Oral presentation 4

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- **Chairs:** Jin Tack Kim (The Catholic Univ.), Sukil Chang (Sung Ae General Hosp.)

OP-34

SOX18 in Chronic asthma

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Background: The transcription factor SOX18 (endothelial-specific transcription factor SRY (sex determining region Y)-box18), involved in blood vessel development and endothelial barrier integrity as well as in wound healing processes. However, the exact role of SOX18 in bronchial asthma remains to be determined.
Objective: In this study we aimed to elucidate the role of SOX18 in the pathogenesis of bronchial asthma.
Methods: Using an established mouse model of ovalbumin-induced chronic allergic asthma, we investigated whether SOX18 is involved in the pathogenesis of bronchial asthma. Moreover, we also determined the levels of SOX18 levels in blood from asthmatic patients (stable and exacerbated state).
Results: The chronic allergic asthma mice showed that the transcript and protein of SOX18 in lung tissue were significantly increased after OVA challenge, and significant increases in mucous gland hyperplasia and collagen deposition. SOX18 protein in human microvascular endothelial cells line was increased following house dust mite treatment. Moreover, we found that SOX18 in blood from exacerbated asthmatics was increased compared with those from stable asthmatics. SOX18 not correlated with WBC count, eosinophil proportion, total attack number during follow up period, smoke amount, FVC and FEV1 % pred. SOX18 correlated with Total IgE ($r=0.256$, $p=0.005$) and claudin 5 ($r=0.185$, $p=0.047$).
Conclusion: These results indicate that SOX18 may be involved in airway remodeling of chronic asthma.

Key Words: SOX18, bronchial asthma, airway remodeling

OP-35

Human Rhinovirus Species Interact with Specific Airway Bacteria to Increase Asthma Symptoms

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Rationale: Several studies suggest that bacteria may play a role in virus-induced wheezing and asthma. Human rhinovirus (HRV) species differentially affect severity of respiratory illness and asthma, and this suggests the hypothesis that distinct patterns of HRV species may interact with bacterial pathogens contribute to the severity of respiratory illness.
Methods: 308 children, 165 with asthma, ages 4-12 years provided five consecutive weekly nasal samples. HRV species (A, B, C) were identified with RT-PCR and sequencing of the PCR products. Quantitative PCR for Streptococcus pneumoniae, Haemophilus influenzae and Moraxella catarrhalis was performed. Children scored cold and asthma symptom burdens based on a 4 point scoring system.
Results: The presence of S. pneumoniae alone was associated with increased cold symptoms compared to samples without bacteria (mean 5.0 vs 3.4, $p=0.004$). The combination of S. pneumoniae and HRV-C was associated with increased cold symptoms compared to virus alone (15.6 vs 5.7, $p=0.0003$); similar findings were noted for HRV-B(mean scores 6.6vs 3.8, $p=0.02$). In contrast, the presence of M. catarrhalis alone was associated with a small increase in asthma symptoms compared to samples without bacteria (mean 3.2 vs2.1, $p=0.05$). HRV-A infection together with M. catarrhalis significantly influenced asthma symptom burden compared to HRV-A alone (mean 6.3 vs 3.2, $p=0.008$).
Conclusions: These results provide evidence of specific interactions between HRV-C and S.pneumonia, and HRV-A and M.catarrhalis that are related to increased cold and asthma symptoms. Further investigation is needed to determine whether interventions targeting these bacteria could reduce the severity of HRV respiratory illnesses.

