Reflab

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Food allergy

Basophil histamine release in children with adverse reactions to cow milk. Comparison with RAST and skin prick test

Allergy 1988

P Prahl, F Krasilnikof, P Stahl Skov, S Norn

Link: https://pubmed.ncbi.nlm.nih.gov/2461108/

Abstract

Basophil histamine release was examined in 26 children suspected of having cow milk allergy (CMA). Following oral challenge with cow milk, the initial adverse reaction reappeared in 20 children, the majority developing urticaria. The urticaria patients showed a high degree of correlation between the results of histamine test, RAST and skin test. Children with gastrointestinal symptoms reacted to milk challenge, but only a few showed a positive histamine test, RAST and skin test. Among the patients with atopic dermatitis, the tests gave mostly negative results, which was in accordance with the lack of response to a milk challenge. The results obtained by removal from and fixation to the cell surface of IgE indicate an IgE-mediated reaction in CMA, which, in connection with the correlation between histamine test and RAST or skin test, suggests basophil histamine release as a suitable method for testing Type I allergy in children suspected of CMA.

Immunochemical cross-reactivity between albumin and solid-phase adsorbed histamine

Agents Actions 1990

L K Poulsen, H Nolte, I Søndergaard, P Stahl Skov

Link: https://pubmed.ncbi.nlm.nih.gov/2386106/

Abstract

For production of an antibody against histamine, this was coupled to human serum albumin (HSA) and used for immunization of rabbits. To test the antiserum, an immunoradiometric assay was developed comprising solid-phase bound histamine, antisera and radiolabelled protein A. Titration and inhibition experiments revealed that histamine adsorbed onto a solid-phase could bind the antiserum. However, neither free histamine nor histamine coupled to unrelated carriers could inhibit the binding of antiserum to the solid-phase histamine. Cross-reactivity was demonstrated between HSA and solid-phase bound histamine, as the immunoradiometric assay was inhibited by HSA. This unexpected cross-reactivity was established, as a commercially available antiserum with specificity to HSA without histamine also bound to the solid-phase bound histamine. It is suggested that preparations of antibodies against histamine are tested for this possible cross-reactivity.

Egg and milk allergy in adults: comparison between fresh foods and commercial allergen extracts in skin prick test and histamine release from basophils

Clinical & Experimental Allergy 1992

A Norgaard, P S Skov, C Bindslev-Jensen

Link: https://pubmed.ncbi.nlm.nih.gov/1281442/

Abstract

The ability of skin prick test (SPT) and histamine release from basophils (HR) to diagnose clinical type I allergy to egg and milk was investigated as compared with double-blind, placebo-controlled food challenge (DBPCFC) in 17 adults suspected of type I egg and/or milk allergy. In both SPT and HR, commercial allergen extracts commonly used for SPT were compared with fresh, standardized foods. With commercial extracts the overall sensitivities of SPT and HR were 0.75 and 0.56 respectively, and none of the tests showed concordance with DBPCFC. With fresh, standardized foods the overall sensitivities of SPT and HR were 1.00 and 0.89 respectively, and both tests now showed a significant concordance with DBPCFC ($P < 0.05$). Specificity was only slightly improved in SPT, and unchanged in HR. Thus, the use of fresh, standardized foods significantly improved the outcome of both tests, as regards to sensitivity and concordance with DBPCFC. The diagnostic ability of SPT and HR appear to be strongly influenced by the allergen quality.

Biomolecular regulation of the IgE immune response. I. Cell-associated IgE and in vitro IgE synthesis

Allergy 1992

L K Poulsen, L Baron, J H Heinig, P Stahl Skov, K Bendtzen

Link: https://pubmed.ncbi.nlm.nih.gov/1485661/

Abstract

Several cell types, including mast cells, basophils, macrophages/monocytes, lymphocytes, platelets and eosinophils, may bind or contain IgE. To investigate the source of cell-associated IgE and its relation to spontaneous IgE synthesis, peripheral blood mononuclear cells from allergic and non-allergic donors were examined. Using a combination of different cell fractionation techniques and varying methods for extraction of cell-associated IgE, data were obtained indicating that monocytes constitute a major source of cell-associated IgE in human blood. The amount of cell-associated IgE in peripheral blood mononuclear cells from allergic and non-allergic donors varied more than 100-fold but correlated closely to the level of serum IgE ($r = 0.84$, $p < 0.001$, $n = 38$). Spontaneous and mitogen-induced in vitro syntheses of IgA, IgE and IgG were compared for allergic and non-allergic donors. Only one donor with very high serum IgE demonstrated spontaneous or mitogen-induced in vitro IgE synthesis despite synthesis of IgA and IgG.

