582 Endotyping of Allergic Rhinitis Clusters in Korean Children through Nasal Cytokines/ Chemokines Analysis

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RATIONALE: Allergic rhinitis (AR) is the most common allergic disease and the prevalence of AR is increasing. As AR is a heterogenous disease, many studies analyzed various biomarkers to identify the endotype. However, studies in Korean children are rare, this study tried to analyze the clusters of AR and the characteristics of nasal cytokines/chemokines in each cluster.

METHODS: Children with AR were enrolled in the Korean Allergic Rhinitis Cohort study (KoARCo) from October 2019 to December 2020. Questionnaires, blood tests, and nasal fluids were collected and analyzed. The variables for cluster analysis were age, gender, BMI, sensitization, onset age, total IgE, eosinophil (%), symptom score, family history, and past history. Among clusters, nasal cytokine/chemokine levels were compared.

RESULTS: A total of 122 patients were clustered into three: female-dominant cluster (cluster 1), male-dominant cluster (cluster 2), and total IgE elevation cluster (cluster 3). In comparison of clusters, CCL4 ($p=0.0107$) and TNF-α ($p<0.0001$) were significantly different. In Bonferroni correction, there was a significant difference in CCL4 between cluster 1 and cluster 3 ($p=0.0153$). In addition, significant differences in TNF-α were observed between cluster 1 and cluster 2 ($p=0.0021$), and between cluster 1 and cluster 3 ($p<0.0001$).

CONCLUSIONS: In this study, children with AR were classified into three clusters and nasal CCL4 and TNF-α were significantly different among clusters. This means that not only a Th2 allergic reaction but also a heterogenous inflammatory mechanism exist in pediatric AR. Further studies will be needed to identify the relevance in cluster and endotype analysis of AR in children.

583 Mechanisms of airway disease development following inhalation exposure to indoor fungal contaminants Stachybotrys chartarum and Aspergillus versicolor

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RATIONALE: Fungi are found ubiquitously in the environment and chronic repeated exposures have been associated with pulmonary and cognitive effects in humans. Previous studies have shown that two prominent indoor fungal contaminants, *Stachybotrys chartarum* and *Aspergillus versicolor*, are associated with allergic airway disease, airway remodeling, and Th2-mediated immunological effects. To understand the mechanisms of disease development, genetically modified murine models are an invaluable tool.

METHODS: To examine the immunotoxicological response to each fungal species, a nose-only acoustical generation system was used to deliver dry aerosolized spores to C57BL/6J (WT) and IL-13 and RAG-2 knockout mice, twice weekly for 4 or 13 weeks. Twenty-four-hours after final exposure, the immune response was examined via flow cytometry, antibody quantification, and histology.

RESULTS: Wild-type mice had a strong inflammatory immune response to repeated fungal exposure by 13 weeks, which was significantly diminished in knockout mice. The response to *S. chartarum* resulted in increased T cells and eosinophils, whereas *A. versicolor* exposure in WT mice was characterized by an increase of B cells in the airways and total serum IgE antibodies. Mice lacking IL-13 or RAG-2 demonstrated a significant reduction of each, indicating that the cytokine IL-13 is necessary for the adaptive immune response to fungal exposure, as are mature B and T cells, as indicated in RAG-2-/- mice.

CONCLUSIONS: This study identifies the mechanistic role of IL-13 during fungal exposure. Understanding the interplay of the immune system and indoor fungal contaminants is vital for identifying potential biomarkers and benchmarks for assessment of disease development.

584 Allosteric inhibition of CXCR1/2 by an orally bioavailable chemokine receptor inhibitor attenuates allergic lung inflammation in mice

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RATIONALE: CXCR1/2 chemokine receptors are expressed on the surface of inflammatory cells. We previously reported that parenterally-administered reparixin, a noncompetitive allosteric inhibitor of CXCR1 and CXCR2, inhibits allergic airway inflammation. Ladarixin is a second generation orally bioavailable dual CXCR1/2 inhibitor that binds an allosteric pocket of the trans-membrane region of both receptors with a 100-fold higher affinity than first generation CXCR1/2 inhibitors. Here we investigated whether oral ladarixin also inhibits allergic airway inflammation.

METHODS: Naïve C57BL/6 mice were sensitized with multiple intranasal doses of cat dander extract (CDE), then challenged with intranasal phosphate buffered saline (PBS) or CDE with or without administration of oral ladarixin via gavage. Allergic lung inflammation was assessed.

RESULTS: CDE-challenge in sensitized mice induced allergic lung inflammation characterized by an increase in the number of BAL and lung neutrophils, eosinophils, RorγT positive cells, macrophages and dendritic cells that produced Il23, upregulation of mRNA expression of Cxcr2, Il4, Il5, IL13, IL6, IL1β, Tgfβ1, IL17, and IL23 in BAL cells, and an increase in the levels of serum total IgE and CDE specific IgE. Oral administration of ladarixin inhibited these parameters of Th2/Th17-associated allergic lung inflammation.

CONCLUSIONS: These observations indicate that allosteric inhibition of CXCR1/2 by an orally bioavailable chemokine receptor-inhibitor is sufficient to attenuate Th2/Th17-associated allergic lung inflammation. Oral ladarixin maybe an effective therapeutic strategy in asthma.