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Key Words: Rhinovirus, Bacteria, Asthma

OP-36

Assessment of lung and gut microbiota of Th2 asthma model and obesity-related asthma model

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Background: It has been suggested that certain compositional changes of gut microbiome contribute to the development of asthma. However, the effect of microbiota dysbiosis on lung-gut axis have not revealed directly.
Objective: We investigated to characterize the dysbiosis in lung and gut microbiota and their association using conventional murine asthma model with ovalbumin (OVA) and diet-induced obesity (DIO) model.
Methods: Bronchoalveolar lavage fluid and stool samples were obtained for microbiome analysis from 10-week old normal C57BL/6 mice; those treated with PBS, mice sensitized/challenged with OVA, and high-fat diet mice. After extracting the metagenome using Mobio's FastDNA™ SPIN Kit for Soil DNA, the V3-V4 of 16s rRNA was amplified using the Nextera XT Index Kit according to the 16S Metagenomic Sequencing Library Preparation Manual of Illumina.
Results: Overall, OVA model showed relatively decreased α-diversity of lung and gut microbiome. In the statistical assessment based on Shannon index, gut microbiome showed marginal difference of α-diversity (p-value = 0.047) while lung microbiome did not show significant difference among three groups. Similarly, β-diversity of gut microbiome also showed significant difference among three groups (p-value = 0.002), but not in lung microbiome (p = 0.068). The obesity group had a significantly higher level of phylum Verrucomicrobia and genus Akkermansia, Clostoridium, Eisenbergiella in gut compared to PBS group. Interestingly, obesity was also associated with enrichment of Akkermansia, and depletion of Bacteroides in lung. OVA sensitization/challenge induced dysbiosis of gut microbiome; genus Parabacteroides, Lactobacillus were more abundant while Roseburia was reduced.
Conclusion: Different compositional differences were observed in lung and gut according to murine asthma models. In OVA model, gut microbiome rather than lung microbiome were more remarkably altered despite primary inflammatory changes of airways.

Key Words: Microbiota, Asthma, Obesity

OP-37

Comparison of the therapeutic effects of dexamethasone on two different murine models of fungus-induced allergic lung inflammation

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Fungus-associated asthma endotype may present more severe disease with frequent exacerbations and eosinophilic inflammation. Among fungi implicated in severe asthma, most severe form of allergic fungal disease is known to be associated with Aspergillus fumigatus (Af). Oxidative stress is closely involved in the development of corticosteroid (CS) resistance in intractable pulmonary disorders and our previous study has shown that generation of mitochondrial reactive oxygen species (mtROS) is implicated in CS-resistant inflammation in the lung. In this study, we compared the therapeutic effects of dexamethasone on various features of fungus-induced allergic lung inflammation between Af- and Alternaria alternata (Aa)-induced form, and that investigated the molecular basis of the fungus-induced CS resistance specifically focusing on oxidative stress originates from mitochondria. Results showed that dexamethasone dramatically improved the Aa-induced increases of eosinophil-dominant inflammatory cell infiltration into the lung, airway hyper-responsiveness, and pulmonary Th2 cytokines (IL-4, IL-5, and IL-13f), while it failed to attenuate the Af-induced allergic lung inflammation. In addition, dexamethasone significantly reduced the Aa-induced increases in the fluorescence intensity of mtROS in bronchoalveolar lavage (BAL) cells, whereas it failed to reduce the Af-induced increase in mtROS generation in these cells. Furthermore, while dexamethasone significantly reduced the Aa-induced increases in the nuclear translocation of nuclear factor (NF)-κB p65, it failed to lower the Af-induced increases in NF-κB p65 in nuclear protein extracts of lung tissues. These findings suggest that the difference in the ability of dexamethasone to control inflammation in the two murine models of fungus-induced allergic lung inflammation is in part dependent on whether CS can regulate fungi-induced generation of mtROS and the related activation of NF-κB in the lung.