Codfish allergy in adults. Specific tests for IgE and histamine release vs double-blind, placebo-controlled challenges

Clinical & Experimental Allergy 1996

T K Hansen, C Bindslev-Jensen, P S Skov, L K Poulsen

Link: https://pubmed.ncbi.nlm.nih.gov/8955577/

Abstract

Background: At present, several in vitro tests for immunoglobulin E (IgE)-mediated food allergy are available. An estimation of the diagnostic accuracy of the various tests used in predicting clinical sensitivity to codfish in a well-characterized allergic material is necessary.

Objectives: To compare the diagnostic value of four specific IgE tests, and histamine release from basophils (HR) in identifying clinical type I allergy to codfish. As a true diagnosis, double-blind, placebo-controlled food challenges (DBPCFC) were employed.

Methods: Eight clinically codfish-allergic adult patients were investigated together with 30 codfish-tolerant control subjects for evidence of codfish-specific reactivity by Phadebas RAST (PHA), Pharmacia CAP System RAST (CAP), Magic Lite (ML) and HR. To characterize the diagnostic properties of a freshly prepared raw codfish extract, experiments were conducted employing an in-house radioallergosorbent test (RAST), the Maxisorp RAST (MAXI) and HR. Finally, protein profile and IgE-reacting allergens were detected by means of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting.

Results: The sensitivities of HR with commercial extract and the three commercially available specific IgE analyses were 0.83 and 1.00 respectively. Specificities were 1.00 (HR) and 0.87-1.00 (specific IgE tests). Freshly prepared codfish extracts improved the sensitivity of HR. SDS-PAGE revealed approximately 29 bands (< 14.3-200 kDa) including a band of 12-13 kDa, and in immunoblotting 18 sera identified 17 IgE-binding bands. The protein migrating at 12-13 kDa was identified in the fresh codfish extract tested with sera from all clinical codfish allergics, while no significant reaction was seen in the control subjects.

Conclusion: Based on the small number of adult patients included in our study, the in vitro assays with commercial and fresh extracts have high sensitivity and are acceptable for screening for codfish allergy. Specificity of Phadebas, CAP, and our in-house RAST was less than unity, whereas ML and strong binding of IgE to a 12-13 kDa protein completely matches DBPCFC results, and thus seems sufficient for establishing the diagnosis.

Allergenic properties of kiwi-fruit extract: cross-reactivity between kiwi-fruit and birch-pollen allergens

Allergy 1997

V Voitenko, L K Poulsen, L Nielsen, A Norgaard, C Bindslev-Jensen, P S Skov

Link: https://pubmed.ncbi.nlm.nih.gov/9105517/

Abstract

Our investigation aimed to produce and characterize a kiwi extract and to use this extract to investigate a possible cross-reactivity with birch pollen. Kiwi was extracted in two buffers: phosphate-buffered saline (PBS) and borate-buffered saline (BBS). Extraction in BBS produced a double amount of protein, and a more stabile extract. Tandem crossed-immunoelectrophoresis showed that the BBS and PBS extracts had several common, but also a few individual, proteins. The mixture of both extracts was assumed to represent the most complete allergen extract. The allergenic properties of the kiwi extract were investigated by immunoblotting (IB), RAST, and histamine-release (HR) test in 15 birch-pollen-allergic patients (eight of them with clinical kiwi allergy) and one with clinical monoallergy to kiwi. All eight birch-pollen-allergic patients with kiwi allergy and the kiwi-monoallergic patient were positive in kiwi IB binding most frequently to proteins of 10-12 and 20-25 kDa. With our extract, RAST was positive in four kiwi-allergic and one non-kiwi-allergic patient, whereas the HR test was positive in five kiwi-allergic patients and negative in all non-kiwi-allergic patients. RAST and IB inhibition demonstrated cross-reactivity between birch-pollen and kiwi allergens due to a 10-12 kDa protein. In conclusion, a kiwi extract with allergenic properties was produced, and, by the methods used, cross-reactivity was demonstrated between birch-pollen and kiwi allergens.

