacity. In addition, we describe how targeting FcRn can be optimized with some clever antibody engineering. Methods: Structure activity relationships are unravelled using both in vitro (e.g. FcRn occupancy experiments) and in vivo (e.g. Tg mice models) experiments. Conclusions: Multiple factors, such as increased affinity at both neutral and low pH, size and conformation of the molecule play a role in which a subtle balance must be maintained.

D39 Opportunistic and Pathogenic Cocci Species Show Similar Abundance in AERD and CRSwNP

Keyword: Microbiome

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Rationale: The microbiota has been shown to play a role in type 2 immunity disorders. However, in aspirin-exacerbated respiratory disease (AERD), the specific involvement of cocci bacteria remains unclear, even though some species are known to contribute to various inflammatory disorders. This study aimed to investigate the role of cocci in AERD polyps.Methods: We performed a metagenomic analysis of nasal polyp samples from 9 AERD patients and 10 chronic rhinosinusitis with nasal polyps (CRSwNP) patients. Samples were collected and analyzed using 16S rDNA sequencing on the Oxford Nanopore platform. Subsequent data analysis included strain and species abundance, diversity, exclusive species identification, statistical significance testing, and relative gene expression, conducted using R packages.Results: The most prevalent cocci species in nasal polyps were Anaerococcus, Staphylococcus, Suicoccus, and Micrococcus, with no clear distribution pattern as dominant species. No significant differences were found between pathogenic and opportunistic cocci species (Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus agalactiae, Streptococcus pneumoniae, etc.) in AERD and CRSwNP. Staphylococcus argenteus was the only species to reach statistical significance, being more prevalent in AERD.Conclusions: No substantial differences were observed in the prevalence of pathogenic or opportunistic cocci species between AERD and CRSwNP. However, Staphylococcus argenteus could serve as a promising candidate for further research in the context of AERD.

D40 Genetic, cellular, molecular, and metabolic factors define the immunopathogenesis of nevirapine-associated Stevens-Johnson syndrome/toxic epidermal necrolysis in South Africa

Keyword: Genomics/Transcriptomics

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Background: Nevirapine (NVP), an antiretroviral which was previously first-line to prevent mother-to-child-transmission in persons-living-with-HIV (PLWH), is associated with treatment-limiting HLA class I and II-restricted skin and liver toxicities. The generalizability of HLA-associations across different populations and additional genetic, molecular, and cellular contributors are unknown. We examined these associations with NVP-associated Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) in a South African population. Methods: HLA, KIR, ERAP, and CYP2B6 genotyping were performed on 20 prospectively RegiSCAR-validated NVP SJS/TEN cases and 46 matched drug-tolerant controls from PLWH in Cape Town, South Africa. Single-cell TCR sequencing (sc-TCRseq)/sc-RNAseq/sc-CITE-seq were performed on blister fluid from two acute NVP SJS/TEN cases. Using conditional logistic analyses, we built a multivariate model to examine genetic associations and functional divergence (R version 4.3.0). Results: Cases had a median drug latency of 18(2-44) days and median CD4+ T-cell count of 258(40-988) cells/mm3. HLA-C*04:01 was present in all cases and was strongly and independently associated with NVP SJS/TEN($p<0.001$). CYP2B6 haplotypes associated with reduced NVP exposure($p=0.02$), normal/underactive ERAP peptide-trimming haplotypes($p=0.004$), and low HLA-C functional divergence($p=0.01$) was associated with HLA-C*04:01+ NVP tolerance. sc-TCRseq of blister fluids revealed expression of a private, expanded TCRalpha/beta on CD8+ T cells that expressed markers of residency (ITGAE, ITGA1, CD69), cytotoxicity (GNLY), and regulation (LAG3, KLRC1). Conclusions: In South African PLWH, HLA-C*04:01 has 100% negative predictive value for NVP SJS/TEN, which is further defined by immunogenomic and metabolic risk factors and mediated at the tissue level by oligoclonal resident effector memory CD8+ T cells expressing markers of cytotoxicity and regulation.

D41 Type 2 Immune Response and Lyme Disease: Can Allergic-type Responses to Bacteria Trigger Infection-induced hypermobility?

Keyword: Immunoglobulin E

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There are currently an estimated 476,000 cases of Lyme disease annually in the US, caused by the bacteria Borrelia burgdorferi (Bb). It is estimated that approximately 10% of people who have had Lyme disease go on to develop an infection-associated chronic illness, the underlying causes and long-term impacts of which remain poorly understood. In our Lyme disease mouse model, we discovered that C3H/HeJ mice mount significantly higher levels of anti-Bb IgE compared to C57BL/6 mice. As IgE is heavily involved in the Type II immune response, we further investigated the IgE epitopes and identified highly conserved epitopes that are conserved across spirochetes. Histological analysis of infected C3H mice also revealed IgE-dependent mast cell degranulation. Furthermore, we found that these mice develop atopic dermatitis (AD) on their tails, in sites with high levels of Bb load. Strikingly, we find that intradermal infection with Bb led to the development of AD followed by a gradual development of hypermobility in the tail, revealing infection-induced hypermobility which was not seen in uninfected age-matched C3H or age-matched B6 regardless of infection status. Moreover, we observed Bb in fresh mouse tissue under simultaneous label-free autoflourescent-multiharmonic microscopy in the connective tissues with extensive mast cell recruitment C3H mice suggesting that IgE-dependent mast cell-mediated connective tissue breakdown can result in infection-induced hypermobility.

D42 Patients with a History of Allergic Proctocolitis Exhibit CD161+ Memory T-Cells and Treg Imbalances Similar to Those Seen in Atopic Dermatitis and Food Allergy

Keyword: Food Allergy

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Rationale: Allergic Proctocolitis (AP) is a non-IgE-mediated disease. However, it has been associated with the later development of IgE-mediated food allergies and eosinophilic esophagitis through a mechanism that is not yet well understood.Methods: Cryopreserved PBMCs from subjects at 12, 24, and 36 months of age, including those with atopy (n=131, with eczema (AD), AP, and/or food allergy (FA)) and without atopy (n=66), were analyzed using spectral flow cytometry (Cytek - Aurora) and the unsupervised algorithm FlowSOM 2.14.0. Statistical analysis was performed using the Wilcoxon test with FDR correction for multiple comparisons.Results: CCR7+CCR9+A4B7+CD38+ Treg cells were significantly lower at 1 year of age ($p<0.03$), compared to non-atopic patients. CD49d+CD27+CD127+CD161+ memory T-cells cell populations increased significantly by 3 years in AP patients ($p<0.02$), similar to peTh2 (CD49d+ CD161+ CD127+ CRTH2+ CD25+) ($p<0.003$) cells as shown separately in hypothesis driven work. AD and FA patients exhibited a comparable but nonsignificant trend, while a combined analysis of AD, FA, and AP patients demonstrated significant shifts across these populations.Discussion: AP patients have an immune profile that aligns partially with other atopic conditions, but also reveals distinct memory T-cell imbalances that persist beyond disease resolution, warranting further investigation of these immune markers as potential predictors of long-term atopic and perhaps other disease risk.

D43 Tissue resident memory cells after early life viral infection contribute to asthma

Keyword: Asthma

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Asthma in childhood is common, mediated by Th2 immunity, and causes significant morbidity. Viral lower respiratory tract infection within the first three years of life is a risk factor for the development of asthma. This association has been demonstrated for several common pathogens, including human metapneumovirus (HMPV). Insight into mechanisms of virus-induced asthma could guide development of novel preventative and therapeutic strategies. To model this phenomenon, we developed a neonatal murine model of HMPV followed by re-infection in adulthood. Neonates infected with primary HMPV mounted an increased Th2 response compared to adult mice. Neonates infected with HMPV and then re-infected in adulthood had features of Th2-driven asthma including eosinophil recruitment, mucus production and airway hyperresponsiveness. In contrast, adult mice primarily infected and then re-infected with HMPV had no Th2-mediated pathology. In neonatal mice re-infected with HMPV, systemic CD4+ T cell depletion mitigated histopathologic evidence of asthma. Anti-CD4 antibody delivered into the airway modestly blunted mucus production and inflammation, suggesting that CD4+ tissue resident memory cells (TRMs) induced by neonatal infection contribute to asthma pathology. Long-lived CD4+ TRMs formed after neonatal HMPV exhibited Th2 skewing. Single cell RNA sequencing of TRM populations showed clonally expanded cells with Th2 features (e.g. GATA3, IL-4, and IL-5 expression) that were abundant after neonatal HMPV infection. The IL-33 receptor, ST2, was highly expressed on this cluster of TRMs. Future studies will interrogate the effect of disrupting IL-33/ST2 signaling on the virus-induced asthma model after neonatal infection.

D44 Focal Adhesion Kinase Activity Affects Mast Cell-Neuron Interactions During IgE-Mediated MC Activation

Keyword: Mast Cell/Basophil

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Rationale: Anaphylaxis is a rapid, life-threatening allergic reaction produced by allergen-induced mast cell (MC) degranulation. MCs cluster near peripheral nerve endings and both cell types activate each other via mediators including neuropeptides and cytokines. Focal adhesion kinase (FAK) regulates focal adhesion complex formation, but whether it plays a role in MC-neuron interactions during anaphylaxis is unclear. We hypothesized that FAK regulates MC-neuron interactions during anaphylaxis initiation.Methods:We developed a co-culture system between rodent dorsal root ganglia (DRG) and the basophilic leukemia cell line RBL-2H3. RBLs were activated with dinitrophenyl (DNP)-IgE and DNP-human serum albumin (HSA) or ionomycin. A beta-hexosaminidase assay was used to measure MC degranulation. Confocal microscopy was used to take images of the co-culture and ImageJ was used to measure neurite length. Results:RBLs degranulate in the presence of DRGs after stimulation with either ionomycin (33%) or DNP-IgE with DNP-HSA (20%). Seventy percent of DRGs had neurite growth after 18h of co-culture with sensitized RBLs and reached a maximum length of 400um, compared with twenty percent of DRGs and a length of 100um with non-sensitized RBLs. The number of IgE-sensitized RBLs associated with each DRG had a positive correlation with neurite length. After DNP-HSA activation, RBL secretion of tryptase and carboxy peptidase A3 directionally favors neurites. Incubation with a FAK inhibitor decreases MC degranulation and changes MC-neuron co-culture cellular morphology.Conclusion:MC sensitization and activation increase MC-neuron interactions. MC directionally secrete granule contents toward neurons. This suggests FAK activity in mast cells is important in neuronal interactions.

