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193 Secretion of Food Allergen Proteins in Saliva

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RATIONALE: Peanut proteins were found to be secreted in 50% of lactating women's breast milk. We wanted to develop a testing method to predict the secretion of peanut protein in breast milk. The secretion of food protein in saliva was hypothesized to be a possible predictor of secretion of foods in breast milk following ingestion.

METHODS: Non-allergic volunteers, some lactating, ingested 50 grams of either whole peanuts, peanut milk or cow's milk and various immunoassays were utilized to analyze for the presence of peanut or cow's milk proteins in saliva and breast milk. Saliva and breast milk samples were subjected to SDS-PAGE, Western blot and ELISA analysis with anti-raw and roasted peanut and anti-alpha-casein antibodies and pooled serum IgE from peanut allergic individuals.

RESULTS: Peanut protein levels in breast milk were undetectable using Western blot analysis and inconsistent with ELISA analysis. However, peanut proteins around 20 and 30 kDa that reacted with anti-roasted peanut antibody were detected, 6-18 hours following ingestion, in saliva of different individuals. An 18 kDa band that reacts with anti-alpha casein antibody was also detected in saliva 6-18 hours following ingestion.

CONCLUSIONS: Secretion of food allergen proteins or peptides in saliva several hours following ingestion may have important implications for delayed allergic reaction by sensitive patients. Also, due to the fact that these proteins or peptides survive digestive enzymes, become absorbed into the blood stream and are subsequently secreted in biological fluids may indicate that they are most likely the sensitizing or tolerizing agent within an allergic food.

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194 IgE Cross-reactivity between the Cysteine Proteases Der p 1 and Act c 1, the Major Allergens from House Dust Mites and Kiwifruit

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RATIONALE: The cysteine proteases Act c 1 and Der p 1 are well-described major allergens in mites and kiwifruit, respectively. However, there are no data about IgE cross-reactivity between these allergens that are taxonomically not related but belong to the same protein family.

METHODS: Crystal structures and sequences of Der p 1 and Act c 1 were used to locate identical residues on the molecules' surfaces. Characterization of IgE binding and inhibition experiments were performed using Act c 1 and Der p 1. Sera of 16 patients were selected who had allergy to kiwifruit and/or sensitization to mites.

RESULTS: Sequence of Der p 1 and Act c 1 showed 30.6% identity and 41.8% similarity. Structural analysis of exterior amino acid side chains showed 18.7% identity and 29.1% similarity. The highest structural similarity was found within and in the vicinity of the active site of the two molecules. All sera tested contained IgE reactive to Der p 1 and 10 sera to Act c 1. The overall range of inhibitory capacity of Act c 1 upon IgE binding to Der p 1 was 20% to 61%. Der p 1 inhibited IgE binding to Act c 1 from 25% to 92%.

CONCLUSIONS: Here we demonstrate that homologous cysteine proteases of diverse sources share common IgE epitopes despite espite low sequence and structural similarity.

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195 Allergenic Fruit TLPs Possess Different Degrees of IgE Cross-reactivity

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RATIONALE: Thaumatin-like proteins (TLPs) are important allergens of a number of plant foods. We aimed to examine the IgE cross-reactivity between the apple TLP Mal d 2 and TLPs from kiwi (Act c2), grape (VvTLP), and cherry (Pru av 2).

METHODS: Amino acid sequences of apple, cherry, kiwi, and grape TLPs were aligned. The TLPs were purified. IgE ELISA and ELISA inhibitions were carried out using sera from 46 apple allergic patients. The relevance of N-glycosylation for IgE-binding was investigated by inhibition with the oligosaccharide BSA-MUXF.

RESULTS: Sequence alignments showed 33% identity of Mal d 2 to Act c 2, 35% to VvTLP, and 69 % to Pru av 2. Mal d 2 was IgE reactive with a prevalence of 39% (18/46). Nine of these 18 patients recognized N-glycan epitopes on Mal d 2, the 9 remaining showed binding to protein epitopes. Two of the 9 protein-specific sera had IgE to all four TLPs, 6/9 to Mal d 2, Act c 2, and Pru av 2, and one to Mal d 2 and Pru av 2. In addition, Mal d 2 and Pru av 2 showed a high extent of cross-reactivity.

CONCLUSIONS: The glycoallergen Mal d 2 shares cross-reactive epitopes with other TLPs that encompass the Mal d 2 polypeptide and its N-glycan structure. Inhibition of IgE-Mal d 2 interactions with Pru av 2 demonstrated that many of the epitopes of Mal d 2 were cross-reactive with epitopes on Pru av 2.

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196 IgE-Reactive Proteins in Cashew Apple Juice Concentrate are Removed by Filtration

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RATIONALE: Cashew apple juice has the potential to be a natural source of vitamin C and sugar in processed foods. The juice of the cashew apple is obtained by pressing the fleshy peduncle, or receptacle, which forms a rounded "apple" that sits above a narrow extension containing the cashew nut. Cashew nut allergy is the second most commonly reported tree nut allergy in the United States. Thus, it is relevant to determine if cashew apple juice contains cashew nut allergenic proteins.

METHODS: Immunoblotting was performed using cashew apple juice 6X concentrate that was further concentrated through dialysis, lyophilization and resuspension.

RESULTS: IgE from sera of 10 of 28 patients allergic to cashew nut bound proteins in the cashew apple juice concentrate. For some patient sera, reactivity could be inhibited by pre-incubation of the sera with either ryegrass pollen or cashew nut extract. Using mAbs specific for cashew nut allergens, the concentrate was found to contain anti-Ana o 1- and anti-Ana o 2-reactive peptides. After 5kd filtration with a cellulose membrane, neither IgE from cashew nut allergic sera nor mAb to Ana o1 or Ana o 2 bound any proteins in the cashew apple juice concentrate.

CONCLUSIONS: Cashew apple juice concentrate contains IgE-reactive proteins, including some also found in the cashew nut. After 5kd filtration, cashew apple juice concentrate contained no IgE-reactive proteins.

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