Key Words: Fungal asthma, Corticosteroid resistance, Mitochondrial ROS

OP-38

The increased expression of TRPV1 and its association with inflammatory cytokines in patients with asthma and rhinosinusitis

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Background: The airway epithelium is exposed to a range of irritants in an environment which can trigger airway inflammation. Transient receptor potential vanilloid 1 (TRPV1) is a Ca2+-permeable cation channel critical for detecting noxious stimuli by sensory neurons. Recently increasing evidence suggests TRPV1 is expressed in non-neuronal cells and involved in the pathophysiology of chronic airway disease.
Objectives: We examined the TRPV1 expression in nasal lavage fluid (NLF) and induced sputum in patients with chronic rhinosinusitis (CRS, n=33), asthma (n=87) and controls (n=20), respectively. The association TRPV1 expression and inflammatory cytokines as well as clinical parameters were analyzed.
Methods: The mRNA of TRPV1 and proinflammatory cytokines were determined by real-time quantitative PCR. ELISA of TRPV1 was performed using nasal lavage fluids and induced sputum of the patients with chronic rhinosinusitis, asthma, and normal controls, respectively.
Results: The expression of TRPV1 mRNA was significantly higher in the sputum of asthmatics compared with that of normal controls. The degree of TRPV1 expression correlated with the clinical phenotype such as airway obstruction and declined lung function with statistical significances. The TRPV1 expression correlated with IL-4, IL-13, IL-25, IL-33, TSLP, and TNFα. In particular, the strong correlation was noted between TRPV1 and Th2 and epithelial driven cytokines. TRPV1 expression was noted in the lavage fluids of the patients with CRS. The cytokines expression of nasal lavage fluids in CRS patients was the similar pattern with asthmatics.
Conclusions: TRPV1 mRNA expression was increased in both CRS and asthma patients. The degree of TPRV1 expression was associated with epithelial driven and Th2 cytokines in both upper and lower airways, suggesting that TRPV1 activation may be involved in Th2 inflammation. ** This research was supported by NFR-2017R1C1B5076565

Key Words: Transient receptor potential vanilloid 1 (TRPV1), Th2, airway inflammation

OP-39

Phosphoinositide 3-kinase-δ influences fungi-induced NLRP3 inflammasome assembly/activation in human bronchial epithelial cells

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Respiratory fungal exposure is known to be associated with severe allergic lung inflammation. Among various species, Aspergillus fumigatus (Af) is frequently implicated in severe asthma. NLRP3 inflammasome and phosphoinositide 3-kinase (PI3K)-δ in airway epithelium are involved in various inflammatory processes. Previously, we reported that fungus-induced activation of PI3K-δ in airway epithelium leads to the generation of mitochondrial reactive oxygen species (mtROS), thereby being involved in the corticosteroid resistance in Af-induced fungal allergy murine model. In this study, we performed in vitro experiments using normal human bronchial epithelial (NHBE) cells to investigate the role and molecular basis of PI3K-δ in the modulation of NLRP3 inflammasome assembly/activation against fungal exposure, especially focusing on mtROS. We also checked NLRP3 expression in lung tissues from allergic bronchopulmonary aspergillosis (ABPA) patients. Expression of NLRP3, caspase-1, and ASC and their cytoplasmic co-localization were significantly increased in Af-stimulated NHBE cells. Furthermore, levels of mature IL-1β were increased in cell lysates from Af-stimulated NHBE cells. Notably, Af-induced increases of NLRP3 inflammasome components and their co-localizations were dramatically lowered by a potent PI3K-δ inhibitor, IC87114, or PI3K-δ specific siRNA. This modulatory role of PI3K-δ was mediated through the regulation of mtROS generation in these cells. In human lung tissue specimens, pulmonary expression of NLRP3 in ABPA patients was increased compared to that in disease control (idiopathic pulmonary fibrosis) or healthy controls. These findings suggest that fungi-induced assembly/activation of NLRP3 inflammasome in airway epithelium may be modulated by PI3K-δ, which is mediated partly through the regulation of mtROS generation. Inhibition of PI3K-δ may have potential for treating fungi-induced severe allergic lung inflammation in humans.

