821 Expression of a Deglycosylated Recombinant Fel d 1 in Pichia pastoris

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RATIONALE: Expression of recombinant Fel d 1 in *Pichia pastoris* results in a mixture of hyperglycosylated and non-glycosylated forms of Fel d 1. Hyperglycosylation could potentially interfere with IgE binding. Our goal was to produce a deglycosylated recombinant allergen that more closely resembles natural Fel d 1.

METHODS: Site-directed mutagenesis was used to substitute asparagine at position 103 to alanine (N103Q), thus disrupting the N-glycosylation consensus motif in Fel d 1 c-DNA. N103Q was purified by affinity chromatography and compared to both rFel d 1 and natural Fel d 1 by SDS-PAGE, mAb ELISA and IgE Ab ELISA.

RESULTS: SDS PAGE showed the rFel d 1 monomer bands at 16kD and 20kD, while the N103Q monomer appeared at 16kD only. This suggests a reduction of glycosylation from 20kD to 16kD in *Pichia* expressed N103Q. The natural Fel d 1 band appeared at 18kD, indicating glycosylation of the natural allergen in cats. Preliminary data show comparable immunoreactivity of rFel d 1, deglycosylated rFel d 1 and natural Fel d 1 in mAb and IgE ELISA assays.

CONCLUSIONS: Disruption of the N-glycosylation motif in rFel d 1 removed the hyperglycosylated forms in *Pichia pastoris* expressed rFel d 1. This improved rFel d 1 will be useful for allergen standardization and the development of improved allergy diagnostics and therapeutics.

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822 In Vitro Evidence For Different Routes Of Sensitization To Peanut In Children

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RATIONALE: Despite the requirement for prior contact with an allergen for sensitization to occur, the majority of peanut allergic children react to their first known peanut ingestion. Evidence suggests that sensitization may occur by contact with allergen through the skin. Individuals thus sensitized may be predisposed to developing peanut allergy, whilst tolerance to peanut may be induced by oral exposure. We employ the use of skin and gastrointestinal homing memory T cell markers (Cutaneous Lymphocyte Antigen (CLA) and $\alpha4\beta7$ respectively) to indicate the likely route of initial sensitization and examine the evidence for this theory.

METHODS: Immunomagnetic beads are used to isolate CLA+ and $\alpha4\beta7$+ memory T cells from peripheral blood mononuclear cells. The cells are stimulated with peanut extract in the presence of antigen presenting cells. Thymidine incorporation is assayed to measure lymphocyte proliferation. Stimulation indices to peanut in the CLA+ cells are compared to those in the $\alpha4\beta7$+ cells in both peanut allergic and peanut tolerant children. Peanut specific cytokine production including IL4, IL5, IFN-γ, TFN-α, IL10 and TGF-β is batched and measured in the cell culture supernatants of both groups.

RESULTS: Higher proliferation is observed in the CLA+ memory T cells relative to the $\alpha4\beta7$+ memory T cells in peanut allergy. This trend appears to be reversed in non-allergic patients.

CONCLUSIONS: In vitro evidence supports the hypothesis that sensitization to peanut via the skin may be associated with the development of peanut allergy, whilst oral sensitization may induce tolerance. Such studies may help to facilitate the diagnosis of peanut allergy.

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823 Recognition of Human Milk Peptides by IgE Antibodies from Infants with Cow's Milk Allergy

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RATIONALE: Occasionally, exclusively breastfed, cow's milk-allergic infants continue to be symptomatic despite strict maternal milk avoidance. We sought to determine whether this phenomenon could be due to sensitization against human milk proteins.

METHODS: We generated ten peptides (10-12 amino acid in length), representing known bovine milk IgE-binding epitopes of alpha-lactalbumin, beta-, and kappa-casein and the corresponding, highly similar human milk peptides (differing by 1 to 5 amino acids) on the SPOT membranes. The peptides were labeled with sera from 9 breastfed milk-allergic infants (milk-IgE <0.35 to 15.9 kUA/L) who were asymptomatic and 6 infants (milk IgE 100 kUA/L) who were symptomatic during maternal milk-elimination diet; aged 3 weeks to 12 months.

RESULTS: Human milk peptides were generally less often bound by IgE from milk-allergic infants than the homologous bovine milk peptides. Nine patients had peptide-specific IgE to human alpha-lactalbumin, four to human beta-casein and twelve to human kappa-casein. At least one human milk peptide was strongly bound by IgE from 4/6 symptomatic infants and by 3/9 asymptomatic infants. Interestingly, two infants who were symptomatic while their mothers avoided cow's milk had IgE specific to a human beta-casein peptide but none or little to the corresponding bovine counterpart.

CONCLUSIONS: These data suggest that human milk proteins are recognized by the IgE from the majority of milk-allergic infants who continue having symptoms despite maternal milk avoidance, although some asymptomatic infants also possess some human milk-specific IgE. The clinical significance of these IgE antibodies requires further investigation in a functional assay.

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824 Clinical and Laboratory Parameters Predicting Severity and Nature of Allergic Reactions After Ingestion of Nuts

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RATIONALE: Although acute allergic reactions after ingestion of nuts are common, fatalities are rare. It is not possible to predict who is most likely to develop severe reactions and therefore unnecessary anxiety and over cautious management of many patients is the norm.

METHODS: From 1992 - 2004, we collected detailed information on the clinical severity and allergy test results of 1094 patients with nut allergy attending a regional allergy center. In a cohort of 122 patients with the most severe symptoms, we assayed sera for activity of enzymes critical for the catabolism of bradykinin.

RESULTS: Different risk factors were associated with the likelihood of life-threatening pharyngeal edema, bronchospasm and circulatory insufficiency. Severe pharyngeal edema was 3.8 (2.1 - 6.9) times more common in patients with severe hay fever, and 2.6 (1.8 - 3.7) more common after ingestion of tree compared with ground nuts. Patients with serum ACE concentrations < 37.0 mmol/L had a 9.6 (1.6 - 57) fold risk of severe upper airway obstruction. In contrast life-threatening bronchospasm was most likely in patients with severe asthma (relative risk 6.8 (4.1 - 11.3) and less so in patients with milder asthma ((2.7 (1.7 - 4.0). Severe circulatory insufficiency was 8.9 (3.9 - 20.0) times more common in adults than children, and also more common in patients with severe eczema (3.1 (1.1 - 8.4)).

CONCLUSIONS: Prevention and management of life-threatening clinical complications need to be tailored to individual risks. Life-threatening airway obstruction may involve bradykinin, explaining why some people have previously not responded to epinephrine.

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