EO1-1  Mepolizumab decreased the levels of serum galectin-10 and eosinophil cationic protein in asthma

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iPOT-5 (Anti-I-L-5 antibody : Prediction of efficacy and Observation of Time course of Inflammatory Markers) investigators89)

Background: Mepolizumab decreases the number of blood eosinophils and the frequency of exacerbation of severe asthma. However, the time kinetics of serum galectin-10, which is derived from eosinophil and component of Charcot-Leyden crystal has not been investigated. This study aimed to clarify the precise time course of the levels of serum galectin-10 together with ECP after mepolizumab treatment and to analyze the relationship between the levels of eosinophil-derived molecules and the clinical background or response to mepolizumab treatment. Methods: This multicenter, prospective open-label study recruited 20 patients with severe eosinophilic asthma. Mepolizumab was administered every 4 weeks for 32 weeks and the levels of various biomarkers were serially analyzed. Results: The serum galectin-10 and ECP significantly decreased 4 weeks after initial administration of mepolizumab. Asthma Control Questionnaire-5, Asthma Health Questionnaire-33, and Lund-Mackay scores significantly improved. Both high ECP and eosinophil count related to better response in FEV1 and measurable ECP level at 4 weeks after administration of mepolizumab related to the further improvement in FEV1, toward week 32. No significant difference in improvement in FEV1, was observed in galectin-10 high group. The level of ECP at baseline was significantly related to the higher prevalence of nasal poly and Lund-Mackay score.

Conclusion: This study was the first to show that the levels of serum galectin-10 decreases after initial administration of mepolizumab. The significant relationship between serum ECP and better response in FEV1, suggested the potential role of serum ECP as a predictive biomarker for the efficacy of mepolizumab.

EO1-2  Parenchymal disease in asthma: Fixed airflow obstruction and lung function trajectory

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Background: Pathophysiological studies have suggested parenchymal abnormalities in non-smokers with asthma, but not elucidated their functional impacts in asthma. Objectives: To examine whether low attenuation area percentage (LAA) % and exponent D characterizing size distribution of low attenuation clusters on computed tomography (CT) affect fixed airflow obstruction (FAO), forced expiratory volume in 1 second (FEV1), and the longitudinal decline in FEV1 in independent of airway diseases. Methods: Asthmatics and mild to moderate chronic obstructive pulmonary disease (COPD) patients from two Hokkaido cohorts were included. In addition to D and LAA %, the wall area percent (WA %) at segmental bronchus and airway fractal dimension (AFD) were measured on inspiratory CT. Results: D was lower and LAA % was higher in COPD (N=42) and asthma with FAO (N=101) than it was in asthma without FAO (N=88). The decreased D and increased LAA % were found in a subgroup of either non-smokers, smokers, non-severe asthmatics, or severe asthmatics. In multivariable analysis, decreased D and increased LAA % were significantly associated with an increased odds ratio of FAO and decreased FEV1, irrespective of WA % and AFD. Moreover, decreased D predicted the longitudinal decline in FEV1 in severe asthmatics independent of smoking status despite no association between D and eosinophil counts in blood and sputum. Conclusions: Asthmatics with FAO showed parenchymal diseases irrespective of smoking status and asthma severity. Parenchymal diseases were associated with an accelerated FEV1 decline, but not with eosinophilic inflammation, suggesting the importance of establishing novel strategies besides Type2 targeting approaches.
EO1-3  
Association between pharyngeal bacterial colonization and antibiotic treatment in acute exacerbations of childhood bronchial asthma

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Background: The current guidelines do not recommend routine antibiotic prescription for bronchial asthma exacerbations. It has been reported that antibiotic therapy in acute exacerbations of childhood asthma might be associated with prolonged hospital stay. Recently, we reported that pharyngeal Streptococcus pneumoniae (S. pneumoniae) colonization in acute exacerbations of childhood asthma was associated with shorter duration of wheezing. To elucidate the effect of antibiotics on asthma, we examined pharyngeal bacterial colonization, aeroallergen sensitization, antibiotic use, and, duration of wheezing.

Methods: Potential bacterial pathogens were investigated in pharyngeal samples of 95 children who were inpatients with moderate exacerbations of asthma (mean/median age: 28/24, respectively). We measured allergen-specific IgE, peripheral white blood cell counts including neutrophils and eosinophils, and, C-reactive protein. Results: Pharyngeal bacterial cultures were positive in 94 of 95 children. The three major bacterial pathogens were S. pneumoniae (31.6%), Moraxella catarrhalis (M. catarrhalis) (9.5%), and Haemophilus influenzae (H. influenzae) (9.5%). Antibiotics were used in 47 (49.5%) patients. In patients with S. pneumoniae, the duration of wheezing was significantly longer in those with antibiotics than those without. In same patients, neutrophils and eosinophils were significantly lower in those with antibiotics than without. Conversely, in patients with M. catarrhalis or H. influenzae, in patients with or without aeroallergen sensitization, no significant difference was found between patients with or without antibiotics. Conclusions: These results suggested that antibiotic treatment in patients with pharyngeal S. pneumoniae colonization was associated with longer duration of wheezing in moderate exacerbations of bronchial asthma.

EO1-4  
Age-specific associations of early-life infections and preterm birth with allergic diseases in childhood

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Background: The effect of infection and developmental adaptations in infancy on the prevalence of asthma and allergic diseases at different ages during childhood is not fully revealed. Objective: To investigate the effects of early-life infection (i.e., daycare, older siblings, and severe airway infection) and developmental adaptations (i.e., preterm birth and overweight gain) on the subsequent asthma, allergic rhinitis/conjunctivitis, and atopic dermatitis at different ages in children. Methods: In the longitudinal cohort study (n=47015), children were followed from 0.5 to 11 years. Potential risk and protective factors including daycare attendance at 6 months, existence of older siblings, history of hospitalization owing to cold/bronchitis/pneumonia during 0.5-1.5 years, preterm birth, and overweight gain at 1.5 years were assessed, using multivariable logistic regression with adjustments for potential confounders. Results: The negative association of early-life daycare attendance with asthma was not significant after 10 years, while the negative association with allergic rhinitis/conjunctivitis was observed consistently and the association with atopic dermatitis was positive during childhood. In contrast, the early-life history of hospitalization owing to cold/bronchitis/pneumonia was a risk of incidence of asthma and allergic rhinitis/conjunctivitis. Preterm birth was a significant risk factor of child asthma. Older siblings and overweight gain in infancy did not have much effect on the development of allergic diseases. Conclusion: Different age-specific patterns were demonstrated in the relationship of early-life daycare, severe airway infection, and preterm birth with each allergic disease in childhood.
EO1-5 Risk factors for the development of asthma at 3 years of age in the general population

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Objective: The purpose of this study is to investigate risk factors for asthma at 3 years of age in the general population. Methods: The questionnaires were distributed to caregivers of children at 3-years-old well-child visits between January 2020 and November 2020 in two cities: Osaka and Hino City, Tokyo. Risk factors for asthma at 3 years of age were assessed by calculating odds ratio (OR) for children with asthma versus children without asthma by logistic regression analysis. Results: A total 1326 questionnaires were administered, and 478 responses were analyzed. There were 27 children (5.6%) with asthma. Significant associations with asthma at 3 years of age were seen for the following factors: history of atopic dermatitis (OR 3.0, 95% confidence interval (CI) 1.4-6.1), history of food allergy (OR 2.8, 95% CI 1.4-5.5), history of antibiotic use (OR 5.5, 95% CI 2.3-16.3), family history (FH) of asthma in the father (OR 7.5, 95% CI 3.6-15.6), FH of asthma in the mother (OR 6.0, 95% CI 3.0-12.1), FH of allergic diseases in the father (OR 3.6, 95% CI 1.9-7.5), and FH of allergic diseases in the mother (OR 2.3, 95% CI 1.2-4.6). There were no statistically significant associations between asthma and cesarean section, pets, smoking or swimming. Conclusions: Several risk factors for asthma at 3 years of age were identified in the general population. FH of asthma was found to be the biggest risk factor for asthma.

