EGCG downregulates mucin gene expression through the MAPK signaling pathway in asthma.

119 Enhancer of Zeste Homolog 2: A Pivotal Role in Pulmonary Artery Smooth Muscle Cell Proliferation
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Background: Pulmonary arterial hypertension (PAH) is a progressive and a devastating disease characterized by excessive proliferation of pulmonary artery smooth muscle cells (PASMCs). The pathogenesis of PAH is not fully understood and treatment options are limited. Studies suggest that PAH and cancers share apoptosis resistant state featuring excessive cell proliferation. Proliferation of cancer cells is mediated by increased expression of Enhancer of Zeste Homolog 2 (EZH2), a mammalian histone methyltransferase that contributes to the epigenetic silencing of target genes. However, the role of EZH2 in PAH has not been studied. In this study, we hypothesized that EZH2 could play a role in PASMC’s proliferation.

Methods: In the present study the effects of EZH2 overexpression on human PASMC’s proliferation were tested. PASMCs were transfected with wild type EZH2 cDNA or GFP using the Lonza 4D nucleofector system. After transfection, cells were incubated for 48 hours at 37°C. PASMCs proliferation and cell cycle analysis were performed by flow cytometry; PASMCs apoptosis was determined using annexin V staining, and cell migration was tested by the wound healing assay. Expression levels of EZH2 were confirmed by real time PCR.

Results: The overexpression of EZH2 in PASMCs enhances proliferation, migration, and decreases the rate of apoptosis when compared to GFP transfected cells. There was a 3.5-fold increase in proliferation and a 1.5-fold increase in the percentage of cells in the G2/M phase in the EZH2 transfected cells while there was a significant decrease in the rate of apoptosis in the PASMCs.

Conclusions: These findings suggest that EZH2 plays a role in the migration and proliferation of PASMCs. It also suggest that EZH2 could play a role in PAH development and serve as a potential target for new therapies for PAH.

120 The Features of Airway Remodeling Are More Severe in Female Mice
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Background: Epidemiological studies have already shown that females are dominant in terms of the sex ratio of adult asthma prevalence and severe asthma. It has also been reported that female mice are more susceptible to the development of allergic airway inflammation and airway hyperresponsiveness (AHR) than males. However, there have been few reports of studies on sex difference in the pathogenesis of severe asthma, especially airway remodeling in an animal model. In this study, we investigated sex difference in formation of airway remodeling using a long-term antigen challenged asthma model.

Methods: Following ovalbumin (OVA)/alum intraperitoneal injection, male or female mice (BALB/c) were challenged with aerosolized 1% OVA on 3 days/week for 5 weeks, and we investigated the sex difference in AHR, airway inflammation, as well as airway remodeling.

Results: In OVA-sensitized and -challenged (OVA/OVA) female mice, AHR, the number of eosinophils and lymphocytes, as well as Th2 cytokines and growth factors in BAL fluid were increased compared with OVA/OVA male mice. On the other hand, there is no significant difference in the level of eotaxin in BAL fluid. The histological features of airway remodeling, including goblet cell hyperplasia, subepithelial fibrosis and myofibroblast hyperpertrophy, were also increased in OVA/OVA female mice. Moreover, serum total and OVA-specific IgE were significantly elevated in OVA/OVA female mice.

Conclusions: These results indicate that female mice are dominant in terms of forming airway remodeling as compared with male mice. The involvement of sex difference for sensitization and growth factor release in lung tissue based on inflammatory cell infiltration is indicated for the mechanism of sex difference of airway remodeling.

121 Ovalbumin-induced Bronchial Asthma is Compromised in Apoptosis Signal-Regulating Kinase-Deficient Mice
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Background: Apoptosis signal-regulating kinase 1 (ASK1), a member of mitogen–activated protein (MAP) kinase kinase kinases (MAP3Ks) protein family, plays a crucial role in the induction of apoptosis and inflammation in some cell types. Allergic asthma is a chronic inflammatory airway disease characterized by airway hyperresponsiveness (AHR), inflammatory cell infiltration, and airway remodeling. In the present study, we examined whether ASK1 is involved in the induction of bronchial asthma using a mouse model of airway inflammation.

Methods: ASK1-deficient (ASK1−/−) and wild-type (WT) control mice were sensitized with ovalbumin (OVA) in saline intraperitoneally on consecutive 7 days. Eighteen days later, mice received intranasal administration of OVA aerosol and were assayed for AHR, cytokine production, cell proliferation, antibody (Ab) production, and lung tissue histopathology at 24 hours after the last serial OVA administration. Levels of Ab and cytokines were determined by enzyme-linked immunosorbent assay (ELISA).