D45 The Role of Cysteinyl Leukotrienes and Extracellular Nucleotides in Regulating Platelet-Mast Cell Interactions in Allergic Inflammation

Keyword: Asthma

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Rationale: Mast cells release mediators including prostaglandin D2 (PGD2) and cysteinyl leukotrienes (cysLTs) to induce allergy. Recent studies have demonstrated that interactions between platelets and other immune cells are involved in proinflammatory responses. We previously revealed that mast cell activation required platelets, type 2 cysLT receptors (CysLT2), and IL-33 in an aspirin-exacerbated respiratory disease (AERD) model. However, little is known about potential crosstalk between platelets and mast cells in allergic inflammation. Methods: A co-culture system of IL-33-stimulated mouse bone marrow-derived mast cells (BMMCs) and mouse platelets was established. The interaction between them was examined using receptor antagonists and several receptor gene knockout mice. The supernatants from IL-33-stimulated BMMCs or leukotriene C4 (LTC4)-treated platelets were collected for mediator identification. Results: Platelets dose-dependently enhanced IL-33-induced mast cell PGD2 generation and became activated in the co-culture. Both platelet-dependent potentiation and platelet activation were absent in ST2 (IL-33 receptor) KO BMMCs and CysLT2 KO platelets. CysLTs were detected in the supernatant of IL-33-stimulated BMMCs, and BMMC-derived LTC4-activated platelets. The supernatant from LTC4-treated platelets, but not LTC4 itself, induced PGD2 release from BMMCs. LTC4-stimulated platelets potently released ATP/ADP and several P2Y1 (purinergic receptor for nucleotides) antagonists significantly attenuated the PGD2 production in BMMCs activated by LTC4-treated platelet supernatants and in the co-culture. The PGD2 release was completely absent in P2Y1 KO BMMCs. Conclusions: CysLTs from mast cells and extracellular nucleotides from platelets were identified as key potential mediators of a platelet-mast cell circuit involved in driving IL-33-dependent cell activation relevant to AERD and allergic inflammation.

D46 Cysteinyl Leukotrienes are Potent Chemoattractants for Platelets in Type 2 Lung Inflammation

Keyword: Asthma

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Both free platelets and platelet-leukocyte aggregates accumulate in the airways of patients with asthma, especially in those with aspirin-exacerbated respiratory disease (AERD). Preclinical models indicate that platelets are necessary to drive type 2 (eosinophilic) airway inflammation (T2I) through various mechanisms, but the basis of their recruitment to the airways is unknown. Cysteinyl Leukotrienes (cysLTs) are potent inflammatory mediators derived from arachidonic acid by the 5-lipoxygenase (5-LO)/leukotriene C4 synthase (LTC4S) metabolic pathway. CysLTs promote bronchoconstriction and features of T2I. Platelets express both the type 1 and type 2 receptors for cysLTs (CysLT1R and CysLT2R, respectively) and CysLT2R signaling elicits platelet activation ex vivo. We hypothesized that cysLTs might be important in platelet recruitment to the lung during experimentally induced lung T2I.Our preliminary results show that leukotriene C4 (LTC4) and Leukotriene D4 (LTD4) (300 nM) potently elicit chemotaxis of both mouse and human platelets in 2D chemotaxis assays ex vivo. This attraction was attenuated by selective CysLT1R (montelukast) and CysLT2R (HAMI-3379) antagonists. Platelets from Cysltr1-/- mice failed to respond to cysLTs. Challenges of AERD-like prostaglandin E2 synthase-1 knockout (Ptges-/-) mice with inhaled lysine aspirin-induced rapid, potent recruitment of platelets to the lung parenchyma, accompanied by increased airway resistance. Genetic deletion of LTC4S and short-term blockade of CysLT1R attenuated both the changes in lung function and platelet recruitment. These studies suggest that cysLTs are physiologically relevant chemotactic factors for platelets in AERD and T2I, likely operating through cooperative functions of CysLT1R and CysLT2R.

D47 Prostaglandin E1 confers protection in Type 2 allergic airway inflammation by targeting EP2/EP4/IP receptor signaling

Keyword: Asthma

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Prostaglandins (PGs) derived from arachidonic acid, like PGE2 and PGD2, are implicated in type 2 airway inflammatory (T2I) disorders like asthma, nasal polyps, and aspirin exacerbated respiratory disease (AERD). Prostaglandin E1 (PGE1) derived from dihomo-γ-linolenic acid (DGLA) is less well studied and yet to be explored for its mechanistic role in T2I. We observed that mice deficient in 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 extract from Dermatophagoides farinae (Df). Intratracheal administration of PGE1 reduced airway inflammation in Df challenged Ptges -/- mice, decreasing both group 2 innate lymphoid cells (ILC2s) and mast cells (MCs) in lung tissues. 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. Studies using selective receptor antagonists demonstrated that EP2, EP4, and IP receptors were likely involved in mediating anti-inflammatory effect of PGE1 under in vitro conditions. PGE1 mediated its regulatory role via cAMP, but not via Protein Kinase A (PKA) or EPAC pathways. PGE1 also strongly affected platelet function and aggregation. The broad impact of PGE1 makes it a molecule of immense interest in context of AERD, where ILC2s, MCs, and platelets all display prominent activation in a setting of impaired cyclooxygenase/PGE2 synthase function and impaired EP2 receptor signaling.

D48 A Key Role for IL-13 Receptor Alpha 1 in an Experimental Model of Eosinophilic Gastritis

Keyword: Eosinophil

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Rationale: Eosinophilic gastritis (EoG) is a chronic, immune-mediated allergicdisorder characterized by local type 2 cytokines, eosinophilia and mastocytosis.Nonetheless, the molecular mechanisms of EoG are largely understudied. We aimedto develop and characterize an experimental model of EoG. Methods: Experimental EoG was induced in wild type (WT), Il13ra1 -/- and Nlrp3 -/-mice as follows: mice were ear sensitized (1% oxazolone in acetone).After six additional skin challenges (0.5% oxazolone), the mice wereintragastrically challenged [200µl oxazolone, 1% (1:2 ratio of olive oil and 95%alcohol, respectively)]. Gastric histopathology was determined by H&E, Gomoritrichrome, anti-Ki67, anti-eosinophil major basic protein, and chloroacetate esterasestaining (CAE). Bulk RNA sequencing was performed on gastric tissues to assesstranscriptomic changes associated with EoG. Results: Induction of EoG in WT mice resulted in increased epithelial thickness,basal cell hyperplasia, infiltration of eosinophils, mastocytosis, and fibrosis. RNAsequencing revealed dysregulation of 1,779 transcripts (885 upregulated, 894downregulated, 2-fold, FDR p<0.05) in experimental EoG including increasedexpression of mast cell-, type 2 immunity-, tissue remodeling- and leaky gut-associated genes (e.g., Mcpt1, Mcpt2, Il4ra, Il4i1, Tff2, Sprr2a, Cldn2, Muc1,Muc4). Il13ra1 -/- mice displayed marked reduction in epithelial mucosalthickness whereas Nlrp3 -/- mice displayed elevated mastocytosis compared to WTmice. Conclusion: Experimental EoG represents a new model that effectively resembleskey molecular and histopathological features of human EoG. Using this model weidentified an important role for IL-13 signaling in EoG and that gastric mast cellsnumbers in EoG are negatively regulated by NLRP3.

D49 Deciphering Human Lung Mast Cell Heterogeneity

Keyword: Mast Cell/Basophil

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Human lung mast cells (HLMCs) are crucial for lung surveillance and implicated in lung diseases like asthma, COPD and fibrosis. Traditionally categorized as MCT and MCTC subsets, HLMCs exhibit greater diversity than this dichotomy suggests. This study explored HLMC heterogeneity using single-cell RNA sequencing on 19,232 mast cells from five donors, dissected from bronchi, parenchyma, and pulmonary vessels (arteries and veins).Analysis revealed five distinct HLMC clusters across lung compartments. Two major clusters account for over 80% of cells: one characterized by high expression of immediate early response genes (IEGs), predominantly in bronchi, suggesting cells poised for rapid responses; another cluster showing low IEG expression but potentially involved in immune regulation and cell survival, proportionally high in parenchyma, possibly representing a stable, homeostatic population. Other clusters include cells high in cytokines and chemokines, mainly in bronchi and arteries, likely involved in inflammatory processes; a cluster with MCTC markers and high IL2RA expression, primarily in parenchyma; and an intriguing cluster with high MEG3 and MEG8 expression, mainly in veins. Characteristic mast cell gene expression varied significantly across lung compartments. Differential expression analysis revealed compartment-specific signatures: bronchi (CCL4, AREG), parenchyma (MT2A, DUSP4), arteries (CCL2), and veins (SSTR2, PTX3).These findings suggest HLMCs adapt to local microenvironments for specialized functions, explaining their multifaceted roles in lung homeostasis and pathology. This study for the first time provides insights into HLMC gene expression profiles across lung compartments, contributing to our understanding of mast cell functions in human lung, potentially informing development of targeted therapies.

D50 CysLT2 receptor signaling in mast cells protects leukotriene-dependent type 2 allergic lung inflammation

Keyword: Asthma

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Rationale: Cysteinyl leukotrienes (CysLTs) are abundant lipid mediators in asthma and eosinophilic (type 2) inflammation (T2I). While CysLTs promote T2I in animal models by signaling through the types 1 and 3 CysLT-specific G protein-coupled receptors (GPCRs) (CysLT1R and CysLT3R), the type 2 cysteinyl leukotriene receptor (CysLT2R) can either protect from or promote T2I in vivo depending on the context. Methods: The study investigates how CysLT2R signaling in mast cells (MCs) affects T2I using in vivo mouse models, in vitro cord blood-derived mast cells, and CysLT2R-transfected cell systems. It examines the mechanisms underlying CysLT2R-mediated protection, including its unique ability to enhance adenylate cyclase (AC) activation and subsequent cAMP-dependent protein kinase A (PKA) activation.Results: In transfected cells, CysLT2R signaling engages Gq, Gi, and G12 proteins. Although it cannot directly activate Gs, sustained CysLT2R signaling markedly potentiates AC activation in response to several classical Gs-coupled GPCRs (AC superactivation), including prostaglandin E2 (PGE2)-selective EP2 and EP4 receptors, via a mechanism requiring Gβ γ proteins. This mechanism is unique among CysLT receptors, and CysLT2R/cAMP/PKA signaling inhibits CysLT1R-induced activation of MCs. Deletion of MC-specific CysLT2R signaling amplifies features of T2I induced by repetitive inhalation of an extract (Df) from house dust mite, and this amplified T2I is blocked by CysLT1R inhibition.Conclusions: MC-intrinsic CysLT2R signaling negatively regulates allergic inflammation by enhancing cAMP/PKA signaling, thereby inhibiting CysLT1R-induced MC activation. This proposes a potential therapeutic strategy to selectively modulate CysLT2R signaling for managing allergic lung inflammation.