EO1-6 Identifying the heterogeneity of severe adult asthmatics by using a real-world longitudinal clinical database in Korea

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Background: Severe asthma is a heterogeneous airway disease in the etiology, pathophysiology, and prognosis. Understanding the heterogeneity of severe asthma is critical for better management of severe asthma. Methods: We aimed to classify distinct lung function trajectories of severe adult asthmatics by a latent class mixed model utilizing the long-term clinical data collected for over 10 years at Ajou University Hospital. The clinical characteristics and laboratory biomarkers were compared according to the subgroups of adult severe asthmatics. Results: A total of 414 severe adult asthmatics were distinguished by 3 latent groups with different lung function trajectories. Patients in groups 1 and 2 preserved their percent predicted of forced expiratory volume in 1 second (FEV1%) throughout the 10 years of follow-up. Conversely, group 3 showed a progressive decline in FEV1% and a persistently higher annual asthma exacerbation rate than group 1 and 2, even with the optimal treatment by asthma specialists. Patients in group 3 were significantly older than those in group 1 and 2 (P =0.002). Blood eosinophil and neutrophil counts (P=0.037, P=0.004) and serum eosinophil-derived neurotoxin levels (P=0.001) were significantly higher in group 3 than in group 1 and 2. No differences were found in sputum eosinophil or neutrophil counts (%) among the 3 groups. Conclusion: In conclusion, we identified 3 latent groups of severe adult asthmatics with distinct lung function trajectories and annual asthma exacerbation rate. Higher serum eosinophil-derived neurotoxin levels may potentially predict the worst clinical outcome in adult severe asthmatics.
EO1-7 The effect of biologics on severe asthma control in real world clinics in Korea

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Background: Real-world data of new biologics for severe asthma comparable to previous controlled clinical trials have been recently gathered. We aimed to analyze the outcomes of patients who received biologics using the data recorded in the multicenter Korea severe asthma registry (KoSAR). Methods: Among patients enrolled in KoSAR (N=831), this retrospective study included patients who received biologics (N=154). We analyzed changes in the incidence of exacerbation and other clinical indicators due to biologics administration and the occurrence of adverse reactions. Good responders to treatment were defined as patients with a 50% or more reduction in oral corticosteroid (OCS) dose or acute exacerbation rate after 4 or more doses. Results: The most commonly prescribed medication was reslizumab. The mean administered dose was 131.85 ± 44.06 mg. The difference in the incidence of exacerbation before and after the administration of four doses was not statistically significant. However, patients treated with biologics had a significant increase in average FEV1 by more than 120 mL and a substantial decrease in blood eosinophils. Asthma control improved significantly. No serious adverse reactions were reported. It was also confirmed that the odds of requiring OCS significantly decreased by 77%. Mepolizumab was more effective in improving lung function, and reslizumab was more effective in controlling asthma symptoms. The OCS-sparing effect of mepolizumab was greater than that of reslizumab. Conclusion: In this real-world study, biologics add-on therapy was remarkably effective without causing serious complications.

EO1-8 Decreasing ten-year (2008-2018) trends of the prevalence of childhood asthma and air pollution in Southern Taiwan

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Background: The prevalence of childhood asthma increased in developed countries. Recently, several reports from different parts of the world showed reversed trend. This study investigated the trend of childhood asthma through serial cross-section surveys in the south part of Taiwan, and identify associated factors related to this trend in elementary school children. Methods: We used the Chinese version ISAAC questionnaire to assess the asthma status of elementary school students aged 6-12 years in Tainan city in 3 independent study periods, namely, 2008-2009, 2010-2012 and 2017-2018. We assessed the trend of “asthma” and “related respiratory symptoms” across three study periods. Results: Of the 19,633 respondents, 17,545 (89.4%) completed the questionnaires. After adjustment for covariates, the prevalence of asthma and related respiratory symptoms was significantly lower in 2017-2018 than in the two earlier periods. Among the protective factors, the increasing rate of breastfeeding might be partly responsible for the observed reduced prevalence of current asthma and exercise-induced wheeze. The presence of pet-keeping was the risk factor that correlated with the prevalence for of nocturnal cough. Pearson correlation analysis showed a significant correlation of the prevalence of physician-diagnosed asthma, current asthma, and exercise-induced wheezing with the concentrations of air pollutant particles with PM10 (r=0.84, 0.77 and 0.81, respectively). Conclusion: The prevalence of asthma and related respiratory symptoms has declined in elementary school-age children in southern Taiwan. The increased prevalence of breastfeeding, decreased rate of pet-keeping, and improvement in outdoor air pollution seem to be related to this decreasing trend of asthma in school children.
EO2-1 A transcription factor Blimp1 in CD4+ T cells promotes Type 2 immune responses in the lungs while it suppresses IgE antibody production in mice

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Background: Allergic immune responses generally involve generation of antigen-specific type 2 CD4+ T cells and production of IgE antibodies. A transcription factor B lymphocyte-induced maturation protein-1 (Blimp1) regulates plasma cell development and effector functions of T cells. However, little information is available regarding the roles of Blimp1 in allergic immune responses. Methods: We crossed Prdm1 (encoding Blimp1)-floxed mice with Cd4 Cre mice to delete Blimp1 specifically in the CD4+ T cell compartment (Blimp1 cKO mice). Mice were exposed intranasally (i.n.) to ovalbumin (OVA) antigen with extract of fungus Alternaria as an adjuvant. Results: When exposed i.n. to OVA plus Alternaria, Blimp1 cKO mice showed higher numbers of T follicular helper T cells and germinal center B cells in draining lymph nodes as compared to wild-type (WT) mice. Accordingly, serum levels of OVA-specific IgE were higher in Blimp1 cKO mice. In contrast, when challenged i.n. with OVA antigen, Blimp1 cKO mice showed marked reduction in type 2 immune responses in the lungs, including production of type 2 cytokines, airway eosinophilia, mucus production and airway hyperreactivity. The number of memory-type CD69+Th2 cells was significantly lower in the lungs of Blimp1 cKO mice. Furthermore, expression of genes associated with effector function and tissue localization was modulated in Th2 cells from Blimp1 cKO mice. Conclusions: Type 2 immune responses in the lungs and IgE antibody production can be regulated reciprocally at the CD4+ T cell level. Blimp1 likely plays critical roles in type 2 airway inflammation during allergic immune responses.

EO2-2 PM2.5 exposure may be involved in the Th2 airway inflammation in a mouse model of bronchial asthma via TSLP and IL-7

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Objective: Group 2 innate lymphoid cells (ILC2s) are involved in the cause of severe asthma resistant to inhaled corticosteroids (ICS). PM2.5 exposure exacerbates bronchial asthma seasonally, but the mechanism is unclear. To clarify the involvement of PM2.5 exposures to the ILC2s and type 2 inflammation in asthma. Methods: Six mite-sensitized asthma mouse model groups (6 weeks old female Balb/c) were prepared: 4 groups were nasally exposed to PM2.5 (low and high concentrations collected in summer and winter) and asthma or control groups without PM2.5 exposures. The lungs were homogenized on 21st day. Various cytokines in the supernatant were compared. Results: The IL-4 level was higher in winter-high group, the IL-13 levels were lower in winter-low and winter-high groups, IL-7 levels were lower in summer-high and winter-high groups, and GM-CSF level was lower in summer-high group than those in asthma group. No correlation between IL-17 and IL-13 was observed in the control or asthma group, but strong positive correlations were observed in the four PM2.5-exposed groups. IL-7 was strongly correlated with both IL-17 and IL-13 in the summer-low group and IL-7 and TSLP in the winter-low group. Conclusion: PM exposure at the onset of atopic asthma linked the IL-17 system to Th2 inflammation of the lungs. It was suggested that IL-7 and/or TSLP may be candidates for the bridging cytokines.
EO2-3  
**Chitin induces steroid-resistant airway inflammation in mice**

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**Background** : Previously we reported chitin, which is the second most abundant polysaccharide on earth, induced airway inflammation in a mouse model. In the current study, we investigated whether inhaled chitin particles induced steroid-resistant airway inflammation in the mouse models. **Methods** : We developed mouse models of inhaled chitin particle-induced airway inflammation and steroid-resistant ovalbumin (OVA) -induced airway inflammation. Some experimental groups of mice were treated additionally with dexamethasone (DEX). Lung-draining lymph node (LLN) cells were cultured to determine the production of cytokines. **Results** : DEX treatment inhibited the OVA-induced airway hyperresponsiveness (AHR) and airway inflammation, but not AHR and airway inflammation induced by chitin or the combination of OVA and chitin, including the increases in the numbers of total cells, macrophages, lymphocytes, eosinophils, and neutrophils in BAL fluids. The airway inflammation induced by OVA or the combination of OVA and chitin developed production Th2 cytokines in LLN cells, and DEX treatment inhibited these productions. In contrast, the production of IL-17A in LLN cells that was induced by OVA or the combination of OVA and chitin were not affected by DEX treatment. **Conclusions** : These results suggest that inhaled chitin induces steroid-resistant airway inflammation and AHR. Inhaled chitin may contribute to features of steroid-resistant asthma.

