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62 Keratinocytic Thymic Stromal Lymphopoietin Plays an Important Role in Epicutaneous Sensitization and the Atopic March
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Background: Atopic dermatitis (AD or eczema) often precedes the development of asthma and allergic rhinitis in atopic subjects, a phenomenon known as the atopic march. An important role of epicutaneous (e.c.) sensitization has been recognized in the atopic march; however, the factors involved in e.c. sensitization remain poorly understood. Our previous studies using mouse models have shown that induced overexpression of Thymic Stromal Lymphopoietin (TSLP) in keratinocytes not only triggers an AD [Li, M. et al. Proc Natl Acad Sci U S A. 2006;103:11736–11741] but also aggravates experimental asthma induced by systemic sensitization and airway challenge of ovalbumin (OVA) [Zhang Z, et al. Proc Natl Acad Sci U S A. 2009;106:1536–1541], suggesting that TSLP represents an important factor linking AD to asthma. However, whether keratinocytic TSLP is essentially required for developing e.c. sensitization and triggering the atopic march remained to be determined.

Methods: We develop a mouse model in which e.c. sensitization of OVA through tape-striped skin is followed by intranasal challenge to induce an allergic asthma. TSLP−/− mice (in which TSLP is selectively ablated in epidermal keratinocytes at adult stage) or TSLP−/−/Vdr−/− mice (in which keratinocytic TSLP overexpression is induced by topical application of MC903, a low-calcemic vitamin D analog) are subjected to this mouse model.

Results: Upon OVA e.c. treatment, TSLP−/−/Vdr−/− mice develop a defective allergen sensitization evidenced by decreased production of OVA-specific IgE and IgG1 and a reduction of the secretion of Th2 and Th17 (but not Th1) cytokines by in vitro OVA stimulated splenocytes. TSLP−/−/Vdr−/− mice also exhibit a decreased OVA-induced skin inflammation. Finally, upon intranasal challenge, TSLP−/−/Vdr−/− mice develop a less severe asthma inflammatory and a reduced airway hyperresponsiveness. In contrast, overproduction of keratinocytic TSLP boosts the e.c. sensitization and triggers an aggravated asthma.

Conclusions: Our results demonstrate an important role of keratinocytic TSLP in developing epicutaneous sensitization, generating allergic skin inflammation and triggering the atopic march. Thus, blocking the expression or activity of keratinocytic TSLP could be helpful to limit epicutaneous sensitization and prevent the atopic march.

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63 Pivotal Role of Intestinal Interleukin-17-Producing Gammadeltat Cells in the Food Allergy
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Background: Food allergy is a serious health problem, which affect 5% of children in westernized countries and evoke life-threatening hypersensitivity, termed anaphylaxis shock. Type 2 helper T cell (Th2) response and immunoglobulin E (IgE) has been implicated in the progression of food allergy, but the roles of specific lymphocyte subpopulations and cytokines remain to be clarified.

Methods: The mucosal adjuvant, cholera toxin (CT) and ovalbumin (OVA) were co-administered orally into mice, while OVA alone could induce oral tolerance. To evaluate the contribution of various cytokines, we used interleukin-17 (IL-17) or IL-23 knockout (KO) and wild type (WT) mice as control.

Results: Here we demonstrate that gamma delta T cells in the intestinal mucosa, as well as the cytokines interleukin-23 (IL-23) and IL-17, have pivotal roles to suppress the induction of serum OVA specific immunoglobulins and anaphylaxis in the food allergy model. The expression of IL-23, which was derived mostly from mucosal macrophages, and IL-17 levels were elevated after CT and OVA sensitization, and this induction of IL-17 was dependent on IL-23.

Conclusions: These data, together with analysis of mice genetically disrupted for IL-17 and IL-23, suggest that IL-23 suppress the food allergy, whereas IL-17 has an important role in the anaphylaxis shock. Moreover, depletion of gamma delta T cells exacerbates the food allergy. We propose that T lymphocytes, including gamma delta T cells, could be a therapeutic target for mitigating the allergic response that evokes the anaphylaxis shock.

64 Effect of Formoterol on Eosinophil Trans-Basement Migration Induced by Interleukin-8-Stimulated Neutrophils
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Background: Neutrophils are often increased in the airways of either chronic severe disease or acute exacerbation of asthma. Neutrophils migrated in response to interleukin-8 (IL-8) may lead eosinophils to accumulate in the airways of asthma and possibly aggravate this disease. In this study, we investigated whether formoterol modify the trans-basement membrane migration (TBM) of eosinophils stimulated with neutrophils and IL-8.

Methods: Neutrophils and eosinophils were isolated from peripheral blood obtained from healthy donors. The TBM of eosinophils was examined using a modified Boyden’s chamber technique. Neutrophils were preincubated with or without formoterol (0.1 μM) at 37°C for 30 minutes. Eosinophils were added to the upper compartment of a chamber with a Matrigel-coated trans-well insert. Medium that contained preincubated neutrophils and IL-8 was added to the lower compartment of the chamber. After 90 minute incubation, migrated eosinophils in the lower chamber were calculated using eosinophil peroxidase assays.