Cross-reactivity within the profilin panallergen family investigated by comparison of recombinant profilins from pear (Pyr c 4), cherry (Pru av 4) and celery (Api g 4) with birch pollen profilin Bet v 2

Journal of Chromatography B: Biomedical Sciences and Applications 2001

S Scheurer, A Wangorsch, J Nerkamp, P S Skov, B Ballmer-Weber, B Wüthrich, D Haustein, S Vieths

Link: https://pubmed.ncbi.nlm.nih.gov/11419723/

Abstract

Profilin is a panallergen which is recognised by IgE from about 20% of birch pollen- and plant food-allergic patients. Little is known about epitope diversity among these homologous proteins, and about the correlation between IgE-cross-reactivity and allergenic reactivity. Plant food profilins from pear (Pyr c 4) and cherry (Pru av 4) were cloned by polymerase chain reaction and produced in Escherichia coli BL21. The profilins were purified as non-fusion proteins by affinity chromatography on poly-(L-proline)-Sepharose and characterized by immunoblotting, IgE-inhibition experiments and histamine release assays. The coding regions of the cDNA of pear and cherry profilin were identified as a 393 bp open reading frame. The deduced amino acid sequences showed high identities with birch pollen profilin Bet v 2 (76-83%) and other allergenic plant profilins. Pyr c 4 and Pru av 4 were investigated for their immunological properties in comparison with profilins from celery (Api g 4) and birch pollen (Bet v 2). Fourty-three of 49 patients (88%), preselected for an IgE-reactivity with Bet v 2 showed specific IgE-antibodies to the recombinant pear protein, 92% of the sera were positive with the recombinant cherry allergen and 80% of the sera were reactive with the celery protein. Inhibition experiments showed a strong cross-reactivity of IgE with profilins from plant food and birch pollen. However, IgE binding profiles also indicated the presence of epitope differences among related profilins. All investigated profilins, Pyr c 4, Pru av 4, Api g 4 and Bet v 2, presented almost identical allergenic properties in cellular mediator release tests. Therefore, cross-reactivities between related profilins may explain pollen-related allergy to food in a minority of patients. The nucleotide sequences reported have been submitted to the Genbank database under accession numbers AF129424 (Pyr c 4) and AF129425 (Pru av 4).

Standardization of food allergen extracts for skin prick test

Journal of Chromatography B: Biomedical Sciences and Applications 2001

K Skamstrup Hansen, C Bindslev-Jensen, P S Skov, S H Sparholt, G Nordskov Hansen, N R Niemeijer, H J Malling, L K Poulsen

Link: https://pubmed.ncbi.nlm.nih.gov/11419728/

Abstract

The aim of the study was to standardize and evaluate technically optimized food allergen extracts for use in skin prick test (SPT). The standardization procedure comprised 36 allergic histories in 32 food allergic patients with 21 healthy, non-atopic individuals serving as controls. The patients had a history of allergic symptoms upon ingestion of either cow's milk (n=3), hen's egg (n=9), wheat (n=4), hazelnut (n=14) or cod (n=6). They also had specific IgE in serum to the food in question and a positive SPT with a fresh preparation of the food. The diagnosis had been confirmed by a double-blind, placebo-controlled food

challenge, except for the hazelnut-allergic patients. The controls were subjected to an open food challenge with all the foods to ensure tolerance. The standardization was performed by means of titrated SPT in accordance with the guidelines on biological standardization from the Nordic Council on Medicine. Regression analysis of the skin wheal areas was performed for each patient and the median protein concentration of allergen preparation (median Ch10) eliciting a wheal area of the same size as histamine 10 mg/ml was calculated. The median Ch10 was 0.56 mg/ml for milk, 0.88 mg/ml for egg, 5.4 mg/ml for wheat, 2.1 mg/ml for hazelnut and 0.017 mg/ml for the cod extract. The sensitivity of the median Ch10 estimated from the SPT data was 1 for milk, 0.98 for egg, 1 for wheat, 1 for hazelnut and 0.87 for the cod extract. The allergenic activity of the hazelnut extract was further investigated by leukocyte histamine release (HR) and immunoblotting experiments using sera from 27 hazelnut allergic patients. The clinical sensitivity of the optimized hazelnut extract evaluated by HR was 0.78 compared to 0.30 for a commercially available hazelnut extract (Soluprick). Immunoblotting results showed a stronger IgE binding capacity and additional IgE-binding bands of the optimized hazelnut extract compared with the Soluprick extract.