EO2-4  
**The role of 27-hydroxycholesterol in the pathogenesis of bronchial asthma**

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**Background** : This study aimed to elucidate the contribution of 27-hydroxycholesterol (27-HC) derived from sterol 27-hydroxylase (CYP27A1) to the pathophysiology of asthma. **Methods** : House dust mite extract (HDM) was intranasally administered to C57BL/6 mice and the expression of CYP27A1 in the airways was analyzed by immunostaining. Bronchoalveolar lavage fluid (BALF) was collected and analyzed for total number of cells and fraction. CYP27A1 inhibitors were administered to the mice and their effects on the cell fraction in BALF were examined. In vitro, BEAS-2B cells were treated with HDM and the expression level of CYP27A1 was examined by immunoblotting. Furthermore, BEAS-2B cells were treated with 27-HC and the expressions of E-cadherin and ZO-1 in the cells were analyzed by immunoblotting. Production of regulated on activation, normal T cell expressed and secreted (RANTES) and eotaxin from the 27-HC-treated cells were analyzed by ELISA. **Results** : HDM induced the expression of CYP27A1 in the airways of mice, and increased the number of eosinophils and lymphocytes in BALF from the mice. Administration of CYP27A1 inhibitors suppressed levels of CYP27A1 expression and the number of eosinophils and lymphocytes in the lungs of HDM-treated mice. HDM increased the expression of CYP27A1 in BEAS-2B cells. Administration of 27-HC to BEAS-2B cells decreased the expression of E-cadherin and ZO-1, and increased the expression of RANTES and eotaxin. **Conclusion** : The results of this study suggest that aeroallergen can enhance the induction of CYP27A1, leading to allergic airway inflammation and disruption of the airway epithelial barrier function through the enhancement of 27-HC production.
EO2-5 Early-life EV-A71 infection augments allergic inflammation in asthma through trained immunity of macrophages

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**Background**: Virus-induced asthma is prevalent among children, but its underlying mechanisms are unclear. Accumulated evidences have indicated that early-life respiratory virus increased the susceptibility to allergic asthma. Nonetheless, the relationship between non-respiratory virus infections, such as enterovirus, and the ensuing effects on allergic asthma development is unknown. The aims of this study is to investigate the effect of early-life non-respiratory virus infection on the development of allergic asthma, and explore the mechanisms that lead to virus-induced allergic asthma. **Methods**: National health database was analyzed for the association between enterovirus infections and subsequent allergic disorders in children. Clinically relevant enterovirus A71 (EV-A71) infection in neonatal mice were used to study the immunological effects of virus-related allergic asthma in later age. **Results**: Our population-based data showed early-life enterovirus infection was also correlated with higher risks of allergic diseases in children. Adult mice exhibited exacerbated mite allergen-induced airway inflammation following its recovery from EV-A71 infection in the neonatal period. Bone marrow-derived macrophages (BMDMs) from EV-A71-infected mice showed a sustained innate immune memory (trained immunity) to drive naive T-helper cells toward Th2 and Th17 cells when contacted with mite allergen. An adoptive transfer of EV-A71-trained BMDMs induced augmented allergic inflammation in recipient naïve mice. This augmented effect of trained macrophage can be inhibited by 2-deoxy-D-glucose (2-DG) pre-treatment of trained BMDMs. **Conclusions**: These results suggested that trained macrophages following enterovirus infection is crucial in the progression of allergic asthma later in life.
**EO3-1**  
Impact of gut microbial diversity in school-aged children on the development of atopic diseases

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**Purpose**: The gut microbiota plays an important role in the immunologic education of the host, and alterations within the gut microbiota are likely to lead to the development of atopic diseases. Here, we sought to clarify the association between gut microbial diversity and the development of atopic diseases by comparing the gut microbiota in school-aged children from two regions. **Methods**: In this cross-sectional study, 540 school-age children from Ogawara (103) and Shinagawa (437) were enrolled from December 2018 to January 2019. Eighty-one fecal samples were randomly collected, and their gut microbiota were studied using 16S ribosomal RNA gene-targeted amplification sequencing. The Shannon index was calculated to measure the richness and diversity of the gut microbiota (α - diversity). Principal-coordinate analyses (PCoA) were performed to compare samples based on bacterial community composition (β - diversity). The prevalence of atopic diseases was also investigated based on the physician's diagnosis. **Results**: Of the 81 fecal samples (42 in Ogawara and 39 in Shinagawa), there was no significant difference in β-diversity between the two regions. There was no association between the α-diversity in the gut microbiota and the development of asthma, atopic dermatitis, and allergic rhinitis. Moreover, a decreased Shannon diversity index was associated with the development of food allergy (FA) (FA (+) 4.61 vs. FA (-) 4.94, p=0.041). **Conclusion**: School-going children with FA had lower gut microbial diversity than school-going children without FA. No association could be found between other atopic diseases and gut microbial diversity.

**EO3-2**  
Protein expression of TRP channels in the esophagus of patients with gastroesophageal reflux associated cough

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**Background**: Excessive activation of afferent nerves in the esophagus is assumedly a major mechanism of cough associated with gastroesophageal reflux (GER), which may lead to sensitization of cough reflex. Transient receptor potential vanilloid (TRPV) channels have an important role for converting external stimuli into biological response. We demonstrated an increased gene expression of TRPV1 and TRPV4 in the esophagus of GER patients with cough. However, association between protein expression of these channels in the esophagus and GER-associated cough is still unknown. **Methods**: We prospectively recruited untreated GER patients with (n=32, C+) and without (n=5, C-) cough. Esophageal endoscopy was performed to determine the presence and severity of reflux esophagitis (RE), and to obtain biopsy specimens, which were examined for TRPV1 and TRPV4 protein expression by western blot analysis. **Results**: Age, sex distribution, smoking history, prevalence/severity of RE, and GER symptom scores did not differ between the C+ and C- groups. TRPV4 expression was significantly increased in the C+ group than in the C- group (p<0.0001), but TRPV1 expression was similar in the two groups. The expressions of both receptors were unrelated to the presence/severity of RE or GER symptom scores. **Conclusions**: Cough in GER patients is associated with the increased expression of TRPV4 in the esophagus but not with RE or reflux symptoms. Further studies are needed to elucidate the pathogenesis and precise roles of the increased TRPV4.
EO3-3  Clinical features of IgG4-related disease with and without bronchial asthma

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Background: Immunoglobulin G-related disease (IgG4-RD) patients often show peripheral blood eosinophilia and elevated serum IgE levels. Asthma is a chronic inflammatory disease of the airway with a heterogeneous pathophysiology including eosinophilic inflammation and IgE-mediated immune responses. Patients with IgG4-RD and asthma share similar serological characteristics, but little is known about the association between IgG4-RD and asthma. Methods: We enrolled 105 IgG4-RD patients at the Tokyo Metropolitan Komagome Hospital between 2004 and 2018. We retrospectively reviewed clinical, laboratory, and imaging data of the patients and compared the features between those with and without asthma. Results: Among the 105 IgG4-RD patients, 22 (21.0%) had asthma history. Those patients with asthma were younger at the time of IgG4-RD onset (mean, 58.6 and 65.0, p<0.05) and involved more organ systems (percentage of more than 4 involved organs was 7.2% and 22.7%, respectively, p<0.05) than those without asthma. Further, those with asthma were more likely to have affected organs of nasopharynx and lung (p<0.05). Peripheral eosinophil and serum IgA levels (p<0.05) were elevated in those with asthma. The number of peripheral eosinophils in IgG4-RD with asthma patients, but not without asthma, was correlated with serum IgG4 levels and number of the involved organs damage (p<0.05). Conclusion: Our retrospective study revealed the significant association between early onset of disease, IgG4-related nasal and lung involvement and asthma histories. The differences between IgG4-RD with and without asthma indicate that asthma might predispose and affect IgG4-RD clinical phenotypes.

EO3-4  Clinical features of asthma with severe adrenal insufficiency - Analysis of 128 cases diagnosed and treated as bronchial asthma

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Rational: Adrenal insufficiency (AI) is often under-estimated due to their indefinite symptoms such as fatiguability, frequency of illness, sporadic cardiac/gastric symptoms often not suspected as AI. Asthmatics may suffer AI after systemic steroid therapy but are not usually examined for AI. Present research attempted to characterize symptoms of severe AI by selecting those with cortisol level below 5μ/dl. Methods: We analyzed 128 asthmatics for possible AI by selecting patients with diurnal cortisol (6am, 11am, 15pm, 23pm), and ACTH provocation test (ACTH-PT). Twenty with diurnal cortisol below 5μ/dl at all points, peak CS under 20μ/dl in ACTH-PT was defined as severe AI an designated as group A. Others were grouped, B=26, C=48, D=34 according to their cortisol level. CMI (Cortel Medical Index), TEG (Todai Egogram), YG were employed for assessment of their health state. Results: Items in CMI prominent in group A was frequency of diseases, somatic discomfort and psychologically sensitive profile with high anger score and frequent skin, nerve problem and high sense of tension. Their somatic symptoms were high including cardiac, gastric symptoms. Negative correlation to achieved ratio (R) was found in somatic symptoms, anger, respiratory, eyes and ear problems, with higher level in inferiority complex. In contrast, in TEG, group A had high “Adult score” compared to C. Conclusion: Those with severe AI had unique discomfort with possible relation with AI, though many were already under treatment at the time of survey. Asthma patients should be screened for AI to avoid adrenal crises which may be the cause of acute exacerbation of symptoms in severe asthma. Items in CMI, YG and TEG monitored for symptoms may be useful to detect vague symptoms in AI.
Skin test based strategy for selecting alternative iodinated contrast media in patients with hypersensitivity reaction: a prospective confirmative study

