of linearized ovomucoid increases IgE-reactivity in patients with persistent egg-allergy in vitro and in skin prick tests. Our data provide evidence that reduction is a novel principle which contributes to the allergenicity of food. This may be relevant for new allergies to modern processed food.

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The Choice of Hypoallergens for Fish and Peach to Develop Food Allergy Specific Immunotherapy (TheFAST Project)
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Background: Classical allergen-specific immunotherapy (SIT), using subcutaneous injections with food extracts, may be effective but dangerous due to anaphylactic side-effects. The FAST project (Food Allergy Specific Immunotherapy) aims at the development of a safe and effective treatment of food allergies, targeting persistent and severe allergy to fish (cod) and fruit (peach). Both are caused by a single major allergen, parvalbumin (Cyp c 1) and lipid transfer protein (Pru p 3), respectively. FAST will apply hypoallergenic recombinant major allergens for SIT.

Methods: Two approaches were evaluated for achieving hypo-allergenicity, i.e. site-directed mutagenesis and chemical modification. Wildtype (wt) natural and recombinant allergens and the hypo-allergens were extensively purified and characterized physico-chemically. Their stability was tested and allergenicity was compared by CAP-inhibition and histamine release experiments while immunogenicity was tested in T-cell proliferation experiments and rabbit and mice immunizations.

Results: For Cyp c 1, the mutant without calcium-binding site showed up to a 1000 times reduced allergenicity, while secondary fold and immunogenicity (tested in human PBMC stimulations and by immunization of laboratory animals) were retained. Chemically modified Cyp c 1 demonstrated a reduced capacity to stimulate T-cells and showed less immunogenicity in rabbits. The calcium-binding mutant has been produced under GMP conditions.

Conclusions: For the Cyp c 1 calcium-binding mutant we are preparing to enter Phase I clinical trials. For Pru p 3, we need to evaluate new molecules to generate a hypoallergenic mutant that retains immunogenicity.

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IFN-α Induces Milk Allergen-Specific IL-10-Producing Regulatory B Cells in Non-IGE Mediated Milk Allergy
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Background: Tolerance induction is one of the most important concerns in the treatment of allergies and autoimmune diseases. 10-producing regulatory B cells (Br1s) seem to have a role in immune tolerance to food allergens. Oral immunotherapy using IFN-gamma has been successful for food allergy treatment. We were to investigate the effects of IFN-gamma on allergen-specific Br1 responses in milk allergy patients and milk-tolerant patients to evaluate the immunomodulatory effects of IFN-gamma on Br1 cell responses.

Methods: The 6 milk allergy patients and 8 milk-tolerant subjects were selected by DBPCFC and clinical characteristics. The PBMCs were stimulated in vitro with casein only, IFN-gamma with casein, or without any stimulant. And the CD19(+) CD5(+)+ B cells were gated and the expression of IL-10 and Annexin V binding were subsequently analysed.

Results: In milk allergy group, the Br1 fraction decreased from 24.4 to 15.0% (P = 0.002) after casein stimulation and it was recovered to 22.6% in the presence of IFN-gamma (P = 0.006). In milk-tolerant group, the Br1 fraction increased from 9.4 to 17.1% after casein stimulation (P = 0.014) and to 15.7% after IFN-gamma were added (P = 0.066). The proportion of apoptotic Br1s among CD5(+) B cells decreased from 16.5 to 8.1% with casein (P = 0.003) and 11.8% with IFN-gamma and casein (P = 0.141) in milk allergy group, while in milk-tolerant group, the proportion of apoptotic Br1 increased from 8.2 to 15.0% after casein stimulation (P = 0.014), but was unchanged by casein with IFN-gamma.

Conclusions: Allergen-specific Br1 responses and the apoptotic Br1 fraction were induced by IFN-gamma in milk allergy patients, but were not changed in milk-tolerant subjects. Finally IFN-gamma induced allergen-specific Br1 responses and immune tolerance to specific allergens in milk allergy patients.

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Analysis of IGE, IGE Rast Value and Prick Test in Wheat or Hen’s Egg-Allergy Infants Treated with Slow Specific Oral Tolerance Induction Therapy
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Background: Food allergy primarily causes anaphylaxis in children. Food such as egg, cow milk, wheat and peanut are common allergen in Japan. Methods: In this study total IGE, IGE RAST value and prick test are evaluated to monitor the efficacy outcome in wheat or hen’s egg-allergy infants treated with slow specific oral tolerance induction (sSOTI) therapy.