D51 Combination treatment of glucagon-like peptide-1 receptor agonist (GLP-1RA) and sodium-glucose cotransporter-2 inhibitor (SGLT2i) have additive effects in inhibiting aeroallergen-induced allergic inflammation in polygenic obese mice.

Keyword: Regulation of inflammation

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Glucagon-like peptide-1 receptor agonists (GLP-1RA) and sodium-glucose co-transporter-2 inhibitors (SGLT2i) are used for the treatment of type 2 diabetes (T2D). In addition, combination treatment of these drugs has been tested in clinical studies for T2D. We have reported that GLP-1RA treatment decreased aeroallergen-induced allergic inflammation in obese mice; however, the anti-allergic inflammatory effects of the combination of GLP-1RA and SGLT2i are unknown. Therefore, we hypothesized that the combination of semaglutide (GLP-1RA) and dapagliflozin (SGLT2i) significantly decreases Alternaria alternata extract (Alt-Ext)-induced allergic inflammation compared to each single drug treatment.Alt-Ext or PBS were administered intranasally in polygenic obese mice (TALLYHO/JngJ) on day 1-3 for sensitization and 17-18 for challenge. Semaglutide or the vehicle were treated subcutaneously on day 1 and 17. Dapagliflozin or the vehicle were intragastrically administered on day 0-4 and 16-19. Bronchoalveolar lavage fluid (BALF) and lungs were harvested 24 hours after the last Alt-Ext- or PBS-challenge.The combination treatment with semaglutide and dapagliflozin additively decreased Alt-Ext-induced IL-5, IL-13, IL-33, CCL11, and CCL24 protein expression in the lung homogenates and the number of eosinophils and lymphocytes in the BALF compared to each drug alone. Further, the combination treatment significantly decreased the number of Alt-Ext-induced Th2, Th17, and ILC2, but not Treg and ILC3 in the lung compared to the vehicle treatment.These results revealed that the combination of GLP-1RA and SGLT2i significantly inhibited airway allergic inflammation in obese mice compared to each single treatment. This may represent a more effective therapeutic strategy to reduce allergic inflammation in obese patients.

D52 Single cell transcriptomic profiling of eosinophils and airway immune cells in childhood asthma

Keyword: Eosinophil

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Rationale: scRNA-seq has revolutionized our understanding of cellular heterogeneity. However, sc technologies have faced challenges in capturing granulocytes, particularly within tissue compartments. In this study, we present scRNA-seq datasets generated from nasal lavage samples of children with asthma, achieving a high recovery rate of airway eosinophils and neutrophils. Methods: Nasal lavage samples were collected from six children with allergic sensitization and asthma, and cells were isolated and processed using the 10x Genomics Flex platform by fixing the cells at the time of sample collection, facilitating capture of fragile cells. A custom data processing pipeline based on the EmptyDrops approach was used to better detect low RNA cells. Clustering analysis was used to identify unique subpopulations of airway eosinophils and other airway cell types. Results: Using this approach, we successfully captured and detected granulocytes, including eosinophils and neutrophils, with up to an 18-fold increase in frequency compared to a standard pipeline. As a result, we captured eosinophils (ranging from 3-27% of cells) and neutrophils in proportions equivalent to other immune and epithelial cell types, closely aligning with cell frequencies identified through histologic and bulk RNA-seq assessments. We identified four eosinophil subpopulations, each characterized by unique transcriptional profiles consistent with distinct homeostatic or inflammatory functions.Conclusions: Our results underscore the complexity of the airway immune cell and epithelial cell landscape in pediatric asthma and highlight the presence of functionally diverse eosinophil subpopulations that may contribute to disease pathogenesis. Our study provides a valuable resource for further investigations into the cellular mechanisms underlying asthma.

D53 Hops-derived bitter β-acid with antibacterial properties induces significant mitochondrial and cilia dysfunction in sinonasal epithelial cells

Keyword: Epithelial cells

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Impaired mucociliary clearance (MCC) and sinonasal dysbiosis may contribute to Chronic Rhinosinusitis (CRS) pathogenesis. Bitter taste receptors (T2Rs) regulate innate immunity mechanisms in vitro, including ciliary beat frequency (CBF), and their polymorphisms are linked to CRS outcomes in vivo. We hypothesized that lupulone, a T2R1 and T2R14 agonist extracted from hops with antibacterial properties, may be a novel treatment for CRS by improving MCC through CBF increase and reducing pathogenic bacterial growth.Primary nasal epithelial cells (pNECs) were acquired from CRS and non-CRS (control) patients undergoing planned surgeries. T2Rs isoforms expression was assessed by qPCR, WB and immunofluorescence in sinonasal tissue, differentiated and undifferentiated pNECs, and in RPMI 2650 cells cultured in vitro. T2Rs activation and subsequent impact on cilia activity poststimulation with lupulone was investigated using calcium imaging and CBF measurement, respectively. Finally, lupulone acute and long-term toxicity on nasal cell models was studied using immunofluorescence, transepithelial electrical resistance measurement, and cell death and cell viability assays.We confirmed relevant T2Rs expression in nasal tissue and cellular models in vitro. Lupulone induced a cytosolic calcium response that appeared mediated by T2R signaling at micromolar concentrations ($p<=0.0337$). However, this response was associated with apoptosis ($p<=0.0080$) and reduced cell proliferation in RPMI 2650 cells ($p<0.0001$), as well as barrier function alteration ($p<0.05$) and ciliostasis in differentiated pNEC cultures ($p<0.0143$).Despite significant antibacterial properties, lupulone compromised nasal

epithelial integrity and induced ciliotoxicity. In CRS patients, this may translate into further deterioration of MCC and sinus disease.

D54 Characterizing GRHL2 as a Key Transcription Factor Driving Epithelial Gene Regulation in Eosinophilic Esophagitis

Keyword: Eosinophilic Esophagitis

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Eosinophilic esophagitis (EoE) is a chronic condition marked by eosinophil-driven inflammation mediated by type-2 cytokines. Even during remission, changes in the epithelium and gene expression suggest underlying epigenetic mechanisms. Our research shows that type-2 cytokines, especially IL-13, downregulate structural proteins critical for the epithelial barrier. This study explores how transcription factor, GRHL2 affects downregulation of epithelial regulatory genes and leads to persistent changes in EoE.Epithelial cells from pediatric biopsies were analyzed to identify transcription factors associated with downregulated structural proteins. Effects of high type-2 cytokines on GRHL2 activity were studied using 3D esophageal organoid cultures. Single-cell RNA sequencing of patient biopsies and IL-13-treated cultures assessed GRHL2 expression dynamics, confirmed by immunofluorescence. IL-13-treated organoids underwent RNA and ATAC sequencing to explore GRHL2-mediated chromatin and transcriptional changes. GRHL2 knockdown was also evaluated through morphological and functional analyses.Motif enrichment analysis identified GRHL2 as a crucial epithelial-specific transcription factor in active EoE biopsies, achieving the highest enrichment score of 7.79 among 125 factors. Single-cell RNA sequencing and immunofluorescence confirmed increased GRHL2 expression in proliferating epithelial cells from patient biopsies and IL-13-treated organoids. IL-13 affected GRHL2-related chromatin accessibility and gene expression, while ATAC sequencing revealed changes in GRHL2 binding to key gene promoters. Knockdown of GRHL2 in 3D organoids led to fewer, smaller, and structurally distorted organoids.Our study demonstrates that GRHL2 is a critical transcription factor driving epithelial changes in EoE, highlighting epigenetic mechanisms underlying EoE and offers potential therapeutic targets for better disease management and treatment outcomes.

D55 Crosstalk between nasal basal cells and mast cells contributes to epithelial barrier defects in allergic rhinitis

Keyword: Respiratory Allergy

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Rationale: The integrity of the airway epithelium is guarded by the airway basal cells that serve as progenitor cells. Previous data from our group revealed phenotypical changes within the basal cell population in allergic rhinitis (AR), suggesting a pro-inflammatory character in AR. We here aim to functionally compare basal cell behavior in health and AR and to explore basal cell-mast cell interactions.Methods: Primary nasal basal cells from controls and AR patients were isolated via FACS. OLINK proteomics and ELISA was performed to measure mediator release by basal cells. Mast cells were localized and quantified in situ via immunofluorescence staining of nasal biopsies. Basal cell mobility, proliferation and barrier formation was assessed in presence of type 2 mediators and mast cell factors via respective cellular assays.Results: AR basal cells released higher levels of mast cell stimulator and chemotactic molecule stem cell factor compared to controls (42.83 pg/mL vs 15.05 pg/mL, $p<0.05$). In addition, histology data showed that in AR a higher fraction of submucosal mast cells reside close to the basal cells (70.02% vs 44.37%, $p<0.05$), suggesting that AR basal cells attract mast cells. Finally, our data showed that IL-4 and histamine alter basal cell mobility, barrier formation and/or proliferation in AR, while their effect on basal cell functions is minimal in controls, suggesting functional alterations in disease.Conclusions: Basal cells and mast cells potentially interact with each other in the nasal submucosa and their interactions can contribute to basal cell dysregulation and chronic epithelial barrier defects in AR.