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**Background**: Due to increased use of computed tomography (CT) scans, iodinated contrast media (ICM) became a common cause of drug hypersensitivity. Although skin test has been suggested as a useful tool for diagnosing IgE-mediated reaction and selecting safe alternatives, its role has never been evaluated prospectively so far. **Methods**: We conducted a multicenter study from July 2018 to December 2020. The patients who had experienced immediate hypersensitivity to ICM were recruited and underwent intradermal test (IDT) with a causative ICM. The safe alternative agent was recommended based on the results of IDT as follows: 1) When culprit ICM showed positive result, further skin tests with other six ICM were conducted and IDT-negative agents were recommended for the next CT scan. 2) When culprit ICM was negative on IDT, any different ICM was recommended without additional skin tests. The recurrence and severity of hypersensitivity was assessed in the following CT exam. Premedication (corticosteroids or antihistamines) was administered according to the severity of index event. **Results**: A total of 213 patients were recruited. Our IDT-based strategy reduced recurrence of hypersensitivity reactions in the following CT scans by 86.4% (vs. 60.7% when our strategy was not applied). The severity of hypersensitivity (15 severe, 4 moderate, and 10 mild initial cases) also diminished substantially even in 29 subjects with recurrent hypersensitivity reactions (5 severe, 5 moderate, and 19 mild reactions). **Conclusion**: We have shown that IDT-based strategy significantly reduced recurrence of hypersensitivity reactions to ICM. This provides evidence to encourage IDT to diagnose ICM allergy and find safe alternatives.
English Session-Oral 4
Food allergy, its management and related pathophysiology

EO4-1 Serum 25 (OH) D metabolite levels in food allergy patients are lower than those in atopic dermatitis patients and children without allergy

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Background: Many studies suggest that Vitamin D (VD) insufficiency contributes to allergen sensitization and allergic diseases, but those findings vary among populations. There are differences in VD metabolite levels among countries. The aim of this study was to assess the differences of serum VD metabolite levels among children with food allergies (FA), children with atopic dermatitis (AD), and children without allergy (NA) in Japan. Methods: Study subjects were doctor-diagnosed FA children (at Chiba University Hospital), doctor-diagnosed AD children (in the Chiba High-risk Birth cohort for Allergy (CHIBA) study) and NA children (in the CHIBA study). Serum VD metabolite (25 (OH) D3, 25 (OH) D2, 3-epi-25 (OH) D3, 24, 25 (OH) 2D3) concentration at 1-year-old, 2-years-old, 5-years-old and school-age were measured using liquid chromatography tandem mass spectrometry. Written informed consents were obtained from subjects’ parents. Results: Median 25 (OH) D concentration (ng/ml) in FA, AD and NA children were 14.3, 21.1, 20.9 at 1-year-old, 19.2, 20.1, 21.4 at 2-years-old, 22.4, 24.2, 22.9 at 5-years-old, 23.9, 25.8 24.0 at school-age, respectively. 25 (OH) D concentration in FA children at 2-years-old was significantly lower than that in AD and NA children (p=0.013). The proportion of VD deficiency was higher in FA children than that in AD and NA children. 25 (OH) D2 concentration in AD and NA children increased with age, but NOT in FA children. In FA children, there were no significant differences of 25 (OH) D levels between seasons at 5-years-old and school-age. Conclusions: Serum 25 (OH) D metabolite levels in FA children were significantly lower than those not only in NA children but also in AD children.

EO4-2 Microbiome analysis in patients receiving oral immunotherapy for severe food allergy

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Background: Growing evidence shows that gut microbiota and short-chain fatty acids (SCFAs) play a role in the resolution of food allergy. We assessed changes in the microbiome during oral immunotherapy (OIT). Methods: Patients who failed an oral food challenge with 102-mg milk, 200-mg egg, 53-mg wheat, and 133-mg peanut proteins were included. The OIT group gradually increased protein intake to a target dose (102, 800, 53, and 133 mg of milk, egg, wheat, and peanut proteins, respectively) and continued target dose ingestion. The control group eliminated the causative food. At baseline and 12 months after OIT initiation, gut microbiota was analyzed using 16S rRNA in the OIT group and serum SCFA analysis in both groups. Results: The median age at baseline was 7.7 years (33 patients) and 6.8 years (30 patients) in the OIT and control groups, respectively. Except propanionic acid levels (10.6 and 9.1 μmol/L, respectively), baseline characteristics did not significantly differ between the OIT and control groups. In the OIT group, alpha diversity has a tendency to increase (p=0.06), and the relative abundance of the family Pseudomonadaceae significantly increased (W=23). Moreover, median levels of iso-butyric acid, propionic acid, acetic acid, and iso-valeric acid in the OIT group significantly decreased from baseline (7.02, 10.6, 113.0, and 12.0 μmol/L) to 12 months (0.70, 4.6, 44.4, and 1.6 μmol/L), respectively (p<0.0001). These changes were not observed in the control group. Conclusion: OIT would induce changes in gut microbiota diversity and composition and a marked decrease in serum SCFA.
EO4-3  Ovomucoid-specific IgD increases in children during natural outgrowing who naturally outgrow egg allergy in a cross-sectional study

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Background and Purpose: The majority of egg-allergic children outgrow hypersensitivity against heated egg and then raw egg over time. The roles of ovomucoid (OVM) - and ovalbumin (OVA) -specific IgD in children who naturally outgrow egg allergy are uncertain. We investigated whether specific IgD to egg white (EW), OVM, and OVA correlate with allergen-specific IgE and can predict the development of immune tolerance to egg allergens. Methods: The tolerated doses of cooked egg white, which were determined by oral food challenge and/or an episode of accidental ingestion and corresponding specific IgE, IgG4 and IgE levels were evaluated in 57 children with egg allergy and 23 non-egg allergic children. Results: Patients avoiding all forms of egg had lower EW-, OVM-, and OVA-specific IgD and IgG4 than those partially avoiding egg, those that had outgrown egg allergy, and non-egg allergic children. The ratio of OVM-specific IgD to OVA-specific IgD increased depending on the ingestible amounts of boiled EW, whereas the ratio of OVM-specific IgG4 to OVA-specific IgG4 did not change. Receiver operating curve analysis revealed that the ratio of OVM-specific IgE to OVM-specific IgD was the best index to discriminate intolerant from tolerant egg-allergic patients. Conclusion: The ratio of OVM-specific IgE to OVM-specific IgD is a useful marker to identify high-risk egg-allergic patients capable of ingesting a low-dose of cooked whole egg who might be a good candidate for low-dose oral food challenge tests.

EO4-4  Fish allergy in Hong Kong and Japan - how does it compare?

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Background: Fish is important in children’s diet because of its nutritional richness. Fish, however, is also a common food allergen in Asia. Objective: To compare the clinical and immunological profiles between Japanese and Hong Kong (HK) fish-allergic patients. Methods: Patients with physician-diagnosed fish allergy presenting to two tertiary hospitals in HK and Japan between 2016 and 2019 were recruited. Clinical information and blood samples were obtained. Results: One hundred fish-allergic patients, 50 each from HK and Japan, participated with the majority being male (69%) & median (range) age of 7 (1-33) years. Timing of first fish-allergic reaction was earlier in HK [9 (6-84) months] than Japan [12 (7-144) months, P=0.05]. The major triggers in Japan were salmon, cod and mackerel, and that in HK were salmon, grass carp (GC) and grouper. Seventy-nine percent was allergic to 1-3 fish species, 15% and 6% allergic to 4-6 and >6 species, respectively. Eight percent experienced cardiorespiratory symptoms. The mean (95% CI) sIgE level to GC was the highest [21.9 (15.6-28.2)] but was lowest for cod [10.5 (7.6-13.4)]. Specific IgE levels were not statistically different between HK and Japan groups, but IgE reactivity to GC was significantly higher than that to cod and tuna. Conclusion: Majority of fish-allergic patients reacted at a young age to multiple fish species. Fish-allergic and IgE reactivity pattern across cultural groups needs to be further studied.