Results: A combination of neutrophils and IL-8 significantly induced TBM of eosinophils. Formoterol by itself did not modify the TBM of eosinophils. However, formoterol significantly attenuated TBM of eosinophils stimulated with neutrophils and IL-8.

Conclusions: These results suggest that formoterol may act as a therapeutic agent on enhanced eosinophil inflammation in acute exacerbation or persistent severe disease of asthma. This effect of formoterol likely involves inhibition of activation of neutrophils.

DRUG ALLERGY

65 Characteristics of Liver Injury in Drug-induced Systemic Hypersensitivity Reactions
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Background: Liver is the second most commonly involved organ in drug-induced systemic hypersensitivity reactions (DiSH). Although liver function is very important indicator in the course of DiSH, there have been few studies about the characteristics of the liver injury. In present study, we investigated clinical characteristics of DiSH associated with liver injury (liver-DiSH).

Methods: We retrospectively reviewed medical records of 38 hospitalized patients who developed liver-DiSH (AST or ALT ≥80 IU/L) from January 2008 to February 2011 in a tertiary referral hospital. We analyzed culprit drugs, the type and degree of liver injury, and the effect of systemic corticosteroids. Fisher’s exact test and Chi-square test and Mann-Whitney test were used for statistical analysis.

Results: Thirty-eight patients of liver-DiSH were enrolled, whose clinical phenotypes were Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) (n = 9, 24%), drug reaction with eosinophilia and systemic symptoms (DRESS) (n = 23, 60%), maculopapular rash (MP rash) (n = 5, 13%) and acute generalized exanthematous pustulosis (AGEP) (n = 1, 3%). Antibiotics (n = 16/38, 41%) was the most common cause of liver-DiSH. Culprit agents of liver-DiSH were allopurinol (n = 3/9, 33%) in SJS/TEN and antibiotics (n = 13/23, 57%) in DRESS. Mortality tended to be higher in SJS/TEN than in DiRESS (22% (2/9) vs 17% (4/23), P = 0.846). Degree of liver injury was statistically more severe in DiRESS than in SJS/TEN (mean peak ALT [423 IU/L vs 144 IU/L, P = 0.062], mean peak AST [428 IU/L vs 156 IU/L, P = 0.013], mean peak ALP (alkaline phosphatase) [252 IU/L vs 85 IU/L, P = 0.002], mean total bilirubin [7.7 mg/dL vs 1.3 mg/dL, P = 0.064], and time required for AST/ALT to drop below 80 IU/L [15.8 days vs 4.1 days, P = 0.049]). Seventy-six percent (29/38) of patients were treated with systemic corticosteroid. The use of corticosteroid did not significantly affect both recovery of liver injury and mortality.

Conclusions: Our results suggest that liver-DiSH has distinguished clinical characteristics according to the disease phenotypes. Further studies are needed to evaluate the role of systemic corticosteroid in liver injury in DiSH.

### 66 Food & Drug Allergy: Late-Breakers

#### 67 Influence of Transglutaminase and the Reducing Agent Glutathione in the Gastric Digestion and Immunogenicity of Beta-Lactoglobulin

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**Background:** Among the milk whey proteins, β-lactoglobulin (β-Lg) is the main allergen. In native state, β-Lg is resistant to pepsin, which is considered an indicator of its allergenic potential. Protein antigenicity can be reduced by treatment with transglutaminase (TG), an enzyme which catalyses inter or intra crosslink between Lys and Glu residues. Reducing agents have been used to increase the access of TG to the Lys and Glu residues. This study aimed at investigating the antigenicity and digestibility by pepsin of β-Lg modified by TG in the presence of the reducing agent glutathione (GSH).

**Methods and Results:** The polymerization of β-Lg (Davisco, MN, USA) by microbial TG (WM, Ajinomoto Ltda, Brazil) was studied using a central-composite experimental design with 2 independent variables, GSH concentration [GSH] (0–0.2 mmol L⁻¹) and enzyme:substrate ratio (E/S) (0–44.1 U g⁻¹). The dependent variable was the response of specific IgE obtained from the serum of BALB/c mice sensitized with native β-Lg (IgE anti-β-Lg) against the modified protein. Polymerization was carried out at 50°C/180 minutes and stopped by heating at 80°C/5 min. Digestion was simulated using pepsin (E/S 1.20 w/w, pH 2, 37°C, 1 hour), and SDS-PAGE and the ELISA assay used to characterize and evaluate the antigenicity of the samples, respectively. The linear and quadratic factors of [GSH] and E/S, and their interaction, showed no significant effect on the response of IgE. No treatment resulted in a decrease in the response of IgE anti-β-Lg, but a significant (P < 0.05) increase was observed in the response of IgE against treatments 1, 7 and center point. Intact β-Lg was observed in the electrophoretic profile of the digested samples, indicating resistance to pepsin. After digestion, the samples not presented difference with respect to the digested native β-Lg sample (213.06 ± 9.59 ng mL⁻¹).

**Conclusion:** Polymerization of the β-Lg with TG in the presence of the GSH did not alter its digestibility by pepsin or decrease the antigenicity. Under some of the conditions studied the antigenicity of the β-Lg increased, indicating that the treatment could have created or exposed epitopes.