and 17 kDa; when a sample of *Penicillium sp*, mycelium was used, IgE reactive bands of 97 kDa, 60 kDa, 40 kDa, 33 kDa, 32 kDa, 29,5 kDa, 28.5 kDa, 24 kDa, 20 kDa, 18 kDa and 16 kDa were detected.

Conclusion: We report 4 cases of food allergy to *Penicillium*. An IgE mediated mechanism was demonstrated in all cases.

Although food allergy due to molds is rare, it should be suspected as hidden allergenic source in reactions with sausages and cheeses.

0327 | The effect of different formulas in children with cow's milk allergy on the occurrence of other allergic manifestations and the time of immune tolerance acquisition: The atopic march II study

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Background: Recent data suggest that the use of extensively hydrolyzed casein formula containing the probiotic *L.rhamnosus* GG (LGG) (EHCF+LGG) reduces the incidence of other AMs and hastens the development of immune tolerance in children with IgE-mediated cow's milk allergy (CMA). To see whether formula choice for CMA treatment could impact the occurrence of other AMs and the time of immune tolerance acquisition.

Method: Prospective open non-randomized trial on a cohort of children with a diagnosis of IgE-mediated CMA in the first year of life, already in follow-up. The patients were treated with one of the following formulas: EHCF+LGG, rice hydrolyzed formula (RHF), soy formula (SF), extensively hydrolyzed whey formula (EHWF) or amino-acid based formula (AAF). All subjects were evaluated during a 36 months follow-up.

The occurence of AMs (atopic eczema, allergic urticaria, asthma and oculorhinitis) was diagnosed Immune tolerance acquisition was evaluated every 12 month by the result of oral food challenge.

Results: A total of 365 subjects completed the study, 73 per group. All children were from families of middle socio-economic status and lived in urban areas. At enrollment, all subjects were in stable clinical conditions without symptoms related to CMA. Demographic and anamnestic features were similar comparing the study cohorts at enrolment. Binomial regression revealed that the estimates of the incidence of the AMs are: EHCF+LGG: 0.22 (Bonferroni corrected 95%CI: 0.09 to 0.34); RHF: 0.52 (Bonferroni corrected 95%CI: 0.37 to 0.67); SF: 0.58 (Bonferroni corrected 95%CI: 0.43 to 0.72); EHWF : 0.51 (Bonferroni corrected 95%CI: 0.36 to 0.66); AAF: 0.77 (Bonferroni corrected 95%CI: 0.64 to 0.89). The incidence of the main outcome in the RHF, SF, EHWF and AAF groups vs the EHCF+LGG group was always higher than the pre-specified absolute difference of 0.25 and significantly higher at the pre-specified alpha-level of 0.0125 (*P* -value <= 0.001 in all cases). The acquisition of immune tolerance was significantly higher in the EHCF+LGG group comparing to the other groups. The rate of immune tolerance acquisition for EHCF+LGG groups was (95%CI): at 12 months = 0.41 (0.30 to 0.52); at 24 months = 0.64 (0.53 to 0.75); at 36 months = 0.81 (0.72 to 0.90).

Conclusion: The results of the study suggest that EHCF+LGG is superior to other formulas for the prevention of AMs and for the acquisition of immune tolerance in children with CMA.

0356 | Strong allergenicity of grass carp is related to high IgE-binding capacity of its major allergen cten i 1

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Background: Grass carp is a major food fish in Hong Kong and a frequent cause of IgE-mediated allergic reaction. We sought to investigate its allergenicity, allergenic components and cross-reactivity with other fishes.

Method: Specific IgE to codfish, salmon and grass carp extracts of 162 fish allergic subjects were analyzed by ImmunoCAP assay. The major IgE-binding protein of grass carp was identified by immunoblotting of raw and steamed grass carp extract using serum samples from grass carp allergic subjects. The identity of the IgE-binding protein was confirmed by peptide mass fingerprinting and the protein was subsequently purified from the extract by anion exchange chromatography and size exclusion chromatography. The IgE-binding capacity of the purified allergen was measured by ELISA and cross-inhibition ELISA was performed to determine the cross-reactivity of grass carp allergen with purified allergens from codfish and salmon.

Results: The majority of subjects (n = 158; 97.5%) had positive (>0.35 kU_A/L) specific IgE against grass carp while 22% of the subjects had negative specific IgE against codfish and salmon. The four grass carp negative subjects were all positive to salmon. IgE levels against grass carp was significantly higher than codfish and salmon. Immunoblotting of raw and steamed grass carp extracts revealed a major allergen of about 9 kDa in size. This allergen was identified as parvalbumin by peptide mass fingerprinting and subsequently registered as an official allergen *Cten i 1* at the WHO/IUIS allergen database. The amino acid sequence of *Cten i 1* was highly similar to the common carp parvalbumin *Cyp c 1* (91.7%), but less similar to codfish parvalbumin *Gad m 1* (79.8%) and salmon parvalbumin *Sal s 1* (68.8%). Similar to the ImmunoCAP results, the IgE-binding capacity of purified *Cten i 1* was also higher than that of *Gad m 1* and *Sal s 1*. Competitive inhibition ELISA showed that *Gad m 1* and *Sal s 1* were able to inhibit 72.6% and 58.6% IgE binding to *Cten i 1* respectively, suggesting a moderate degree of cross-reactivity among the three parvalbumins.

Conclusion: Grass carp is a highly allergenic fish in our study cohort. The strong allergenicity of grass carp is related to the high IgE-binding capacity of its major allergen *Cten i 1* rather than the