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EO4-5 The value of oral food challenge test in the long-term management of food allergy in children

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Objective: To investigate the application value of oral food challenge test in long-term management of children with food allergy by reviewing the long-term follow-up of children with food allergy. Methods: To review the results of 215 times of oral food stimulation test in 104 children with food allergy in our department. Results: The age range ranged from 9 months to 15 years, with an average age of 5.1 and 26 years. Follow-up lasted from 1 month to 168 months, with an average of 41.6 28.2 months. There were 215 oral food challenge tests, of which 122 were positive, 92 were negative, and 1 was unsure. In the 92 times of negative oral food challenge test, the food types tolerated were milk 38 cases (41.3%), eggs 26 cases (28.3%), wheat 14 cases (15.2%) and the other 14 cases (15.2%). 9 children were treated with anti-IgE monoclonal antibody. Oral food stimulation test proved tolerance in 3 cases after treatment, and the remaining 6 cases remained allergic. Conclusion: Oral food stimulation test provides an important basis for the determination of tolerance or the addition of hypersensitive food in allergic children.

EO4-6 Effect of E-B-FAHF-2 and 7,4’-Dihydroxyflavone (DHF) on TNF-α and IL-8 production, inflammatory markers of non-IgE-mediated food hypersensitivity

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Background: Pathophysiology of non-IgE-mediated gastrointestinal food hypersensitivity, including food protein-induced enterocolitis syndrome (FPIES) remains poorly understood. Increased TNF-α and IL-8 have been detected in FPIES reactions. We sought to determine the effect of natural plant-derived products E-B-FAHF-2 (ethyl acetate and butanol purified food allergy herbal formula-2) and 7,4’-Dihydroxyflavone (DHF) on TNF-α and IL-8 production, respectively using in vitro cell lines. Methods: RAW 264.7 mouse macrophage cells that produce TNF-α were treated with E-B-FAHF-2 ranging 0-120 μg/mL and stimulated with lipopolysaccharides (LPS, 1 μg/mL). Human epithelial cell line, CACO2 that produces IL-8 was treated with DHF (0-40 μg/mL) for 24 hours followed by IL1-β (10 ng/ml) stimulation for 24 hours. TNF-α and IL-8 levels in supernatants were measured by ELISA. Cytotoxic effect was evaluated by trypan blue exclusion or MTT assay. Quality control of compounds was monitored by HPLC. Results: E-B-FAHF-2 treatment significantly reduced TNF-α levels in a dose-dependent manner in RAW 264.7 cells (p<0.001 vs vehicle). It essentially eliminated TNF-α production at a dose of 120 μg/mL. No cytotoxicity was observed at any tested doses. DHF treatment significantly reduced IL-8 production by CACO2 cells (p<0.001 vs vehicle) without cytotoxicity at any tested doses. These effects were associated with reduction of phosphorylated IκB α. Conclusion: E-B-FAHF-2 and DHF either alone or in combination may be a potential intervention for non-IgE mediated food hypersensitivity. Studies on inhibitory effects of cross-treatment or combined treatment of E-B-FAHF2 and DHF in RAW 264.7 and CACO2 cells on TNF-α and IL-8 are underway.
EO4-7  The efficacy and consumers’ perception of precautionary allergen labelling : Enzyme-linked immunosorbent assay (ELISA) for allergens detection and a survey of parents of children

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**Background** : Korean food allergen regulation currently requires 22 foods to be reported on label and precautionary allergen labelling (PAL) is legally regulated. This study aims to evaluate the efficacy of PAL and assess the perception of consumers on PAL. **Methods** : Prevalence of PAL on 305 products were assessed and 30 were randomly selected from 3 supermarket chains in Korea for measuring cow’s milk and egg white protein concentration. Enzyme-linked Immuno Sorbent Assay (ELISA, Veratox® Total Milk kit, Total Egg kit, Neogen, Michigan, USA) and VITAL® 3.0 were used to validate the application of PAL. Surveys (207 in total) about preferences on current and developed PAL statements were conducted among parents of children with food allergy (p-FA, n=103), other diseases (p-control, n=52) and no diseases (control, n=52). **Results** : PAL was used on 91.8% of products. Of 30 products, 2 products (6.7%) contained detectable amount of cow’s milk protein and none contained detectable levels of egg white protein. Only 1 product was measured above the reference dose and qualified to use PAL. The rate of PAL checking was highest in the p-FA group (87.4%), followed by p-control (40.4%) and control (38.5%) groups. All were not satisfied with the current PAL statement and “OO Free” statement was most preferred. The p-FA group preferred statement with allergen concentration and reliability and favorability on statement were relatively high compared to control groups. **Conclusions** : A risk assessed PAL system is necessary in order to provide consumer-transparent and accurate food allergy information concerning cross-contamination risks.
EO5-1  Pollen shells and soluble components play non-redundant roles in the development of allergic conjunctivitis

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Background: In the previous meeting, we reported a newly-developed murine model of allergic conjunctivitis induced by repeated topical application of ragweed pollen suspension, where the IL-33 receptor ST2 plays a role. However, the exact role of the particulate property of allergens remains elusive. Methods: We administered various pollen suspension, ragweed pollen extract, ragweed pollen shell, particulate air pollutants, or their mixture to the mouse eyes five days a week without prior sensitization. Clinical signs were scored. Histology, cellular components, mRNA expressions, lymph node-recall responses, and serum immunoglobulin levels were investigated. Immune cell-depleting antibodies were used to assess the cellular requirements. Results: The ragweed pollen suspension, but not the extract or the shell alone, induced eosinophilic conjunctivitis. A combination of pollen extract and their shells completely restored eosinophil accumulation. The conjunctivitis was inducible by pollens of other species such as cedars and cypress. Furthermore, eosinophilic conjunctivitis was induced by a mixture of particulate air pollutants and pollen extract. ST2/CD4+ T cell numbers were well-correlated with those of eosinophils. The inflammation was abolished by depleting CD4+ T cells. Pollen shells, but not the extract, induced IL-33 release from conjunctival epithelial cells in vivo. Conclusions: Our results indicate the non-redundant roles for the allergens’ particulate properties and soluble factors in the development of allergic conjunctivitis.

EO5-2  Intralymphatic immunotherapy with tyrosine-adsorbed allergens

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Background: Most previous studies used aluminum hydroxide-absorbed allergen extracts in evaluating the potential therapeutic roles of intralymphatic immunotherapy (ILIT). In this study, we evaluated the therapeutic efficacy and safety of ILIT with L-tyrosine-adsorbed allergen extracts of Dermatophagoides farinae, D. pteronyssinus, cat, dog, or mixtures thereof, in patients with allergic rhinitis induced by these allergens. Methods: In this randomized, double-blind, placebo-controlled trial, study subjects received three intralymphatic injections of L-tyrosine-adsorbed allergen extracts (active group) or saline (placebo group) at 4-week intervals. Results: Although ILIT reduced rescue medication use and skin reactivity, and partially improved the quality of life at 4 months after treatment, all outcomes did not differ between the treatment and control groups. Intralymphatic injection was more painful than a venous puncture, and pain at the injection site was the most frequent local adverse event (12.8%): dyspnea and wheezing were the most common systemic adverse events (5.3%). Conclusions: ILIT with L-tyrosine-adsorbed allergen extracts does not exhibit profound therapeutic efficacy in allergic rhinitis and can provoke moderate-to-severe systemic reactions and cause pain at the injection site.

Trial registration: clinicaltrials.gov, NCT02665754: date of registration, 28 January 2016.
EO5-3  CCL4 expression is upregulated in the nasal polyps of eosinophilic chronic rhinosinusitis patients

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Background: Eosinophilic chronic rhinosinusitis (ECRS) is a subtype of chronic rhinosinusitis with nasal polyps in which the activated eosinophils are infiltrated. The interaction between eosinophils and airway epithelial cells may contribute to the pathogenesis of ECRS. However, little is known about the mechanisms of this interaction. In a recent study, we showed that CCL4 induces eosinophil recruitment into the inflamed tissues. Thus, we hypothesized that CCL4 might play a critical role in the interaction between eosinophils and airway epithelial cells in ECRS. Methods: We investigated the expression of CCL4 in nasal polyps from ECRS patients using immunofluorescence and quantitative polymerase chain reaction (qPCR). To elucidate the interaction between eosinophils and airway epithelial cells, peripheral blood eosinophils and BEAS-2B were co-cultured with CCL4 or anti-CCL4. CD69 expression, as a marker of activated eosinophils, and adhesion molecules were then evaluated by flow cytometry and qPCR, respectively. Results: The nasal polyps of ECRS patients showed significantly increased expression of CCL4 compared with non-ECRS tissues. In co-culture with CCL4, CD69 expression in eosinophils and adhesion molecules of BEAS-2B were both upregulated. Conclusion: CCL4 derived from airway epithelial cells is involved in the accumulation and activation of eosinophils in nasal polyps. These findings might provide a new therapeutic option for eosinophilic airway inflammation in conditions such as ECRS.