Allergenic components of a novel food, Micronesian nut Nangai (Canarium indicum), shows IgE cross-reactivity in pollen allergic patients

Allergy 2002

Eva Sten, P Stahl Skov, S B Andersen, A M Torp, A Olesen, U Bindslev-Jensen, L K Poulsen, C Bindslev-Jensen

Link: https://pubmed.ncbi.nlm.nih.gov/11972478/

Abstract

Background: New foods may present a risk for food hypersensitive patients. Several examples exist of allergic reactions caused by cross-reactive plant-derived foods, and new foods should be scrutinised before introducing them to the market. We have evaluated the clinical and serological relevance of cross-reactivity between Nangai and pollen allergens.

Methods: Cross-reactivity was examined with Maxisorp RAST (radioallergosorbent test), RAST inhibition and Western blot, using sera from patients allergic to grass, birch and mugwort pollen. None of the patients reported having seen or eaten Nangai previously. To determine the biological and clinical relevance of the cross-reactivity, histamine release (HR) test, skin prick test (SPT) and food challenge were used.

Results: There was prevalence for reactivity against Nangai in the group of pollen allergic patients. This cross-reactivity seems to be related--at least in part--to carbohydrate epitopes. Three out of 12 patients tested with Nangai were positive upon open challenge, but using double blind placebo controlled food challenge (DBPCFC) this could not be confirmed in two patients. The biological effects of Nangai on allergic patients were confirmed using HR and SPT.

Conclusion: The Nangai specific IgE found among pollen allergic patients addresses the need for control of new or changed foods before introduction to the market.

Assessment of the potential allergenicity of ice structuring protein type III HPLC 12 using the FAO/WHO 2001 decision tree for novel foods

Food and Chemical Toxicology 2003

C Bindslev-Jensen, E Sten, L K Earl, R W R Crevel, U Bindslev-Jensen, T K Hansen, P Stahl Skov, L K Poulsen

Link: https://pubmed.ncbi.nlm.nih.gov/12453731/

Abstract

The introduction of novel proteins into foods carries a risk of eliciting allergic reactions in individuals sensitive to the introduced protein. Therefore, decision trees for evaluation of the risk have been developed, the latest being proposed by WHO/FAO early in 2001. Proteins developed using modern biotechnology and derived from fish are being considered for use in food and other applications, and since allergy to fish is well established, a potential risk from such proteins to susceptible human beings exists. The overall aim of the study was to investigate the potential allergenicity of an Ice Structuring Protein (ISP) originating from an arctic fish (the ocean pout, Macrozoarces americanus) using the newly developed decision tree proposed by FAO/WHO. The methods used were those proposed by FAO/WHO including amino acid sequence analysis for sequence similarity to known allergens, methods for assessing degradability under standardised conditions, assays for detection of specific IgE against the protein (Maxisorb RAST) and histamine release from human basophils. In the present paper we describe the serum screening phase of the study and discuss the overall application of the decision tree to the assessment of the potential allergenicity of ISP Type III. In an accompanying paper [Food Chem. Toxicol. 40 (2002) 965], we detail the specific methodology used for the sequence analysis and assessment of resistance to pepsin-catalysed proteolysis of this protein. The ISP showed no sequence similarity to known allergens nor was it stable to proteolytic degradation using standardised methods. Using sera from 20 patients with a well-documented clinical history of fish allergy, positive in skin prick tests to ocean pout, eel pout and eel were used, positive IgE-binding in vitro to extracts of the same fish was confirmed. The sera also elicited histamine release in vitro in the presence of the same extracts. The ISP was negative in all cases in the same experiments. Using the proposed decision tree, we demonstrated the safety of the ISP to patients already sensitised to fish, as well as to individuals potentially susceptible to producing IgE responses to proteins. Furthermore, the practicability of the new decision tree was confirmed.