Results: The 3 infants suffered from IgE-mediated food allergy (wheat: 2 years 8 or 10 months old boy [threshold dose 25 g] and girl [7.7 g], hen’s egg: 4 years 9 months old girl [1.8 g], diagnosed, by food challenge, as allergy to wheat and egg. Then, the patients were treated with sSOTI either with hard-boiled egg or wheat noodle at home daily starting with 0.1 g, respectively, increased to a dose of 60 g egg or 100 g wheat, every one to 2 weeks in double dose of the weight, until tolerance was taken on. The daily maintenance dose was 10 g for each food. Four weeks later confirmed was evolution of tolerance by re-challenge. The safety and efficacy of the sSOTI therapy were confirmed in these infants. Total IGE levels were increased after SOTI therapy whereas IgE RAST value to causative antigen such as egg and wheat, contrastingly reduced. IgE RAST value to some other food as cow’s milk, reduced coincidently by bystander inhibition. IgE RAST value to a food, negative in prick test, was increased again, whereas that to a food, positive in the test, was carried on.

Conclusions: The results indicates that sSOTI therapy induced causative antigen-specific IgE-mediated tolerance in children with wheat or egg allergy, and the set of total IGE increased, reduced IgE RAST value and positive prick test was of service to evaluate evolution of tolerance in slow SOTI therapy.

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Efficacy of Slow Oral Immunotherapy for Cow’s Milk Allergy
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Background: Oral immunotherapy (SIT), using subcutaneous injections with food extracts, may be effective but dangerous due to anaphylactic side-effects. The FAST project (Food Allergy Specific Immunotherapy) aims at the development of safe and effective treatment of food allergies, targeting persistent and severe allergy to fish (cod) and fruit (peach). Both are caused by a single major allergen, parvalbumin (Cyp c 1) and lipid transfer protein (Pru p 3), respectively. FAST will apply hypoallergenic recombinant major allergens for SIT.

Methods: Two approaches were evaluated for achieving hypo-allergenicity, i.e. site-directed mutagenesis and chemical modification. Wildtype (wt) natural and recombinant allergens and the hypo-allergens were extensively purified and characterized physico-chemically. Their stability was tested and allergenicity was compared by CAP-inhibition and histamine release experiments while immunogenicity was tested in T-cell proliferation experiments and rabbit and mice immunizations.

Results: For Cyp c 1, the mutant without calcium-binding site showed up to a 1000 times reduced allergenicity, while secondary fold and immunogenicity (tested in human PBMC stimulations and by immunization of laboratory animals) were retained. Chemically modified Cyp c 1 demonstrated a reduced capacity to stimulate T-cells and showed less immunogenicity in rabbits. The calcium-binding mutant has been produced under GMP conditions.

Conclusions: For the Cyp c 1 calcium-binding mutant we are preparing to enter Phase I clinical trials. For Pru p 3, we need to evaluate new molecules to generate a hypoallergenic mutant that retains immunogenicity.
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Background: Efficacy and safety of slow oral immunotherapy (SOIT) is not yet clear, especially regarding tolerance acquisition.

Methods: We recruited 32 cow’s milk (CM) allergy confirmed by oral food challenge (OFC). Twenty-five subjects were enrolled as SOIT group, and remaining 7 were as control. The inclusion criteria were as follows; (1) CM allergy without anaphylaxis confirmed by OFC just before the trial, and (2) children >4 years. In SOIT group, they were asked to take small amount of CM at home after the OFC. The initial dose was about 1/4 of the threshold eliciting positive objective symptoms, and it was built up to 200 mL. CM depends on the symptoms (build up phase). After reaching 200 mL, they were asked to take 200 mL CM daily until the asymptomatic duration lasting for more than 3m (maintenance phase). The subjects, who completed maintenance phase, underwent OFC to confirm the tolerance acquisition after the cessation of CM ingestion for 2w (confirm-OFC). In control group, they had eliminated CM completely and underwent the confirm-OFC after 9.8 ± 2.9 m (mean ± SD). We investigated the endpoints (adverse reactions, medical treatments, results of confirm-OFC, and laboratory findings), prospectively.

Results: In SOIT group (n = 25) and control group (n = 7), the average age was 6.6y and 4.7y, respectively. The average threshold was 52 mL and 17 mL, and the CM specific IgE was 17.6 Ua/mL/9.9 Ua/mL, respectively. The average period of build up and maintenance phases in SOIT group was 80d (n = 25) and 98d (n = 15), respectively. The frequency of adverse reactions in all of build up (1973d) and maintenance phases (2924d) were 13.5% (mild symptoms)/23.3% (moderate symptoms) and 1.7% (mild)/0.3% (moderate), respectively. No patient had administered adrenaline during SOIT. Fifteen subjects in SOIT and 7 in control underwent the confirm-OFC. In SOIT, 8 subjects (53.3%) passed the confirm-OFC, whereas 2 (28.6%) passed in control. There was no statistically significant difference regarding tolerance acquisition between these 2 groups (P = 0.277).