EO5-4  MT3 expression reflects intramucosal zinc level in nasal mucosa

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Introduction: Recently mucosal zinc depletion was found to be involved in several aspects of the pathophysiology of nasal polyps. No clinical useful marker, however, has been established to monitor mucosal zinc levels. Previous data showed serum zinc level did not reflect mucosal zinc level. Zinquin fluorescence has been used as a marker for mucosal zinc level, but only for research purposes. Metallothionein (MT3) is one subtype of metallothionein, zinc chelator genes. Metallothionein depend on intracellular zinc concentration for their expression. In this study, we examined whether MT3 expression reflects intracellular zinc levels in the nasal mucosa. Material and Methods: Primary nasal epithelial cells (HNECs) were cultivated in zinc-depleted medium for 48 hours. MT3 mRNA and protein expression were examined in the zinc-depleted HNECs by qPCR and immunofluorescence microscopy. MT3 expression and tissue zinc level were measured on a tissue microarray (TMA) slide consisting of nasal mucosa taken from 36 patients. Results: MT3 mRNA expression and fluorescence intensity were significantly decreased in zinc-depleted cells (mRNA: 0.45 ± 0.26 folds change, p<0.02, and protein: control 57.83 ± 10.83 vs. zinc-deplete cells 36.68 ± 4.51, p>0.01). A significant positive correlation was found between zinquin and MT3 fluorescence intensity within individual cells (r=0.59, p>0.001). TMA analysis showed a significant positive correlation between zinquin and MT3 fluorescence intensity in nasal mucosa (r=0.45, p=0.007). Conclusion: MT3 expression reflects intramucosal zinc level in the nasal mucosa, suggesting the zinc chelator gene could be useful as a clinical biomarker for monitoring intracellular zinc levels in the nasal mucosa.
EO5-5 Detection of Aspergillus fumigatus antigen in nasal polyp tissues from patients with CRSwNP

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Objective: To investigate the involvement of Aspergillus fumigatus antigen. Asp f 1 in the pathology of CRSwNP in terms of local type 2 immune response. Materials and Methods: We obtained 103 nasal polyp tissues from patients with CRSwNP collected during surgery. Nasal polyp tissues were homogenized and the supernatant were measured the levels of Asp f 1, Aspergillus specific IgE, total IgE, IgG, IgM, IgA, galectin-10 using ELISA assay kits. Tissue infiltrated eosinophils were also counted. Results: Eighteen samples (15.9%) were positive for the Asp f 1. The level of Asp f 1 had a significant positive correlation with that of Aspergillus specific IgE (r=0.89, p<0.0001). Samples were divided into two groups according to Asp f 1 level. The levels of Aspergillus specific IgE, IgG, or IgE in nasal polyp homogenates were significantly higher in high Asp f 1 group than low group respectively. There was no significant difference in IgA or IgM level among groups. Asp f 1 level had positive correlation with galectin-10 level (r=0.89, p<0.0001) but not with tissue eosinophil count (r=-0.27, p=0.28). Conclusion: This is the first report to show the existence of Aspergillus antigen in nasal polyps from patients with CRSwNP. Increased Asp f 1 level was correlated with Aspergillus specific IgE and galectin-10 in nasal polyps. The detection of Asp f 1 may explain the involvement of Aspergillus fumigatus in allergic reaction to Aspergillus fumigatus in CRSwNP.

EO5-6 Distinct subsets of innate lymphoid cells in nasal polyp tissue

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Background: Group 2 innate lymphoid cells (ILC2s) contribute to the pathogenesis of eosinophilic chronic rhinosinusitis with nasal polyps (CRSwNPs). However, the role of other subsets of ILCs and the differentiation of ILCs in CRSwNPs is not well understood. Objective: To characterize the ILC subsets and evaluate the differentiation of ILCs from ILC precursors (ILCPs) in eosinophilic and non-eosinophilic NP tissue. Methods: Relative frequency of ILC subsets and ILCPs were evaluated by flow cytometry in fresh sinonasal mucosa from patients with CRSwNPs and control subjects. Subsets were compared based on clinical variables and immunological features of the patients. Sorted ILCPs (Lin-CD127+CD117+CD45RA+IL 1R1+) were cultured with cytokines. Results: The frequency of ILC1s and IFN-γ-producing ILC1s increased in non-eosinophilic NPs, whereas that of ILC2s and IL-5-producing ILC2s increased in eosinophilic NPs, particularly in patients with comorbid asthma. The frequency of ILC1s and IFN-γ-producing ILC1s, and frequency of ILC2s and IL-5-producing ILC2s positively correlated with that of neutrophils and eosinophils, respectively. The frequency of IFN-γ-producing ILC1s positively correlated with levels of IFN-γ and IL-8, whereas that of IL-5-producing ILC2s positively correlated with levels of IL-5, CCL24, and total IgE. ILCPs were identified in NP tissue and differentiated into IFN-γ-producing or IL-5-producing ILCs responding to IL-12 and IL-18 or to IL-25 and IL-33, respectively. Conclusion: ILC1s and ILC2s contributed to inflammation (neutrophilic and eosinophilic, respectively) in CRSwNPs. In addition, ILCPs located in the sinus mucosa could differentiate into IFN-γ- or IL-5-producing cells in response to local cytokine stimuli.
EO6-1  EETs are structurally resistant to DNase and induce platelet adhesion

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\textbf{Background} : Eosinophil extracellular traps (EETs) have previously shown to be consist with bolder threads than NETs. Our recent study showed the presence of EETs in the thrombus. \textbf{Aim} : The aim of the current study is to assess the stability and platelet adhesion capacity of EETs in vitro. \textbf{Methods} : The characteristics of EETs (ultrastructure, spontaneous spreading, stability against DNase) were compared to neutrophil extracellular traps (NETs). Platelet adhesion was assessed by quantification of fluorescence-labeled platelets adhesion to EETosis cells. \textbf{Results} : EETs were associated with lytic cells and did not spontaneously spread, whereas NETs were more prone to expand, showing a larger DNA area. EETs showed bolder chromatin threads and were more aggregated than NETs, confirming the well-conserved nucleosome structures. In the presence of DNase, NETs showed faster dissolution than EETs, with approximate half-lives of 42 and 120 minutes, respectively. The platelets were significantly adhered to the EETs. \textbf{Conclusions} : EETs are structurally resistant to degradation by DNase and induce platelet adhesion. These properties might contribute to the pathogenesis (i.e. immunothrombosis) in eosinophil-associated disorders.

EO6-2  Multiomics analysis revealed regulatory phenotype of all-trans retinoic acid-stimulated human eosinophils

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\textbf{Background} : Eosinophils possess various types of inflammatory cellular functions. All-trans retinoic acid (ATRA) is recognized as a homeostatic molecule in the immune system with potent prosurviving effect on human eosinophils. However, the role of ATRA in the regulation of eosinophils remains fully uncovered. \textbf{Methods} : Multiomics analysis was performed using blood eosinophils of healthy subjects stimulated with ATRA for 3 days. Surface antigen expressions and apoptosis were evaluated using flowcytometry. \textbf{Results} : Transcriptomic analysis identified a total of 19,809 genes in both groups. In ATRA-stimulated eosinophils, the gene expression of PTGES, HIC1, and SPRY2 was increased, and that of IL5RA, IL17RA, CD44, SELL, NOD2, SELPLG, HLA-DRB1, CD69, CCL23, and IL1RL1 was decreased. Proteomic analysis identified and quantified a total of 7,101 proteins. ATRA-stimulated eosinophils expressed increased amounts of TGM2, CXCR4, HIC-1, ICAM1, SELL, and CCR3, and decreased amounts of NOD2, HLA-DRA, HLA-DRB1, IL3RA, CD69 and IL1RL1. ATRA inhibited IL-5-induced upregulatory effects on PSGL-1 and HLA-DR expressions on eosinophils. Also, ATRA downregulated the expression of IL3RA and IL1RL1 on cell surface and induced hyporesponsiveness to IL-3 and IL-33. \textbf{Conclusion} : These findings suggest that upregulation of ATRA-signaling in eosinophils might be of therapeutical potential in eosinophilic inflammatory diseases.
**EO6-3 miR103a-3p in extracellular vesicles from activated human mast cells enhances IL-5 production by group 2 innate lymphoid cells**