D56 The role of tryptophan metabolism in the pathogenesis of eosinophilic esophagitis

Keyword: Epithelial cells

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Rationale: Eosinophilic esophagitis (EoE) is a food-driven allergic disease of the esophagus. Dietary tryptophan and its metabolites are implicated in inflammatory diseases and can stimulate the aryl hydrocarbon receptor (AHR) with ligand-specific cellular responses. We hypothesize that dietary tryptophan promotes EoE pathogenesis in an AHR-dependent manner.Methods: We supplemented dietary tryptophan in mice with specific deletion of the Ahr gene (Ahr-KO) in the squamous epithelium and wildtype mice. We measured AHR activation by cell-based luciferase assay, metabolomics, transcriptomics, and microbiome analyses. We induced an EoE model by Alternaria alternata challenges and analyzed esophageal gene expression and eosinophilia.Results: Fecal samples from a high-tryptophan diet increased AHR activation compared to a low-tryptophan diet (p=0.047) in wildtype mice but not in Ahr-KO mice. The tryptophan metabolites and AHR-agonists, kynurenic and xanthurenic acid (p=<0.0001), were increased in fecal samples of wildtype mice following a high-tryptophan diet compared to a low-tryptophan diet, but remained low in Ahr-KO mice regardless of diet. Esophageal transcriptomic analysis revealed differentially expressed genes (n=15) between the high- and low-tryptophan fed wildtype mice. Two distinct genes were differentially expressed between high-and low-tryptophan fed Ahr-KO mice. EoE induction in wildtype mice dysregulated transcription of key genes in the tryptophan metabolism pathway(KMO,p=0.004;AFMID,p=0.031). High-tryptophan fed mice had increased esophageal eosinophilia compared to low-tryptophan diet (p=0.034).Conclusion: These findings indicate that esophageal epithelial cells have a role in tryptophan sensing and metabolism (kynurenic and xanthurenic acid generation), AHR activation, and gene expression. Additionally, excessive tryptophan metabolism may have a pro-inflammatory role in EoE pathogenesis.

D57 IFNγ promotes Treg-derived TGFβ production which exacerbates eosinophilic esophagitis.

Keyword: Eosinophilic Esophagitis

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Eosinophilic Esophagitis (EoE) is a chronic food allergy that is characterized by esophageal eosinophilia and results in dysphagia, fibrosis, and food impactions. Though the prevalence of EoE is 1:2000 and increasing by ~15% each year, our understanding of EoE immunopathology is limited. For example, regulatory T cells (Tregs) are elevated in the inflamed EoE mucosa though the phenotype and functional characteristics of Tregs are unknown. Further, though EoE is characterized as a type-2 mediated allergy, type-1 immune features are present including interferon (IFN) γ. This is relevant as IFNγ signaling has been implicated in the modulation of Treg effector functions. To investigate the role of Tregs in EoE, we utilized an established murine model. We found that esophageal Treg frequency and number were increased in EoE, and that Tregs expressed increased levels of TGFβ. When Tregs were ablated using FoxP3DTR mice, reduced esophageal eosinophilia was observed suggesting that Tregs exacerbate EoE-like inflammation. Given known increased IFNγ during EoE, we evaluated the effects of IFNγ on Treg function using mice lacking IFNγ signaling on Tregs (Foxp3iCreIfngr1fl/fl). Tregs that lacked the ability to respond to IFNγ produced less TGFβ, and Foxp3iCreIfngr1fl/fl mice displayed reduced esophageal eosinophilia compared with littermate controls. Finally, Foxp3iCreTgfb1fl/fl mice also displayed less esophageal eosinophilia compared with littermate controls. Together, these data suggest that IFNγ signaling on Tregs promotes Treg production of TGFβ and contributes to EoE-like inflammation.

D58 Allergic Inflammation and SARS-CoV-2: how HDM-induced asthma protects from severe COVID-19 in mice

Keyword: Viral Infection

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Epidemiological, clinical, and experimental data have emerged, showing that allergic asthma may be a protective factor associated with less severe COVID-19 outcomes. Type 2(T2) inflammation has been implicated as an unexpected mechanism by which the host response reduces the severity of disease following SARS-CoV-2 infection. The goal of this project is to characterize the mechanisms immune and epithelial cells respond differently to SARS-CoV-2 in an asthmatic host.Balb/C mice were exposed intranasally to House Dust Mites (HDM) over two weeks. CD90, IL-4, IL-5, IL-13, and IL-4ra neutralizing antibodies, and clodronate liposomes were used to delineate the mechanisms involved. Human airway liquid interface cultures were used to investigate the mechanism in a human cell model.Using the allergic model of Balb/c mice repeatedly exposed to HDM, induced allergic inflammation characterized by eosinophilia, alternatively activated macrophages, increased and type 2 cytokines. HDM mice displayed lessened disease compared to control mice with reduced viral titer, weight loss, and lethality when challenged with both SARS-CoV-2 Beta and Omicron variants. Following infection, the mice with less severe disease had increased expression of IL-13, Arg1, Muc5b, Muc5ac, and Chi3l3. Using neutralizing antibodies towards CD-90, IL-4, IL-5, IL-13, IL-4r, and clodronate liposomes to deplete monocytes, it was determined that macrophages, eosinophils, and secreted mucins were critical for anti-viral activity and reduced disease observed in vivo.Airway M2a macrophages, eosinophils, and mucin secretion in allergic asthma models protect from severe COVID-19 disease in mice and human ALI cultures.

D59 Food Allergen-Induced Eosinophil Activation in Eosinophilic Esophagitis: The IgG4 Pathway

Keyword: Eosinophilic Esophagitis

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Eosinophilic esophagitis (EoE) is a chronic allergic condition, characterized by increased eosinophil infiltration in the esophagus. IgG4 is found in the esophageal tissue of patients with active EoE and co-localizes with both known food triggers and eosinophil-associated proteins. The mechanisms by which IgG4 contributes to allergic inflammation in EoE, however, remain unclear. Peripheral blood samples were collected from active EoE patients to generate food allergen-specific IgG4 monoclonal antibodies (mAbs). Solid-phase immune complexes (ICs) were formed by incubating IgG4 mAbs (Bos d 8-specific) with purified Bos d 8-coated (5µg/ml) ELISA plates and soluble ICs were formed by mixing IgG4 mAbs with purified Bos d 8 in solution before incubating with the eosinophils. Human peripheral blood-derived eosinophils were isolated using Miltenyi magnetic separation and incubated with these ICs for 1h. Eosinophil activation was determined by quantifying Eosinophil-Derived Neurotoxin (EDN) release in culture supernatants. Bos d8-specific IgG4 mAbs were successfully generated from antibody-secreting B cells of active EoE patients using the hybridoma technique. Naive eosinophils incubated with solid-phase IgG4-Bos d 8 ICs released significantly more EDN compared to eosinophils incubated with IgG4 alone (477.3+-28.1 vs 243.5+-8.8, $p<0.01$) in a dose-dependent manner. Moreover, eosinophils pre-treated with Fc-blocker did not show activation upon subsequent incubation with the IgG4-Bos d 8 ICs (278.3+-21.3 vs 307.57+-7.3). Interestingly, eosinophils incubated with soluble ICs did not show any activation. These findings suggest that the solid phase IgG4-Bos d 8 ICs activate eosinophils via an Fc-gamma receptor. Whether this process is occurring in EoE remains unknown and warrants further study.

D60 Tuft cells sense protease-rich allergens and direct distinct olfactory and respiratory remodeling programs

Keyword: Epithelial cells

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Rationale: Tuft cells (TC) are rare epithelial cells in the respiratory nasal and tracheal epithelium of mice and humans, but abundant in the olfactory epithelium. They produce a unique cassette of eicosanoids, IL-25 and acetylcholine. While TCs mediate lung inflammation upon allergen inhalation, how allergens are recognized by TCs and the consequences of allergen-elicited activation of the abundant olfactory TCs are unknown. Methods: TC ligands were defined by whole mount Ca2+-imaging of noses and tracheas from Chatcre-GCaMP6f mice. TC-specific mediators after allergen stimulation of whole mounts of WT- and TC-deficient mice (Pou2f3-/-) were measured by ELISA, Lipidomics and HPLC. Allergen-elicited compositional olfactory changes were assessed by FACS and bulk RNA sequencing of sorted epithelial and neuronal populations in Pou2f3-/-, Chatcre-Ltc4sfl/fl (with TC-specific deletion of cysteinyl leukotrienes (CysLTs), and mice with deletion of each CysLT receptor. Results: The protease-rich house dust mite allergen Dermatophagoides pteronyssinus (Dp), the mold allergen Alternaria, the protease papain, a protease receptor 2 agonist and ATP trigger acute increases of [Ca2+]i in both olfactory and tracheal TCs. The allergens Dp and Alternaria induce TC-dependent generation of CysLTs and prostaglandin D2 in the nose and trachea, while Alternaria induces TC-dependent tracheal release of acetylcholine. Inhalation of Alternaria and ATP induces TC-dependent proliferation of olfactory stem cells, which is diminished in Chatcre-Ltc4sfl/fl and Cysltr2-/- mice. Conclusions: Allergens activate respiratory and olfactory TCs to generate a unique cassette of lipid and neuromediators. While TC allergen sensing and mediators are shared, the downstream mucosal responses are distinct in nose and lung.

D61 Cholesterol 25-hydroxylase has an important role in the recruitment of mast cell progenitors during type 2 inflammation.

Keyword: Mast Cell/Basophil

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Basophils, eosinophils, and mast cells (MCs) are 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. While the chemotactic signals recruiting basophils and eosinophils to inflamed tissue are well characterized, the signals directing MC progenitor (MCp) recruitment are unknown. Single-cell RNA sequencing (scRNA-seq) analysis of human peripheral MCps reviled high expression levels of Gpr183 transcript, encoding the oxysterols receptor EBI2. Venous endothelial cells isolated from human sinus and asthmatic tissue exhibit high expression levels of the cholesterol 25-hydroxylase (ch25h) transcript, a necessary enzyme to produce EBI2 ligands. Mouse MCps and mouse endothelial cells exhibit similar transcript expression pattern of Gpr183 and ch25h, respectively. To further assess the role of the ch25h/EBI2 axis in pulmonary MC expansion, we adapted an approach in which mice were IV-injected with FITC-labelled anti-CD45 to distinguish immune cells within the vasculature versus immune cells within the lung parenchyma to track the dynamics of MCp recruitment following aeroallergen challenge. 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. In contrast, in ch25h deficient mice, MCp entry to the lung parenchyma was reduced by 95% and no phenotypic maturation is observed. Ch25h deficiency had no effect on basophil or eosinophil recruitment, suggesting a selective role for the Ch25h, possibly through EBI2, in selectively directing MCp recruitment from the vasculature during T2I.