Results: Control WT mice showed inflammatory infiltrates in airways in response to OVA to a greater extent than ASK1−/− mice. The number of cells, especially eosinophils accumulating in airways, was reduced in ASK1−/− mice relative to control mice. OVA-induced AHR is also compromised in ASK1−/− mice. Anti-OVA IgE Ab production in ASK1−/− mice was substantially reduced, although levels of other isotypes were comparable to those in control mice. Levels of some Th2 cytokines (IL-5 and IL-13) and pro-inflammatory cytokine TNF-a in BAL fluid from ASK1−/− mice were substantially diminished relative to control, although a comparable level of a typical Th2 cytokine IL-4 and anti-inflammatory cytokine IL-10 was produced. Although the BAL fluid TNF-a levels from ASK1−/− mice were severely diminished, lymph node cells from ASK1−/− mice produced comparable levels of TNF-a to WT in vitro. Intranasal administration of recombinant TNF-a caused a comparable increase in AHR between ASK1−/− and WT mice, whereas the TNF-a–induced accumulation of inflammatory cells was severely reduced in ASK1−/− mice.

Conclusions: ASK1 appears to be involved in the induction of OVA-induced bronchial asthma, probably through cytokine production such as TNF-a and IL-13. Moreover, TNF-a sensitivity in response to OVA is also regulated by ASK1.

122 Role of the CC—Chemokine Receptor CCR9 in the Regulation of Inflammatory Process During Allergic Airway Inflammation
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T cell responses to Timothy grass allergens are directed to William Kwok, PhD Tetramer Guided Epitope Mapping was involved in asthma. It is not known whether CCR9 may have a regulatory role of the development of this disease. Our aim was to analyze the expression of CCR9 in a murine model of allergic airway inflammation (WT) and compared to CCR9 deficient (KO) mice. Methods: Four groups of 6 to 8 weeks female CCR9-deficient mice were sensitized by intraperitoneal injections of 10 micrograms of ovalbumin (OVA) in alum (ALOH3) diluted in PBS, on days 1 and 8 of the established sensitization protocol. Aerosolised OVA was administered (1% in PBS) on days 15, 20 and 34. 24 hours after last OVA exposure, mice were sacrificed and bronchoalveolar lavage (BAL) fluid and cells were obtained. Total and differential cell numbers were obtained and characterized cell subpopulations by FACs analysis. Cytokine/chemokine levels were quantified by ELISA and qRT-PCR respectively.

Results: Total cell numbers in BAL were no significantly different between WT and KO mice. Interestingly, reduction in the numbers of eosinophils was observed in CCR9 KO mice compared to WT mice. Histological analysis of lung tissue demonstrated a reduction in the granulocytic population (eosinophils) in CCR9 KO mice. Analysis of cell subpopulations by FACs demonstrated that CD4+ lymphocytes were significantly reduced but CD8+ and CD19+ lymphocytes numbers were not different between WT and CCR9-deficient mice. A population of CCR9+ Gr1+ was altered in KO mice and it correlated with cytological analysis. Furthermore, histological analysis demonstrated alteration in mucous production in allergic airway in CCR9 deficient mice, accompanied with a no-significant reduction of OVA-specific anti-IgE antibodies in serum at the time of analysis.

Conclusions: Altogether, these results suggest that CCR9 may be involved in recruitment of granulocytic cell subpopulation into the allergic airways and have an impact in the regulation of the chronic inflammatory process.

MECHANISMS OF ASTHMA AND ALLERGIC INFLAMMATION

124 Heterogeneity of Allergen Epitope-specific CD4+ T Cells Responses: Steps Toward Optimal Composition for Peptide-based Immunotherapy

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Background: Peptide-based allergen immunotherapy is a promising alternative to conventional allergy vaccine. However, the optimal composition of such vaccines, in terms of the choice of the appropriate peptides, has remained unclear. Knowledge of the epitope-specific T cell responses to allergens can give important information on the pathogenesis and regulation of allergic inflammation. In this study we sought to identify candidate allergen-epitopes that can be used to improve peptide-based allergen immunotherapy.

Methods: Tetramer Guided Epitope Mapping was first used to identify CD4+ T cell epitopes for group 1 and group 5 timothy grass pollen allergens. MHC class II tetramer technology was then used in an ex vivo approach to assess the grass pollen-specific CD4+ T cell responses in allergic and non-allergic individuals. The frequency, surface marker phenotype and cytokine profile of these cells were directly analysed by flow cytometry.

Results: CD4+ T cell responses to Timothy grass allergens are directed to a broad range of epitopes characterized by defined immunodominance hierarchy patterns. We observed heterogeneity of phenotype within the allergen-specific CD4+ T cells that depends on the epitope for which the cells are specific. T cell epitopes associated with production of IL-10 or IFN-γ are recognized at low frequencies in both allergic and healthy individuals. In contrast, allergy-associated epitopes are only recognized in allergic individuals by high frequency, terminally differentiated allergen-specific CD4+ T cells, which are susceptible to deletion by repeated stimulation with high doses of antigen. Allergen-specific immunotherapy caused significant changes in the epitope hierarchy of the grass pollen allergen-specific memory CD4 T cell pool.

Conclusions: The ability to evaluate epitope-specific T cell responses to allergens can give important information on the pathogenesis and regulation of allergic inflammation and could be of great use in designing peptide-based allergy vaccination strategies. Some epitopes may play a prominent role in