Key Words: Fungal asthma, PI3K-δ, NLRP3 inflammasome

OP-40

Phosphorylation of PKR in airway epithelial cells contributes to asthmatic exacerbation through enhancing type 2 responses linked to ER stress

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Most asthma exacerbations are triggered by viral infections. The double-stranded RNA (dsRNA)-activated serine/threonine kinase R (PKR) is well characterized as an essential component of the innate antiviral response. However, to date, the role of PKR phosphorylation in bronchial epithelial cells is controversial. In this study, we investigated whether PKR phosphorylation in epithelial cells is involved in acute exacerbation induced by poly (I:C) and we aimed to define the interaction between PKR pathway and ER stress in bronchial epithelium leading to epithelial cell activation. We found that PKR inhibition using C-16 decreased severe asthmatic features; the number of airway inflammatory cells in bronchioalveolar lavage (BAL) fluids, airway hyperresponsiveness, and the expression of Th2 cytokines, IL-17, KC and IFN-γ in lung tissues. Interestingly, the expression of PKR, ER stress markers including p-eIF2α, and epithelial derived cytokines IL-25,IL-33, TSLP was increased in lung tissues from mice sensitized with ovalbumin (OVA) and lipopolysaccharide (LPS) and challenged with OVA (OVALPS-OVA mice). Moreover, the expression of PKR and ER stress markers in LPS-stimulated normal human bronchial epithelial (NHBE) cells. These findings were attenuated significantly by the treatment with c-16. In addition, the administration of poly (I:C) aggravated the all measurements compared to those in OVALPS-OVA mice. These exacerbated asthmatic features were suppressed by the administration of C-16. This study indicates that PKR phosphorylation plays an important role in acute asthma exacerbation, highlighting the potential of PKR inhibitor as a potent controller of bronchial epithelial cell activation in severe asthma and its acute exacerbation.

Key Words: PKR, Epithelial cells, Asthma exacerbation

OP-41

Pathophysiological function of eosinophil extracellular traps

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Background: Activated eosinophils release extracellular traps (DNA and cytotoxic granule complexes) through a unique cell death. Although eosinophil extracellular traps (EETs) are involved in allergic diseases, the role of EETs in the development of asthma is still unclear.
Objectives: To investigate the role of EETs in airway mechanisms of asthma.
Methods: EETs were isolated from peripheral blood eosinophils of patients with non-severe asthma. The effect of EETs on tissue damage and immune responses in wild-type BALB/c mice was investigated.
Results: Mice treated with EETs generated lung dysfunction. EETs significantly induced epithelium-derived cytokine production such as IL-33 and TSLP, leading to enhanced Th2 response.
Conclusions: EETs are involved in the pathogenesis of asthma through stimulating epithelial cells which further exacerbate immune responses in the lungs. Regulation of EETs-mediated inflammation may be a novel therapeutic approach for eosinophilic asthma.

Key Words: Asthma, eosinophil extracellular traps, epithelium

OP-42

Adjuvant Effect of Silver Nanoparticles in Allergic Inflammation

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Background: The effects of silver nanoparticles (SNPs) on allergic airway inflammations have been remained as a controversy. In this study, we aimed to investigate the size-specific effect of SNPs on inhalation toxicity and allergic airway inflammation in asthma development and exacerbation using mouse model.
Methods: Three experiments were conducted using 6 week-old BALB/c female mice. First, as a model for identifying respiratory toxicity, 10 nm and 100 nm sized SNPs were instilled intra-nasally for 4 weeks (twice a week). Second, SNPs (10 nm or 100 nm) and ovalbumin were instilled intra-nasally together for 4 weeks. Third, two sized SNPs were administered intra-nasally to the systemically induced OVA-asthma model (intra-peritoneal sensitization with OVA). Then, body weight, airway hyper-responsiveness, cytological, histologic and immunologic changes were measured.
Results: There was no difference in body weight between SNP treated and sham mice. In second experimental model, mice treated with OVA and 100 nm sized SNPs showed marked neutrophilic and eosinophilic airway inflammation compared with OVA administered mice. In this group, both Th2 and Th1 cells were activated and cytokines (IL-33, TSLP, IL-1β, and INF-γ) was increased in lung tissue. However, airway-hyper-responsiveness was not induced in all groups. In third experiment model, eosinophilic and neutrophilic airway inflammation was augmented when 100 nm sized SNPs administered to the systemically induced OVA-asthma model. In this group, both Th2 and Th1 cell activities and inflammatory cytokines (IL-33, TSLP, and INF-γ) were increased in lung tissue.
Conclusions: SNPs can induce pro-inflammatory cytokines and have adjuvant effect on allergen sensitization. The effects are more prominent in 100 nm SNPs rather than 10 nm.