D62 Mast cell adhesion and focal adhesion kinase activity increase with mast cell activation and anaphylaxis

Keyword: Mast Cell/Basophil

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Rationale: While crosslinking of the high-affinity IgE receptor is well-characterized in mast cell degranulation, the role of adjunctive processes remains understudied. RNA sequencing of human samples collected during anaphylaxis revealed significant up-regulation of the focal adhesion kinase (FAK) pathway, thus we sought to investigate the role of FAK and related mast cell (MC) adhesion molecules in allergen-mediated MC activation.Methods: RBL-2H3 (rodent) and LAD2 (human) MC models were sensitized by incubation with monoclonal immunoglobulin E (IgE), and degranulation induced by anti-IgE antibody or ionomycin was quantified by beta-hexosaminidase release and CD63/CD107a expression. FAK activity was modulated by small molecule inhibition, degradation, and activation. Adhesion was assessed using cell adherence to fibronectin-coated plates and calcein-AM staining after washing off non-adherent cells. Cellular structure was visualized by immunofluorescence. Following stimulation, LAD2 cells underwent single cell RNA sequencing (scRNAseq).Results: Cell adhesion positively correlated with the degree of degranulation following stimulation with anti-IgE or ionomycin. FAK antagonism reduced anti-IgE-induced beta-hexosaminidase release (31%-33%;$p<0.0001$) and adhesion (33%;$p<0.0001$) preferentially over ionomycin-induced beta-hexosaminidase release (19-22%;$p<0.0001$) and adhesion (17%;$p=0.004$) in a dose-dependent manner without cytotoxicity, as well as reduced surface CD63/107a expression with normalization of cell morphology. scRNAseq of LAD2 MCs revealed correlation between degranulation signature and FAK pathway genes with significant variation between anti-IgE and ionomycin-induced activation.Conclusion: MCs demonstrate a positive functional and gene expression association between degranulation and adhesion which is preferentially disrupted by FAK antagonism during IgE/anti-IgE-induced activation, suggesting a role for adhesion mediated by FAK in MC activation during allergic reactions.

D63 Distinct Roles for Thymic stromal lymphopoietin (TSLP) and IL-33 in Experimental Eosinophilic Esophagitis

Keyword: Eosinophilic Esophagitis

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Rationale: Eosinophilic esophagitis (EoE) is a Th2-associated allergic disease. Thymic stromal lymphopoietin (TSLP) and IL-33 are alarmins implicated in EoE pathogenesis by activating multiple inflammatory cells including dendritic cells, mast cells, Th2 cells and ILC2s. Nonetheless, whether TSLP and/or IL-33 have a role in EoE, especially in vivo requires further investigation.Methods: Experimental EoE was induced in wild type (WT) and Il33-/- mice by skin sensitization with oxazolone followed by intraesophageal challenge. Expression of TSLP, IL-33, and their receptors was determined (immunohistochemistry and flow cytometry). The role of TSLP in experimental EoE was examined using neutralizing antibodies (clones M702 and 28F12). Esophageal histopathology was determined by H&E, anti-Ki67, anti-CD31 and anti-MBP staining. RNA was subjected to bulk RNA sequencing.Results: Induction of EoE led to increased expression of TSLP and IL-33 in lamina propria and epithelial cells. Mast cells displayed the highest expression of TSLPR and ST2 compared to other esophageal immune cells. Neutralizing TSLP decreased esophageal inflammation/remodeling as shown by decreased epithelial thickness, edema, vascularization, basal cell hyperproliferation and eosinophilia. Despite reduced esophageal eosinophilia in Il33-/- mice, no alterations in tissue remodeling were observed compared to WT mice. RNA sequencing of anti-TSLP-treated esophagi revealed differential expression of key genes associated with human EoE (e.g. eotaxins, Il19, Klk5, Flg, Il36rn, Il1r2). Bioinformatics analyses revealed a role for TSLP in regulating IL-1 signaling, barrier integrity and epithelial cell differentiation.Conclusion: TSLP may have a broader role than IL-33 in the pathophysiology of experimental EoE highlighting its potential as a therapeutic target.

D64 The role of the aryl hydrocarbon receptor in esophageal epithelial barrier function

Keyword: Epithelial cells

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Rationale: Eosinophilic esophagitis (EoE) is an allergic disease characterized by eosinophilia and esophageal barrier dysfunction, partially mediated by the loss of the protease inhibitor, SPINK7. Proton pump inhibitors (PPIs) are a first-line treatment for EoE patients; however, the mechanism of action is not completely resolved. We hypothesized that PPIs improve barrier function by activating the aryl hydrocarbon receptor (AHR) in esophageal epithelial cells.Methods: We used an air-liquid interface model of human immortalized esophageal epithelial (EPC2) cells deficient of AHR (KO) and control (WT) cells. Barrier function was measured using a FITC-Dextran assay, trans-epithelial electrical resistance, and gene expression following treatment with the AHR agonists, FICZ, or the PPI omeprazole. Expression of downstream AHR target genes was analyzed from esophageal biopsies of PPI-treated EoE patients.Results: AHR depletion impaired the barrier function indicated by increased barrier permeability ($p=0.0048$), decreased electrical resistance ($p=0.0003$), and reduced SPINK7 expression ($p<0.0001$). Omeprazole or FICZ exposures improved the electrical resistance ($p=0.0496$; $p=0.0171$) and SPINK7 expression ($p=0.0069$; $p=0.0313$) compared to vehicle exposure in WT but not AHR KO cells. Analysis of esophageal biopsies from EoE patients revealed that omeprazole-treated patients had increased barrier gene expression (SPINK7, FLG, DSG1) compared to patients treated with other PPIs.Conclusions: AHR activation is important for barrier function. We suggest that omeprazole treatment enhances barrier function in an AHR-dependent manner in the esophageal epithelium and increases SPINK7 expression. Further study is necessary to evaluate the clinical effectiveness and AHR dependence of all PPIs.

D65 Lower Baseline CXCL10 In the Epithelium of Asthmatic Children with Severe Exacerbations Permits Enhanced Rhinovirus Replication and Inflammation

Keyword: Asthma

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Rationale: Host factors influencing the risk of human rhinovirus (RV)-triggered exacerbations remain poorly characterized.Methods: Bronchial epithelial cells (BECs) from asthmatic children (n=37) were differentiated to an organotypic state, infected with RV-A16, then harvested at 2-, 4-, 7-, and 10-days post infection, and compared to uninfected BECs. Viral load and bulk RNA-sequencing were performed, comparing children with severe exacerbation history (SE; n=23) vs. without severe exacerbation (NSE; n=14). Generalized additive mixed models (GAMMs) were used to compare kinetic differences between groups. Differentially expressed genes were grouped into coexpression modules using WGCNA and biologically interpreted through pathway enrichment analysis. BEC protein secretion was measured via Luminex.Results: RV load associated with SE history; BECs from SE donors demonstrated higher viral load across timepoints compared to NSE donors(P<1e-4). CXCL10 secreted by uninfected BECs was lower in SE donors (P=1.51e-3), associated with viral load (P=1.83e-2), and was inversely associated with secreted type I and III interferons and CXCL10 protein 2-days following RV infection. RNA-seq analysis identified gene modules increased by RV with the SE group enriched for modules associated with anti-viral interferon response, remodeling and inflammation, and unfolded protein and stress response. Modules with decreased expression in the SE group were enriched for cellular metabolism and cellular transcriptional activity genes. Conclusions: Lower resting CXCL10 tone in uninfected bronchial epithelium from children with asthma permits enhanced RV replication and resultant excessive secondary interferon responses that mediate a cascade of

inflammatory pathways which may increase risk of future severe exacerbation and promote airway remodeling.

D66 Investigating the role of allergic asthma in viral respiratory infections in mice

Keyword: Viral Infection

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In most viral respiratory infections, such as those caused by influenza and rhinovirus, asthma is a risk factor for developing serious complications. Surprisingly, multiple studies have shown that asthmatic patients are not over-represented in severe COVID-19 cases, and some studies have even shown that asthma protects individuals from severe COVID-19. We aim to understand the mechanism by which allergic asthma results in drastically different outcomes during respiratory infection with different viruses. Understanding the varied host responses to viral infection in asthmatics could lead to therapeutics for these diseases. We have previously established a house dust mite induced allergic asthma model in Balb/c mice. These asthmatic mice, along with control non-asthmatic mice, were challenged separately with SARS-CoV-2 (B.1.351 variant) and H1N1 influenza (influenza A/Netherlands/602/2009). In the SARS-CoV-2 challenge, the asthmatic mice had reduced weight loss as compared to the controls. In the influenza challenge, the asthmatic mice had increased weight loss and higher mortality rates as compared to the controls. Interestingly, there was no significant difference in viral lung titer between the asthmatic and control mice in the influenza challenge, but significant reduction in titer in the SARS-CoV-2-infected asthmatic mice. Overall, asthmatic mice exhibited less severe disease upon SARS-CoV-2 infection as compared to controls, while asthmatic mice exhibited more severe disease upon influenza infection. Host immune cell and lung epithelial cell evaluation is ongoing to determine the factors responsible for this difference.

D67 Interleukin-5 enhances the anti-viral response of human mast cells

Keyword: Mast Cell/Basophil

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RATIONALE: In addition to their role in allergic disease, mast cells are immune sentinel cells. Exacerbations of allergic asthma are often associated with respiratory viral infections. Elevated levels of interleukin-5 (IL-5) are associated with allergic disease and targeted therapeutically. We examined the ability of IL-5 to modify human mast cell responses to viral infection.METHODS: Human mast cells were derived from umbilical cord-blood stem cells, treated with IL-5 or control and infected with either human coronavirus OC43, or respiratory syncytial virus (RSV). Expression of interferons and interferon-stimulated genes was evaluated. Total RNA sequencing was performed on mast cells derived from 8 donors to determine the impact of IL-5 on transcription and related functional assays were performed.RESULTS: IL-5 treated mast cells infected with OC43 coronavirus (n=10) produced significantly more type I and III interferons ($p<0.001$) and CXCL10 ($p<0.0001$) than untreated controls. Similar results were obtained for RSV infections (n=18). Mechanistically, IL-5 signaling upregulated the expression of the pro-survival factor B-cell lymphoma 2 (BCL2, $p<0.01$) and protected mast cells from apoptosis ($p<0.0001$). These responses were related to IL-5-induced upregulation of expression of Endothelial PAS Domain Protein 1 (EPAS1). Analysis of published transcriptomic data from peripheral blood obtained during clinical trials of IL-5 blockade also revealed a decrease in EPAS1 expression ($p<0.0001$) suggesting similar impacts of IL-5 on other cell types.CONCLUSIONS: IL-5 signaling regulates mast cell survival and interferon responses to respiratory viral infection. These observations may need to be considered in the context of IL-5 blockade therapies.