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**Background**: Mast cells (MCs) are key regulators of IgE-mediated allergic inflammation. Cell-derived extracellular vesicles (EVs) contain bioactive compounds such as microRNAs (miRNAs). EVs can transfer signals to recipient cells, thus employing a novel mechanism of cell-to-cell communication. However, it is unclear whether MC-derived EVs are involved in FcεRI-mediated allergic inflammation. **Objective**: We sought to investigate the effect of EVs derived from FcεRI-aggregated human MCs on the function of human group 2 innate lymphoid cells (ILC2s). Methods: Human cultured MCs were sensitized with and without IgE for 1 hour, and then incubated with anti-IgE antibody (Ab). IL-33 or medium alone for 24 hours. EVs in the MC supernatant were isolated using ExoQuick-TC. **Results**: Co-culture of ILC2s with EVs derived from the FcεRI-aggregated MCs significantly enhanced IL-5 production and sustained up-regulation of IL-5 mRNA expression in IL-33-stimulated ILC2s, but IL-13 production and IL-13 mRNA expression were unchanged. miR103a-3p expression was up-regulated in IL-33-stimulated ILC2s that were co-cultured with EVs derived from anti-IgE Ab-stimulated MCs. Transduction of an miR103a-3p mimic to ILC2s significantly enhanced IL-5 production by IL-33-stimulated ILC2s. miR103a-3p promoted demethylation of an arginine residue of GAT A3 by down-regulating protein arginine methyltransferase 5 (PRMT5) mRNA. Reduction of PRMT5 expression in ILC2s by using an siRNA technique resulted in up-regulation of IL-5 production by IL-33-stimulated ILC2s. Furthermore, miR103a-3p expression was significantly higher in EVs from sera of patients with atopic dermatitis than in non-atopic healthy control subjects' EVs. **Conclusion**: Eosinophilic allergic inflammation may be exacerbated due to ILC2 activation by MC-derived miR103a-3p.

**EO6-4 The role of the principal cysteine persulfide synthase in the innate immunity of type 2 airway inflammation**

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**Introduction**: Group 2 innate lymphoid cells (ILC2s) play pivotal roles in the innate immunity of asthma. Airborne allergens such as *Alternaria alternata* induce the release of IL-33 from mucosal barriers and rapidly activate ILC2s, leading to the production of type 2 cytokines and eosinophilic inflammation. Reactive sulfur species (RSS), including cysteine persulfide, are novel biological redox regulators, which also exist in human lungs. We recently found cysteinyl-tRNA synthetase (CARS2) acts as the principal cysteine persulfide synthase (CPERS). However, the role of CARS2/CPERS and RSS in the innate immunity of asthma is unknown. **Purpose**: To investigate the role of CARS2/CPERS in the innate immunity of type 2 airway inflammation. **Methods**: C57BL/6 background wild-type (CARS2+/-) and CARS2 heterozygous deficient (CARS2-/-) mice were used. We performed in vivo experiments using mouse models of type 2 airway inflammation induced by A. alternata and IL-33. **Results**: There was no difference in the amounts of IL-33 in BALF between A. alternata-treated CARS2+/- and CARS2-/- mice. However, the number of eosinophils and the amounts of type 2 cytokines in BALF were significantly increased in A. alternata-treated CARS2+/- mice. Intranasal administration of IL-33 significantly increased the number of eosinophils and the amounts of type 2 cytokines in CARS2+/- mice. Flow cytometric analysis revealed the number of lung ILC2s in IL-33-treated CARS2+/- mice was increased compared to those in CARS2-/- mice. **Conclusion**: Heterozygous deficiency of CARS2/CPERS in mice may aggravate fungal allergen and IL-33 induced type 2 airway inflammation. A deficiency of CARS2/CPERS could enhance IL-33-mediated ILC2 activation and proliferation.
EO6-5  Transcriptional repressor Gfi1 induces IL-7/IL-33-dependent innate-like Th2 responses by activating cellular metabolism

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**Background**: IL-33 receptor-positive (IL-33R+) Th2 cells produce IL-5/IL-13 in response to combination of IL-7 and IL-33 (innate-like Th2 response) and induce chronic type 2 airway inflammation. We previously reported that Gfi1 plays an important role in the differentiation of IL-5-producing Th2 cells and subsequent onset of Th2-type airway inflammation. However, the role of Gfi1 in the regulation of innate-like Th2 response remains to be elucidated. **Methods**: The role of Gfi1 in IL-33R+ Th2 cell differentiation and activation was determined by using CD4 T cells from T cell-specific Gfi1-deficient (Gfi1 KO) mice in vitro. **Results**: The expression of IL-33R and IL-5/IL-13 production in Th2 cells were decreased in Gfi1 KO Th2 cells. The metabolic analyses revealed that the glycolysis was low in Gfi1 KO Th2 cells compared to that of wild-type Th2 cells. IL-7-dependent glycolytic activation was also impaired in Gfi1 KO Th2 cells. Furthermore, the activation of Srebp1 and Srebp2, which regulate fatty acid synthesis and mevalonate pathway respectively, was decreased in Gfi1 KO Th2 cells. We found that IL-7/IL-33-dependent expression of IL-33R, and IL-5 and IL-13 production were suppressed by the pharmacological inhibition of glycolysis or Srebps activation. In addition, the activation of Srebps was suppressed by the inhibition of glycolysis. **Conclusion**: These results suggest that Gfi1 induces IL-5-producing Th2 cell differentiation and IL-7/IL-33-induced innate-like Th2 response by activating glycolysis and subsequent fatty acid synthesis and mevalonate pathway. Thus, the fatty acid and/or cholesterol synthesis pathways may be potential target for treating chronic type 2 inflammation.

EO6-6  Antigen specific Treg suppression can be mediated just by dendritic cells

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**Background and Purpose**: Initial studies have shown that mature DCs are specialized antigen-presenting cells for stimulating CD4+T cells including Foxp3+CD4+ T cells (Tregs). Here we discuss the suppressive properties of DCs that have been co-cultured with Treg cells. **Methods**: DCs pulsed with two kinds of antigen peptides, A: ova, or B: LCMV peptides were co-cultured for 18 hr with either A-specific Tregs or B-specific Tregs. DCs were separated from the co-culture and then stimulated in the second co-culture for 4 days with either of the two kinds of naive CD4 T cells specific to A or to B peptides. Suppressive functions of the separated DCs from the first co-cultures were determined by the proliferation of the naive CD4 T cells in the second co-cultures. **Results**: A-specific naive CD4 T cells were suppressed by DCs that had been co-cultured with A-specific Tregs, but not suppressed by DCs that had been co-cultured with B-specific Tregs in the first co-culture. B-specific naive CD4 T cells were not suppressed by DCs that had been co-cultured with A-specific Tregs, but suppressed by DCs that had been co-cultured with B-specific Tregs in the first co-culture. **Conclusion**: Even in the absence of Tregs, DCs were able to retain suppressive properties once gained from Foxp3+Tregs. Retained suppression was antigen specific as if the DCs had memorized which antigen specific Treg they had interacted with.
**EO7-1**  
Basophil-derived IL-4 induces the generation of M2 macrophages which dampen skin chronic allergic inflammation via the effecory clearance of dead cells

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**Background**: Basophils are shown to play essential roles in the induction of delayed-onset skin allergic inflammation (IgE-CAI) in mice. Moreover, they contribute to the termination of allergic inflammation via the induction of anti-inflammatory M2 macrophages. In IgE-CAI, M2 macrophages are differentiated from blood-circulating inflammatory monocytes through the action of basophil-derived IL-4. However, it remains elusive how M2 macrophages dampen allergic inflammation. **Methods**: WT and CCR2-deficient mice were sensitized with hapten-specific IgE and challenged with intradermal administration of hapten-conjugated OVA to induce IgE-CAI. Ear swelling was monitored for 5 days, and allergen-challenged ear skin was subjected to flow cytometric analysis, histological analysis, and single-cell RNA-seq analysis on day 3 or 5 post-challenge. **Results**: CCR2-deficient mice showed defective monocyte recruitment and hence impaired the generation of M2 macrophages, leading to prolonged ear swelling and massive neutrophil infiltration in IgE-CAI. Histological analysis of IgE-CAI skin lesion in CCR2-deficient mice revealed the presence of neutrophil-rich leukocyte clusters, which contained abundant necrotic cells. Necroptosis inhibitor ameliorated ear swelling and neutrophil infiltration in CCR2-deficient mice, suggesting that impaired clearance of dead cells triggers aggravated neutrophilic inflammation. Indeed, single-cell RNA-seq analysis revealed that M2 macrophages at late-phase (day 5) displayed upregulated gene expression associated with phagocytosis, including Gas6. In accordance with this, inflammatory monocytes stimulated in vitro with culture supernatants of activated basophils showed enhanced phagocytosis of dead cells, which was blocked by anti-IL-4 antibody. **Conclusion**: Basophil-derived IL-4 contribute to the resolution of allergic inflammation through the generation of M2 macrophages that possess high effector capacities.