Key Words: Asthma, Silver Nanoparticle, Toxicity

OP-43

Effect of phthalate exposure to severity of atopic dermatitis in children

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Background: Previous studies have reported the association of phthalates, one of the indoor pollutants everywhere, with allergic diseases using date from questionnaires. The aim of this study was to investigate the direct and significant effect of phthalate exposure in children on the severity of atopic dermatitis through objective tests.
Method: As part of the 2017 Seongnam Atopy Center Study, 448 children aged 10-12 years were selected for study, except for children who did not complete the questionnaire or did not undergo urinary phthalate sampling or eczema area and severity index (EASI) scoring. In addition, we measure transepidermal water loss (TEWL) and allergic sensitization in serum. The questionnaire was used to measure the presence of atopic dermatitis, combined allergic diseases, family history, and symptom score as visual analog scale (VAS).
Results: There were 84 children (18.8%) clinically diagnosed as AD and 364 (81.3%) as control group. Compared with the two groups, the parents\\\' AD family history and combined allergic disease were significantly higher in AD group. The concentrations of phthalates were not significantly different between the two groups, but the median values of MCPP and MBZP were higher in the atopic group. However, there was a significant positive correlation between VAS in AD patients and MECPP and MEHHP among phthalates (P = 0.023 and 0.033, respectively) and the EASI score was also positively correlated with MECPP, MEHHP, and MEOHP (P = 0.030, 0.041 and 0.039, respectively). TEWL showed a positive correlation with almost all phthalates. In the analysis adjusted for multiple factors, both VAS and EASI score showed significant positive association with previous phthalate metabolites in urine.
Conclusion: Exposure to phthalates is a risk factor that affects the severity of atopic dermatitis.

Key Words: atopic dermatitis, phthalate, EASI score

OP-44

Innate Type 2 Response to Aspergillus Fumigatus in a Murine Model of Atopic Dermatitis-Like Skin Inflammation

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Atopic dermatitis (AD) is a chronic and relapsing inflammatory skin disease mediated by Th2 cells in acute phase. Group 2 innate lymphoid cells (ILCs) play a role in the initiation of the Th2 response. Although mold exposure is associated with the development of AD, studies on the underlying mechanisms are lacking. This study investigated whether group 2 ILCs are involved in skin inflammation of AD-like skin induced by Aspergillus fumigatus (Af). When Af extract was applied to the dorsal skin of BALB/c and Rag1-/- mice five times per week repeatedly with 2 week intervals, Clinical scores and TEWL were higher in Af-treated group than in control group. Histologic findings showed thickening of the epidermal and dermal as well as eosinophil and mast cell infiltration into the skin of Af-treated group. The levels of IL-13 in the supernatants of cultures of skin-draining LNs stimulated with Af. Populations of Lin-CD25+IL-33R+ group 2 ILCs in the skin were significantly higher in the Af-treated group than those in control group. In addition, expression levels of IL-33 mRNA were significantly higher in the lesional skin of the Af-treated group. In the Rag1-/- mice lacking mature lymphocytes, AD-like skin lesions were still induced by Af and ILCs depletion using anti-CD90.2 mAb reduced Af-induced inflammatory response. This study suggests that group 2 ILCs may have a role in a murine model of Af-induced AD-like skin lesions.

Key Words: Atopice dermatitis, Innate Lymphoid cells