D68 Mast cells impact the acute innate immune response to RSV reducing viral load and inflammatory mediators

Keyword: Mast Cell/Basophil

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Rationale:Respiratory Syncytial Virus (RSV) is a major health concern for vulnerable populations, including infants, the elderly, and immunocompromised individuals. Despite its prevalence, early immune responses to RSV infection remain poorly understood. Mast cells are known to produce mediators in response to viral infections and are strategically located in the respiratory system. While previous studies have shown that mast cells can respond to RSV in vitro, their role in vivo during RSV infection remains unclear.Methods:To evaluate the role of mast cells in RSV infection, we used a c-kit-independent mouse model of selective mast cell deficiency, the Cpa3-Cre; Mcl-1fl/fl (Hello Kitty) mouse. Mast cell-deficient mice were compared to mast cell-containing wildtype (WT) controls in terms of viral load assessed by droplet digital PCR, weight loss, and lung inflammation, assessed through flow cytometry and histology, following RSV infection.Results:Mast cell-deficient mice exhibited a significantly higher viral load ($P < 0.05$, n=14 /group) in the lungs and experienced greater weight loss compared to their wildtype counterparts ($P<0.001$, n=5-12 per group). Additionally, mast cell-deficient mice demonstrated increased levels of inflammatory mediators such as CCL4, CXCL10 and TNF in the lung ($p< 0.05$, n= 6-7 per group), indicating a heightened inflammatory response in the absence of mast cells.Conclusion:These findings reveal a previously unrecognized protective role for mast cells in RSV infection, suggesting that mast cells help control viral replication and modulate the inflammatory response. These insights could inform the development of therapeutic strategies aimed at preserving or enhancing mast cell function.

D69 Induction of CRTH2 on draining lymph node CD4 T cells of helminth-infected mice is Chi3l1-dependent

Keyword: Respiratory Allergy

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RationaleChitinase 3-like protein 1 (Chi3l1) is a chitin-binding protein that is strongly associated with allergic disease activity. We previously determined Chi3l1 promotes TH2 priming in an acute gastrointestinal parasitic infection with Heligmosomoides polygyrus (Hp). CRTH2 is a marker of highly pathogenic activated TH2 cells in allergic patients and is posited to serve as a receptor for Chi3l1. We investigated CRTH2 expression on IL-4+ CD4 T cells of wildtype and Chi3l1-/- mice acutely infected with Hp and in mice sensitized to Aspergillus fumigatus (Af) conidia. MethodsHp infection: mice were infected with 200 L3 Hp larvae by gavage. Mesenteric lymph node cells were analyzed by flow cytometry on day 14. Af sensitization: anesthetized mice were administered 107 Af conidia intratracheally on day 1 followed by 106 Af conidia daily on days 7-9. Mediastinal lymph node cells were analyzed by flow cytometry on day 10. ResultsCD4 T cell CRTH2 expression is restricted to draining lymph node IL-4+ CD4 T cells in helminth-infected mice and in Af-sensitized mice. IL-4+CXCR5-PD-1- and IL-4+CXCR5+PD-1+ CD4+ cells express CRTH2. CRTH2 mean fluorescence intensity is reduced on Chi3l1-/- CD4+ CXCR5+PD-1+ cells. DiscussionCRTH2, a marker for activated TH2 cells in allergic patients, is also expressed on murine draining lymph node IL-4+ CD4 T cells in acute Hp infection and after allergic sensitization to inhaled Af conidia. Chi3l1 deficiency reduces CRTH2 expression on draining lymph node IL-4+ CD4 T cells. The interrelationship between Chi3l1 and CRTH2 may provide insight into future studies of allergic disease.

D70 Neuropeptides substance P, neurokinin B, and calcitonin gene-related peptide disrupt nasal epithelial integrity by modulating tight junctions

Keyword: Epithelial cells

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Rationale: Epithelial barrier integrity is crucial for physiological functions and protecting the host against harmful airborne stimuli. Neuropeptides like substance P (SP), neurokinin B (NKB), and calcitonin gene-related peptide (CGRP) are implicated in neurogenic inflammation. Since barrier dysfunction and neurogenic inflammation often co-occur in upper airway diseases, this study examined the effects of these neuropeptides on epithelial barrier integrity in Calu-3 cells and primary human nasal epithelial cells (pNECs). Methods: Cultured Calu-3 cells and pNECs were stimulated with SP, NKB, and CGRP, with and without receptor antagonists. Epithelial barrier integrity was assessed by measuring transepithelial electrical resistance (TEER) and evaluating tight junction proteins zona occludens 1 (ZO-1), occludin, and claudin 4 using quantitative PCR and immunohistochemistry. Results: Immunohistochemistry revealed that pNECs express the neurokinin 1 receptor for SP, the neurokinin 3 receptor for NKB, and the calcitonin receptor-like receptor for CGRP. Administration of SP, NKB, and CGRP significantly decreased TEER values in cultured Calu-3 cells within 15 minutes, indicating disturbance of epithelial barrier integrity. Specific antagonists reversed this dysfunction by upregulating tight junction proteins ZO-1, occludin, and claudin 4. These results were confirmed using pNECs, showing clinical relevance. Conclusion: This study shows that neuropeptides SP, NKB, and CGRP compromise epithelial barrier integrity by modulating tight junction proteins. Specific antagonists effectively reverse these effects, highlighting the interplay between barrier dysfunction and neurogenic inflammation in upper airway diseases. These insights provide potential strategies for treating diseases characterized by barrier dysfunction.

D71 OX40L, CD30L, and ICOSL Control Allergen Reactive Tissue-resident Memory CD4 T cells responsible for Asthma Exacerbations

Keyword: Asthma

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Background: Tissue memory CD4 T cells are considered critical in asthma exacerbations; thus, their modulation may have the potential to achieve airway tolerance. Objective: To modulate allergen-reactive memory CD4 T cells by targeting potentially active co-stimulatory molecules. Methods: Using single-cell RNA-sequencing analysis of CD4 T cells from asthmatic lungs of humans and mice, a combination of co-stimulatory molecules was identified expressed on many cells, and blocking antibodies to these molecules were examined in a mouse model of disease exacerbation. Results: Transcriptomic analysis revealed the co-expression of ICOS, TNFRSF4 (OX40), and TNFSF8 (CD30L) on allergic asthmatic CD4 T cells. Inhibiting ICOSL with OX40L or CD30L efficiently limited the accumulation of lung-localized memory effector T cells and ablated all aspects of lung inflammation during an asthma exacerbation. Importantly, transient therapeutic inhibition of these molecules together resulted in greatly reduced numbers and activity of memory CD4 T cells maintained in the lungs over time, even when mice were further challenged repeatedly with allergen. This led to a state of hyporesponsiveness such that subsequent exposure to allergen failed to re-exacerbate asthmatic lung tissue inflammation. Conclusion: Transient inhibition of ICOSL along with OX40L or CD30L limits the accumulation of memory CD4 T cells in allergic lungs, revealing potential therapeutic strategies for asthmatics.

D72 Evaluating allergen-mediated MCT and MCTC activation during immunotherapy

Keyword: Mast Cell/Basophil

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Mast cells (MCs) can be categorized into two distinct populations: subepithelial MCs that co-express tryptase and chymase (MCTC) and epithelial MCs that express tryptase alone (MCT). MCT are the principal phenotype found in airway epithelium, suggesting they are central drivers of aeroallergy. However, all current in-vitro systems give rise to MCTC. MC subsets are transcriptionally distinct from each other and differentially express key genes associated with IgG-driven inhibition, suggesting differential sensitivity to allergen-mediated desensitization. To test this, we selectively differentiated MCT and MCTC from peripheral blood-derived CD34+ cells, sensitized them using patient serum samples, and evaluated: 1) if differences in activation exist between MC subsets, and 2) whether immunotherapy improves MC subset activation. For this study, we utilized serum samples from a recent study of cat allergic individuals evaluating effects of subcutaneous immunotherapy (SCIT) on patient responses to cat dander. Our initial studies indicate that in-vitro differentiated MCT exhibit a 2-fold increase in degranulation compared with MCTC following activation with cat dander antigen. This enhanced MCT activation is especially pronounced when sensitized with serum from patients with low cat-specific IgE titers. Sensitizing MC subsets with SCIT treated patient serum samples improved MCT, but not MCTC activation, suggesting that MC subsets may exhibit differential sensitivity to activation during desensitization.

D73 LIGHT-Lymphotoxin Beta Receptor Activity in Lung Fibroblasts Regulates Collagen Synthesis and is Crucial for Lung Fibrosis in a Mouse Model of Severe Asthma

Keyword: Asthma

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Rationale: Airway remodeling is a feature of severe asthma, characterized by peribronchial fibrosis. Fibroblasts produce collagen and other matrix components but essential factors that act on fibroblasts to drive fibrosis in asthma are not well understood. LIGHT (TNFSF14)-deficient animals exhibit impaired fibrosis in models of severe asthma, suggesting that signals through its receptor, LTβR, in lung fibroblasts might directly control collagen deposition in the lungs.Methods: Mice with conditional deletion of LTβR in fibroblasts were used to determine its role in lung inflammation and fibrosis during an asthmatic response to house dust mite allergen. Recombinant LIGHT and TGFβ were assessed together to understand their activity in human pulmonary fibroblasts (HPF) in vitro.Results: LTβR deletion in fibroblasts led to reduced numbers of CD4 T cells, macrophages and neutrophils in the lung following multiple challenges with allergen. Histological assessment also showed significant reduction in cellular infiltration and importantly collagen deposition in the lungs was almost ablated even though numbers of fibroblasts were unaltered. In vitro, HPF treated with TGFβ and LIGHT/TNFSF14 synergistically upregulated inflammatory and profibrotic transcripts, including many collagen isoforms, such as COL4A1, COL4A2, and COL8A2, as well as other molecules associated with fibrosis and inflammatory activity, such as TNFSF4, ACTA2, and MYH11.Conclusions: LTβR activity in fibroblasts is crucial for inflammatory and fibrotic activity and regulates collagen deposition in the peribronchiolar regions of the mouse lung. LIGHT amplifies the effect of TGFβ and strongly drives a profibrotic phenotype in HPF relevant for development of severe asthma.