**EO7-2**  
Antimicrobial peptide human β-defensin-3 ameliorates atopic dermatitis in a mouse model through autophagy activation

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**Background**: Antimicrobial peptide human β-defensin-3 (hBD-3) is known to improve the skin barrier function; however, its role in the development of atopic dermatitis (AD), which is characterized by impaired skin barrier, remains unclear. **Methods**: We established a skin-specific autophagy knockout AD mouse model (K14CreAtg7l/f AD mice). Following treatment with mouse β-defensin (mBD) -1,4, a mouse homolog of hBD-3, the dermatitis score, ear thickness, scratching behaviour and transepidermal water loss (TEWL) were evaluated. The expression of autophagy markers such as LC3 and p62 and tight junction (TJ)-related proteins in the skin was evaluated by Western blot and immunofluorescence, while the autophagic structure was assessed using electron microscopy. **Results**: We observed downregulation of LC3, enhancement of p62 deterioration of autophagic structure and decrease of TJ-related proteins in wild-type AD mice. Moreover, mBD-14 treatment resulted in recovery of autophagy status and TJ barrier in AD mice. Interestingly, although K14CreAtg7l/f mice showed significant loss of body weight and increased TEWL during the first month after birth, these mice recovered to the normal levels as those in the wild-type mice in the second month. Interestingly, K14CreAtg7l/f AD mice displayed increased ear thickness, severe dermatitis and scratching behaviour compared to the wild-type AD mice. Most importantly, mBD-14 failed to improve dermatitis in K14CreAtg7l/f AD mice, suggesting that activation of autophagy is required for the mBD-14-mediated improvement of AD symptoms. **Conclusion**: Collectively, our findings provide evidence that hBD-3 might be a therapeutic target for the treatment of AD, a skin disorder with dysfunctional autophagy and skin barrier.
EO7-3 Investigation of role of hemokinin-1 in sera of patients with chronic spontaneous urticaria

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**Background**: We previously reported upregulation of expression of Mas-related G protein-coupled receptor X2 (MRGPRX2) on mast cells (MCs) in the skin of patients with severe chronic spontaneous urticaria (CSU). Serum levels of substance P (SP) were reportedly significantly elevated, in correlation with the severity of CSU. Hemokinin-1 (HK-1) reportedly induced histamine release from LAD2 cells via MRGPRX2. **Objective**: We aimed to investigate HK-1’s role in CSU. **Methods**: The concentrations of HK-1 and SP were measured using ELISAs. Skin and synovium derived cultured MCs were generated by culturing dispersed skin and synovial cells, respectively, with stem cell factor. MRGPRX2 expression in the MCs was reduced using a lentiviral shRNA silencing technique. **Results**: Anti-SP Ab used in the SP ELISA showed 100% cross-reactivity to HK-1, but anti-HK-1 Ab showed 0% cross-reactivity to SP. The serum level of HK-1 was significantly lower in patients with CSU (n=151) than in non-atopic healthy control (NC) subjects (n=114). The EC50 of histamine release from MCs induced by HK-1 (5056 nM) was 12-fold higher than by SP (426 nM). Degranulation by HK-1 was significantly reduced in the MCs transduced with MRGPRX2 shRNA compared with the non-targeted shRNA-transduced MCs. Brief pretreatment of MCs with HK-1 at concentrations of 3.0 to 10 μM significantly reduced histamine release by 0.1 μM SP. However, brief incubation of MCs with HK-1 did not elicit rapid MRGPRX2 internalization. **Conclusion**: In NC subjects, high HK-1 concentrations may desensitize MRGPRX2-mediated MC activation, thereby preventing MC degranulation by SP.

EO7-4 Microbial analysis of the skin and feces using spontaneous atopic dermatitis mice

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**Background**: Many studies have been performed about microbiomes of atopic dermatitis. The relationship between microbiomes of and serum cytokines or dermatitis was still obscure. **Purpose**: The aim of this study was to examine the relationship between the microbiomes and serum cytokines or clinical status of dermatitis. **Materials and Methods**: Serum cytokines of NC/Tnd mice, spontaneous atopic dermatitis mice, were measured by Multiplex immunobead assay. The relationship between bacterial DNA in the skin and feces using 16s ribosomal RNA and cytokines were analyzed using MaAsLin2 analysis. **Results**: Clinical score of dermatitis, serum IL-4, IL-5, IL-13 showed positive association with some microbiomes of the skin, Staphylococales, Campilobacterales and negative association with Rhizobiales and Chloroplast. Some microbiome of the feces, Staphylococales, and Campilobacterota showed the similar positive association with clinical score. Bacteroidales of the feces showed positive association with serum IL-5 and IL-13. **Conclusion**: Some microbiomes of the skin and feces showed pro and con-inflammatory association with dermatitis.
EO7-5  Canonical TGF-β signaling via SMAD3 and SMAD4 suppresses Th1 and Th17 differentiation in psoriasis

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**Background**: Th1, Th17 and Th22 cells are the major pathogenic effector T cells in psoriasis. Transforming growth factor (TGF)-β plays pivotal roles in effector T cell differentiation, which is abundantly expressed and activated in the psoriatic lesions. Canonical TGF-β signaling pathway is mediated through TGF-β-specific receptor-regulated SMADs (SMAD2 and SMAD3) and the common SMAD, SMAD4. Signaling mechanisms how SMAD-mediated TGF-β signaling regulates pathogenic T cell responses in psoriasis remain largely unknown. **Objectives**: We sought to determine the mechanisms how canonical TGF-β signaling regulates the pathogenicity of effector T cells in psoriasis. **Methods**: We generated T cell-specific SMAD4-deleted mice using Cre-loxP system (Cd4Cre: Smad4f/f; ΔEx10-14). They were treated with 5% imiquimod (IMQ) cream for 6 days on ear and shaved back. The clinical course is assessed using the Psoriasis Activity and Severity Index (PASI) scale which ranks severity of erythema, induration and desquamation, histology and immunophenotyping. **Results**: IMQ-induced psoriasis was significantly exacerbated in Cd4Cre: Smad4f/f mice compared with the Cd4Cre: Smad4f/f littermates. Th1 and Th17 cells increased in the draining lymph nodes and skin lesions of IMQ-treated Cd4Cre: Smad4f/f mice. SMAD4 in complex with SMAD3 (SMAD3/4) suppressed Th1 differentiation by repressing the transcription of T-bet and IFN-γ. In contrast with linker phosphorylated SMAD2-induced Th17 differentiation, we found that SMAD3/4 rather suppressed Th17 differentiation by upregulating the negative regulators of STAT3 signaling such as SOCS3, SHP1 and SHP2. **Conclusions**: SMAD3/4 suppresses Th1 and Th17 cell differentiation in the pathogenesis of psoriasis, suggesting that enhancing SMAD3/4 signaling in the skin lesion could be a therapeutic strategy for psoriasis.

EO7-6  SMAD3/4-mediated TGF-β signaling suppresses allergic contact dermatitis by inhibiting cytotoxic T lymphocyte-induced Th1 apoptosis

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**Background**: Allergic contact dermatitis (ACD) is a cutaneous delayed-type hypersensitivity immune response. Effector T cells induced in the sensitization phase are activated to cause inflammation in the elicitation phase. TGF-β is crucial for effector T cell differentiation. TGF-β signaling is mediated through receptor-regulated-SMADs (SMAD2,3) and the common-SMAD, SMAD4. Signaling mechanisms how TGF-β regulates pathogenic T cell responses in ACD remain largely unknown. **Objectives**: We sought to determine how TGF-β signaling regulates the effector T cell responses in murine contact hypersensitivity (CHS) and ACD. **Methods**: We generated 1-fluoro-2,4-dinitrobenzene (DNFB) -induced CHS in T cell-specific SMAD4-deleted mice (Cd4Cre: Smad4f/f; ΔEx10-14). CHS was assessed by immunohistochemistry and immunophenotypes. Skin biopsy samples from ACD patients were evaluated by immunohistochemistry. **Results**: DNFB-induced CHS was exacerbated in Cd4Cre: Smad4f/f, Th1 was increased at the early elicitation phase, whereas Th2 and cytotoxic T lymphocytes (CTLs) were increased at the late elicitation phase in the draining lymph nodes and skin lesions of Cd4Cre: Smad4f/f. Cytotoxic molecules were upregulated in Smad4-deficient CTLs. Th1 was prone to apoptosis in Cd4Cre: Smad4f/f, resulting in Th2 predominance and CHS exacerbation, CD8+ cell depletion suppressed Th1 apoptosis, Th2 skewing and CHS, SMAD3/4 repressed the transcription of T-bet and IFN-γ in Th1 and Eomesodermin in CTLs. Consistent with CHS, infiltration of CTLs, Th1 apoptosis and Th2 predominance were observed in the skin lesions of ACD patients. **Conclusion**: Th1 in the early elicitation phase induced CTL differentiation, which induced Th1 apoptosis and Th2-skewed responses in the late elicitation phase of CHS. SMAD3/4-mediated TGF-β signaling suppresses CHS through chronological regulation of Th1 and CTL and CTL-induced Th1 apoptosis.