Conclusions: This study suggests that SOIT for about 1/2 year induces desensitization effectively for CM allergy without severe adverse reactions. Further and longer study would be required to prove accelerated tolerance acquisition by SOIT.

76 Factors Associated with Development of Food Allergy in Liver-Transplanted Children
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Background: The development of food allergy (FA) after transplantation has been described mainly about liver transplantation in children (Pediatr Allergy Immunol. 2009; 20: 741–747). It has been becoming important issue in this population. Although tacrolimus immunosuppressive therapy has been considered a significant risk factor (J Allergy Clin Immunol. 2011; 127: 1296–1298), other risk factors are not identified yet. This study was undertaken to evaluate the risk factors other than tacrolimus immunosuppressive therapy.

Methods: This study was a retrospective analysis of pediatric liver transplant recipients in our hospital. We reviewed the medical records of all patients who underwent liver transplantation during study period. Data collected including preceding-hepatic diseases, the number of previous surgeries, age at liver transplantation and etc.

Results: Between November 2005 and May 2010, 106 children received liver transplantation. The most common indication for liver transplantation was biliary atresia (BA; 47 patients, 44.3%). The other conditions were: congenital metabolic diseases in 27 patients, fulminant hepatic failure in 19, liver cirrhosis in 6, congenital absence of portal vein in 3, congenital hepatic fibrosis in 2 and hepatic tumor in 2 patients. After transplantation, all the patients received immunosuppressive therapy based on tacrolimus regimen. Fifteen patients (10 female and 5 male) developed new-onset FA (14.2%). The average age at transplantation was 10 months and FA has been developed within 2 years (median 11 months, IQR, 4.5–19.0). Eleven patients with BA (23.4%) and 4 patients with the other conditions (6.8%) developed new-onset FA (P = 0.023). Among the patients who developed FA, the number of previous surgeries was significantly higher in patients with BA (P = 0.008).

Conclusions: New-onset food allergy after liver transplantation is now becoming a significant problem. We observed a trend toward an excess of FA in patients with BA compared to patients with other indications for liver transplantation. Patients with BA received surgical operations in several times before liver transplantation. Frequent operations might add some stimulation to generate new-onset FA and should be considered as a susceptible subgroup that requires specific attention.

GLOBAL ASTHMA EPIDEMIOLOGY

77 Recurrent Wheezing in Infancy: Epidemiological Changes Between EISL Phase i and iii
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Background: Prevalence of allergic diseases has increased in the last years. Data on recurrent wheezing (>3 episodes) in infancy is scarce. The aim of this study was to verify changing in prevalence of recurrent wheezing infants in the south of Brazil.

Methods: Cross-sectional study using a standardized and validated questionnaire (EISL: Estudio Internacional sobre Sibilancias en Lactantes) with questions: Has your baby had wheezing or whistling in the chest area or bronchitis in the first 12 months of life? Has your baby had 3 or more wheezing episodes in the first year of life? Parents of infants, ages 12 to 15 months that attended to Health Centers for routine immunization were interviewed between August 2005 to December 2006 (EISL Phase I) and September 2009 to September 2010 (EISL Phase III). Categorical variables are shown as proportion and differences verified by chi-square test, and continuous variables were expressed as mean ± SD and analyzed by Student t test.

Results: Three thousand three parents of infants answered questionnaire in the EISL Phase I, and 45.4% had had at least one wheezing episode; 50.7% were male, and 22.6% had recurrent wheezing episode starting at 5.5 ± 3.1 months. Five years later, in the EISL Phase III, 1003 parents participated in the survey: 40.6% had at least one wheezing episode (P = 0.46), 51.1% were male, and 19.8% had recurrent wheezing (P = 0.1) starting at 6.1 ± 3 months (P = 0.06). Conclusions: Recurrent wheezing in infancy is highly prevalent and starts early in life. In our population, recurrent wheezing rates did not modify in the time period of study.

78 Asthma Admission Rates in Germany: An Analysis of the Nationwide DRG-Statistic of the Year 2009
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Background: Within the OECD Health Care Quality Indicators (HCQI) Project up to 21 countries participated in calculations of 6 indicators on care for chronic conditions. Those so-called Health Promotion, Prevention and Primary