D74 Meningeal mast cells exacerbate Alzheimer's disease by controlling cerebrospinal fluid movement along the bridging veins.Tornike Mamuladze1,2, Zachary Papadopolous1,2 , Leon CD Smyth1,2, Siling Du1,2, Daviti Abramishvili1,2, Igor Smirnov1,2 and Jonathan

Keyword: Mast Cell/Basophil

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Cerebrospinal fluid (CSF) plays a vital role in maintaining brain homeostasis, and its disruption has been linked to the development of neurodegenerative and neuroinflammatory conditions, such as Alzheimer's disease. Recently, novel anatomical structures, termed arachnoid cuff exit (ACE) points, were identified. These structures facilitate CSF outflow through the perivascular space from the subarachnoid space into the dural meningeal layer, while also allowing dural interstitial fluid (ISF) to flow back into the subarachnoid space. This bidirectional flow raises the possibility that pathogens could exploit ACE points to invade the brain, increasing the risk of brain infections. However, the mechanisms regulating fluid flow at ACE points and bridging veins remain poorly understood. Our research reveals that the dura contains specialized immune cells-specifically, mast cells-located at ACE points. We demonstrate that mast cell degranulation at these points impairs fluid flow dynamics. The release of histamine from degranulated mast cells induces vasodilation of bridging veins, disrupting the perivascular space and altering CSF flow. Moreover, during bacterial meningitis, these meningeal mast cells help trap bacteria in the dura, limiting pathogen spread from the dura to the brain via ACE points. Removal of mast cells significantly increases bacterial burden in the brain. Our findings position dural mast cells as key regulators of meningeal immunity and CSF/ISF dynamics. Targeting these cells pharmacologically could represent a novel therapeutic strategy to enhance brain clearance and mitigate bacterial invasion of the brain.

D75 Characterizing the Relationship Between HLA-E mRNA Expression Levels and Cell Surface Protein Expression Levels in B Lymphoblastoid Cell Lines

Keyword: Transcriptional Regulation

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HLA-E is a non-classical class I HLA heterodimer that regulates immune cell effector activities by binding to the inhibitory CD94/NKG2A receptor on natural killer (NK) cells and activating the CD94/NKG2C receptors, which in turn activates NK cells. HLA-E is expressed by most cell types and is loaded with nonamer peptides derived from signal peptides (SPs) of HLA-A, HLA-B, and HLA-C. The Carrington lab has shown that HLA class I SP polymorphism affects HLA-E cell surface expression and CD94/NKG2 receptor recognition. However, analysis of B lymphoblastoid cell lines (BLCLs) as well as peripheral blood cells via flow cytometry has demonstrated that cell surface HLA-E expression levels can vary among individuals with identical HLA SP genotypes, suggesting that HLA-E can also be transcriptionally regulated. To test this hypothesis, I quantified HLA-E mRNA expression in a large set of BLCLs (N=70), for which HLA-E cell surface expression measurements are available. HLA-E mRNA expression levels will be tested for correlation with the cell surface protein expression. Preliminary data suggested that there is no correlation between HLA-E mRNA expression levels and cell surface expression levels, but further investigation is necessary. Currently, these experiments are being repeated in human cell lines. The resulting data will be used to further elucidate the genetic factors contributing to the regulation of HLA-E function, providing insights into immune cell function.

D76 T lymphocytes with adherent platelets demonstrate increased activation and altered metabolic profile in healthy controls and asthmaSindhu Manivasagam, Dawn Newcomb, Courtney Lehman, Taneem Amin, Katherine Cahill

Keyword: Asthma

Sindhu Manivasagam[1], Dawn C. Newcomb[2], Courtney Lehman[3], Taneem Amin[4], Katherine N. Cahill[5]

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In states of chronic inflammation, such as asthma, there is evidence of increased numbers of circulating platelet leukocyte aggregates (PLAs). Platelets when cultured with T cells have been reported to alter T cell cytokine production and proliferation. However, platelet-T cell interactions have not been characterized in asthma. We hypothesize that adherent platelets increase activation of lymphocytes at baseline and during inflammatory states. Peripheral whole blood was collected from patients with asthma and controls and processed to preserve PLAs. Samples were run and analyzed with a mass cytometry panel to characterize T cell subsets, activation status, and platelet adherence. In a cohort of eleven individuals (n = 5 controls and n=6 patients with asthma), approximately 5% of circulating T cells had adherent platelets. Platelet adherence, identified by CD61+, was globally associated with significantly increased expression of T cell activation markers (CD44 and CD38), proliferation marker (Ki-67), and immune checkpoint markers (PD1 and CTLA4) on CD4+T cells ($p<0.05$). Expression of glucose transporters (GLUT1 and GLUT3) and electron transport chain proteins (ATP5a and GRIM19) was also globally upregulated in CD61+ cells compared to CD61- cells in all CD4+ T cell subsets, including Th1, Th2, Th17 cells ($p<0.05$). The effect of platelet adherence was similar across clinical phenotypes. These data show that platelet adherence correlates with increased expression of specific activation markers and metabolism markers on circulating T cells, suggesting there may be potential for PLAs as new therapeutic targets to modulate inflammation.

D77 Proteomic Analysis Reveals Omeprazole's Modulation of Inflammatory, Proliferative, and Metabolic Pathways in Eosinophilic Esophagitis

Keyword: Eosinophilic Esophagitis

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Background: Eosinophilic esophagitis (EoE) is a chronic allergic disease characterized by esophageal dysfunction, type-2 inflammation, and eosinophilic infiltrate in the esophagus. Although proton pump inhibitors (PPIs) like omeprazole are used to manage EoE, their underlying mechanisms remain unclear.Methods: Whole-cell proteomics of air-liquid interface (ALI) cultures of esophageal epithelial cells (EPC2-hTERT) was used to investigate the mode of action of omeprazole in IL-13-treated cells. EPC2 cells grown in ALI were treated with 100 ng/ml IL-13 and/or 50 µM acid-activated omeprazole. Cell pellets were lysed, digested using S-Trap, and peptides were desalted, dried, and reconstituted with iRT peptides. Proteomic analysis was conducted using an Exploris 480 mass spectrometer coupled with an Ultimate 3000 nano UPLC, employing data-independent acquisition (DIA).Results: Unsupervised clustering revealed distinct separation, with the first principal component (PC1) accounting for 37.81% of the variation, primarily due to omeprazole's impact. Differentially accumulated proteins (DAPs) were identified using FDR < 0.05 and log2FC >= |1|. We identified 108 DAPs in IL-13-treated versus untreated ALI, 190 DAPs in omeprazole-treated versus untreated ALI, and 155 DAPs in IL-13 and omeprazole-treated versus IL-13 only-treated ALI. Omeprazole significantly downregulated pathways associated with inflammation (e.g., Interferon Responses), cell proliferation (e.g., E2F Targets, G2M Checkpoint). Concurrently, it upregulated pathways related to cellular metabolism and function, including cholesterol homeostasis, oxidative phosphorylation, fatty acid metabolism, xenobiotic metabolism, and hypoxia response.Discussion: These findings suggest that omeprazole modulates pathways involved in inflammation, proliferation, and metabolism, necessitating further studies to clarify their contribution to its therapeutic effects in EoE.

D78 Human Siglec-9 expression on mast cells contributes to modulation of mast cell function in homeostasis and in mouse models of allergic disease

Keyword: Mast Cell/Basophil

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Background: Mast cell activation is critical for the development of allergic diseases. Ligation of Sialic acid-binding immunoglobin-like lectins (Siglecs) such as CD33, Siglec-6, -7 and -8 have been shown to inhibit mast cell activation. We have demonstrated that human mast cells express functional Siglec-9. Based on this evidence, we aimed to investigate the relevance of Siglec-9 for modulation of mast cell function in homeostasis and disease.Methods and Results: To address whether Siglec-9 can impact mast cell activation state in vivo, we generated a transgenic mouse that express human Siglec-9 exclusively in mast cells (Cpa3-Cre+; hSiglec-9+). We observed that peritoneal mast cells from Cpa3-Cre+; hSiglec-9+ mice exhibited a reduction in LAMP-1 surface expression and serum mast cell protease-1 levels indicating reduced spontaneous mast cell degranulation at baseline. We also observed that Cpa3-Cre+; hSiglec-9+ but not mice with mast cells expressing human Siglec-8, exhibited attenuated allergic airway immunopathology after sensitization and challenge with house dust mite (HDM). In contrast, mice with Siglec-9 expression in myeloid cells were not protected from HDM treatment. Finally, antigenic liposomes displaying a synthetic Siglec-9 ligand inhibited passive systemic anaphylaxis in mice with global Siglec-7 and Siglec-9 expression without causing an impairment in host immune response against a systemic infection with Staphylococcus aureus.Conclusions: Our study indicates that Siglec-9 can contribute to ameliorate allergic disease via modulation of mast cell function.

D79 Assessing Transcriptomic Heterogeneity of Human Neutrophils in Eosinophilic and Non-eosinophilic Asthma using Single-cell RNA-sequencing

Keyword: Asthma

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Introduction: Gaps remain in our understanding of granulocytes' mechanistic roles in asthma. Although technically challenging, recent advances in transcriptomics have allowed pioneering single-cell studies of neutrophils and eosinophils in animal models and cancer. Here we address our hypothesis that heterogenous populations of neutrophils exist in asthma, correlate with clinical asthma endotypes, and may help refine these endotypes and predict asthma treatment responses.Methods: We isolated neutrophils from blood of 6 healthy donors, and blood and sputum of 12 donors with eosinophilic (n= 6) or non-eosinophilic (n=6) asthma. We performed single-cell RNA-sequencing (10X Genomics) and analyzed ≈300,000 neutrophils collected from sputum samples. After filtering low-quality (< 250 gene/cells, <10 % Mitochondrial genes) and doublets cells, we performed clustering analysis (resolution of 0.5) to characterize distinct neutrophil populations. We examined for quantitative (cell count) and qualitative (gene expression) association of neutrophil clusters with eosinophil-high or low asthma endotype.Results: We identified 5 transcriptionally distinct clusters of neutrophils in matched blood and sputum samples. Strikingly, from blood we identified asthma-associated clusters with unique gene signatures, one expressing ARG1. Migration from circulation to airway dramatically impacted neutrophil transcription, with enrichment in pro-inflammatory transcripts including CCL3, CXCL16, IL4R, and IL6ST in sputum neutrophils. Eosinophil-low status was linked to transcripts associated with prostaglandin production (PTGS2) and survival (BCL2A1).Conclusions: These preliminary data suggest heterogeneity between blood and sputum neutrophils. Proportions of neutrophil subclusters vary based on blood eosinophil count. Furthermore, this study suggests asthma-specific neutrophil sub-clusters exist, and ongoing work will help determine this with certainty.

D80 Genome-wide Multi-omic Analysis of the Genetic Basis of Asthma

Keyword: Asthma

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Background: The genetic origins of asthma are incompletely understood, with over 140 susceptibility loci identified to date explaining only a minor portion of the disease heritability. To address this gap, we employed a multi-omic approach investigating the genetic architecture of asthma by integrating genome-wide association study (GWAS) data with single-cell chromatin accessibility profiles from fetal lung tissue.Methods: We utilized RefMap, a machine learning framework that combines genetic signals with functional genomic profiling to map risk regions associated with complex disease. GWAS results from the Trans-National Asthma Genetic Consortium, a multi-ancestry meta-analysis of 23,948 asthma cases and 118,538 controls, were used as the training dataset for RefMap. Functional annotations were derived from sci-ATAC-seq3 data from 59 fetal lung samples (89 to 125 days postconceptual age).Results: Our analysis has identified 5,018 high-confidence 1-kb genomic regions associated with asthma risk (probability > 0.99), including 3,582 positively and 1,436 negatively associated regions. These regions showed distinct chromosomal distribution patterns, with strongest signal clusters concentrated on chromosomes 2, 17, and 6.Conclusions and Future Directions: This integrated analysis provides novel insights into the genetic architecture of asthma in the context of lung development. Further characterization of these regions and their cell-type-specific expression aim to uncover regulatory elements influencing asthma susceptibility during lung development, revealing potential new targets for therapeutic intervention and prevention strategies.

D81 Differential regulation of viral entry-associated genes modulated by inflammatory cytokines in the nasal epithelium

Keyword: Epithelial cells

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Background: This study aimed to investigate the impact of different types of nasal inflammation on the regulation of entry-associated genes of respiratory viruses, including SARS CoV-2, MERS-CoV, HCoV-229E, and influenza virus, in the nasal epithelium.Materials and Methods: Subjects were classified into three groups: the control, eosinophilic chronic rhinosinusitis (ECRS), and non-eosinophilic CRS (NECRS) groups. ACE2 and TMPRSS2, ANPEP, DPP4, and ST6GAL1 and ST3GAL4 were selected as key entry-associated genes for SARS-CoV-2, HCoV-229E, MERS-CoV, and influenza, respectively, and were evaluated. Brushing samples obtained from each group and human nasal epithelial cells cultured using an air-liquid interface system were treated for 7 days with typical inflammatory cytokines and analysed using real-time PCR. Western blotting and confocal microscopy were performed.Results: The entry-associated genes showed distinct regulation patterns in response to each IL-4, IL-13, TNF-α, and IFN- γ. Specifically, ACE2 significantly decreased in type 2 cytokines (IL-4 and -13), while TMPRSS2 significantly decreased in type 1 cytokines (TNF-α and IFN- γ). ANPEP significantly decreased in both types of cytokines. Remarkably, DPP4 significantly increased in type 2 cytokines and decreased in type 1 cytokines. Moreover, ST6GAL1 and ST3GAL4 significantly increased in type 2 cytokines and decreased in type 1 cytokines, particularly IFN- γ. These findings were supported by western blotting and confocal imaging results, especially for ACE2 and DPP4.Conclusions: The findings regarding differential regulation suggest that patients with ECRS, primarily mediated by type 2 inflammation, may have lower susceptibility to SARS-CoV-2 and HCoV-229E infections but higher susceptibility to MERS-CoV and influenza infections.

D82 TCF1 expression defines functional Th2 heterogeneity in tissues with Type 2 inflammation

Keyword: Asthma

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Repetitive exposure to antigen in chronic infection and cancer drives T cell exhaustion. In contrast, ongoing antigen exposure in allergic disease results in aberrant, sustained T cell responses. We recently defined a human Th2 cell state, the Th2 multipotent progenitor (Th2-MPP) through single cell RNA sequencing (scRNAseq), single cell TCR sequencing, and ex vivo function studies. These tissue resident cells co-express TCF1 and LEF1, transcription factors critical for chronic and memory responses, and can differentiate into effector cells while maintaining self-renewal capacity. We propose that this chronically activated tissue memory population may sustain chronic inflammation in type 2 disease. To understand the factors that modulate Th2 progenitor differentiation and maintenance, we established a mouse model of chronic pulmonary type 2 inflammation with house dust mite and Alternaria alternata. We found that type 2 inflammation was sustained over 8-16 weeks despite chronic stimulation. In contrast to the acute phase response, Th2 cells during chronic inflammation were heterogeneous and included a TCF1(+) compartment. These cells could self-renew for weeks without contribution from the draining lymph node. scRNAseq analysis of acute and chronic responses defined the transcriptional signature of tissue Th2 progenitors. After adoptive transfer, parenchymal Th2 progenitors could self-renew and differentiate into effector cells. Th2 progenitors, but not Th2 effectors or ILC2s, were sufficient to initiate and sustain allergic inflammation over several weeks. Our findings reveal tissue lymphocyte adaptations to chronic allergic inflammation and define the function of a progenitor Th2 population with the capacity to sustain chronic type 2 inflammation.

D83 GPR15-GPR15L Axis and MAdCAM1 Facilitate T Cell Homing to the Esophagus in Eosinophilic Esophagitis (EoE)

Keyword: Eosinophilic Esophagitis

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Background: - Eosinophilic esophagitis (EoE) is a food-allergen driven Th2-skewed chronic 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 were also detected in the esophagus,indicating a role in T cell migration in EoE. This study investigates the involvement of GPR15 and its ligand GPR15L in T cell migration, along with the roles of adhesion molecules PECAM1 and MAdCAM1, given their reported higher expression in the esophagus of active EoE patients.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. However, in the presence of MAdCAM1, there was a notable enrichment of CD38+ memory CD4+ T cells and CD38+ GPR15+ memory CD4+ T cells.Conclusion: The results suggest that GPR15L functions as a chemoattractant, with MAdCAM1 enhancing GPR15-GPR15L-mediated trafficking of memory CD4+ T cells to the esophagus. These findings highlight the potential of targeting GPR15 and MAdCAM1 pathways to modulate T cell migration in EoE.

D84 Mast cell responses in a mouse model of food allergy are regulated via a ST2/IL-4 axis

Keyword: Mast Cell/Basophil

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Allergen-induced mast cell (MC) activation is critical for the development of food allergy. The alarmin cytokine, IL-33, plays a pivotal role in regulating MC responses. MCs constitutively express the IL-33 receptor, ST2, and genetic polymorphisms in IL-33 and ST2 are strongly linked to disease susceptibility. We assessed the role of IL-33 in regulating MC function using a well-established model of systemic sensitization to food antigens. Briefly, wild-type and ST2-/- mice were immunized with chicken egg ovalbumin (OVA), followed by intragastric challenges with OVA. Oral challenge with OVA resulted in a robust IgE-mediated response leading to the development of intestinal anaphylaxis and accompanied by increased numbers of intestinal MCs as well as increased MC activation in WT mice. In contrast, ST2-/- mice exhibited attenuated responses presenting with decreased allergic diarrhea, reduced intestinal MC expansion, and decreased MC activation. Interestingly, systemic T cell responses were also reduced in ST2-/- mice, resulting in diminished levels of IL-4. Furthermore, both OVA-sensitized T cells and bone marrow-derived MCs also exhibited decreased IL-4Ra expression. Lastly, treatment with rIL-4 significantly enhanced allergic inflammation in both WT and ST2-/- mice and restored MC responses in allergic animals. Collectively, our data demonstrate that IL-33 plays a critical role in mediating MC responses to food allergic sensitization, with effects on both MC expansion and activation. Furthermore, these effects are mediated via induction of IL-4, resulting in restoration of MC responses in ST2-/- mice.

D85 Cholesterol 25-Hydroxylase (CH25H) Regulation of Airway Inflammation and Remodeling

Keyword: Respiratory Allergy

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RATIONALE: CH25H is an ER resident enzyme that generates 25-hydroxy cholesterol (25-HC) expressed in macrophages, fibroblasts, and epithelial cells (EpCs). Recent studies have demonstrated that CH25H may play a role in inflammation, but the mechanisms are poorly understood.METHODS: WT and Ch25h-/- mice were assessed in a model of airway inflammation and remodeling elicited by the fungal aeroallergen Alternaria alternata (ALT). Lineage tracing studies were performed in a Krt5CreRosa26tdT+ reporter strain crossed to Ch25hflox/flox mice or littermate controls. Transcriptional analysis was performed on airway basal cells (BCs) from ALT-challenged WT and Ch25h-/- mice and on 25-HC-stimulated primary human airway EpCs in the presence or absence of pharmacologic inhibitors. RESULTS: Ch25h deletion conferred protection against ALT-elicited tracheal and lung inflammation. Ch25h deficiency prevented the development of ALT-elicited tracheal squamous metaplasia and promoted differentiation towards club and ciliated cells by upregulating genes involved in EpC differentiation. In the BC-specific deletion model, Krt5CreERT2 Rosa26tdTCh25hflox/flox mice had heightened EpC regeneration and differentiation in response to ALT exposure. In air liquid interface (ALI) cultures, 25-HC stimulation inhibited BC differentiation into club and ciliated cells-an effect mitigated by a liver X receptor (LXR) antagonist. Moreover, activation of LXR with an agonist mimicked the effects of 25-HC stimulation.CONCLUSION:Our findings demonstrate that 25-HC thwarts normal epithelial differentiation and promotes inflammation. These findings demonstrate that targeting the CH25H/25-HC axis could offer a novel therapeutic strategy for diseases of airway inflammation and remodeling.