Cross-reactivity to eel, eelpout and ocean pout in codfish-allergic patients

Allergy 2004

E Sten, T K Hansen, P Stahl Skov, S B Andersen, Am Torp, U Bindslev-Jensen, C Bindslev-Jensen, L K Poulsen

Link: https://pubmed.ncbi.nlm.nih.gov/15461598/

Abstract

Fish allergy is one of the most common food allergies in both children and adults and patients with allergic reactions to one fish species have in many cases been given the advice to avoid all fish, without further evaluation. The possible common reactivity between different fish species is not well studied. Because of this and a possible exploitation of fish species hitherto not much used in the Scandinavian diet ocean pout,

eelpout and eel were evaluated. We examined the serological and biological cross-reactivity of these species in double-blind challenged-confirmed codfish-allergic patients using CAP, Maxisorp-radio allergosorbent test (RAST) inhibition, western blot, skin prick test (SPT) and histamine release (HR). All 18 codfish allergic patients had specific IgE to ocean pout, eelpout and eel determined by Maxisorp-RAST. All four fish species could induce basophil HR using blood from 16 of 18 patients and all patients tested reacted in SPT. This study demonstrates that patients with a verified clinical allergy to codfish in a high frequency express biological cross-reactivity to other fish species. By RAST inhibition this common reactivity was shown to be a true cross-reactivity.

A comparative study of the allergenic potency of wild-type and glyphosate-tolerant gene-modified soybean cultivars

APMIS 2004

Eva Sten, Per Stahl Skov, Sven B Andersen, Anna Maria Torp, Annette Olesen, Ulla Bindslev-Jensen, Lars K Poulsen, Carsten Bindslev-Jensen

Link: https://pubmed.ncbi.nlm.nih.gov/14961970/

Abstract

A large proportion of soybean cultivars grown in the USA are now genetically modified varieties and concern has been raised about the safety of these products for consumers. A study of the impact on allergenic potency in soybeans, comparable except for the newly introduced gene (CP4 EPSPS), was performed using soybean-sensitized patients. The allergenicity of 18 different (10 GM and 8 WT) soybean extracts was examined blindly by the following three methods: A) Sera from patients with specific IgE against soybean were used to determine concentrations inducing 50% RAST inhibition; B) Histamine release induced by the extracts was examined using blood from sensitized patients; C) SPT was performed on sensitized patients with all 18 extracts. All three methods showed variations in the allergenic potency between the individual extracts but allergenic potential was not affected by presence of the transgene. By using standard in vitro methods and SPT for determination of allergenicity we were not able to detect any significant difference in the allergenic potency between GM and WT soybeans.

Food allergy to apple and specific immunotherapy with birch pollen

Molecular Nutrition & Food Research 2004

Kirsten Skamstrup Hansen, Marianne Søndergaard Khinchi, Per Stahl Skov, Carsten Bindslev-Jensen, Lars K Poulsen, Hans-Jørgen Malling

Link: https://pubmed.ncbi.nlm.nih.gov/15508179/

Abstract

Conflicting results concerning the effect of specific pollen immunotherapy (SIT) on allergy to plant foods have been reported. The aim of this study was to investigate the effect of SIT using a birch pollen extract on food allergy with focus on allergy to apple. Seventy-four birch pollen-allergic patients were included in a double-blind, double-dummy, and placebo-controlled comparison of sublingual-swallow (SLIT) and subcutaneous (SCIT) administration of a birch pollen extract. Sixty-nine percent of these patients reported

allergy to apple. The clinical reactivity to apple was evaluated by open oral challenges with fresh apple and a questionnaire. The immunoglobulin E (IgE)-reactivity was assessed by skin prick test (SPT), specific IgE, and leukocyte histamine release (HR). Forty patients were included in the final evaluation of the effect of SIT. The challenges were positive in 9 (SCIT), 6 (SLIT), and 8 (placebo) patients after treatment compared to 10, 4, and 10 patients, respectively, before SIT. The symptom scores to apple during challenges decreased in all groups, but only significantly in the placebo group ($p = 0.03$). As evaluated by the questionnaire, the severity of food allergy in general did not change and there were no differences between the groups. In spite of a significant effect on seasonal hay fever symptoms and use of medication and decrease in IgE-reactivity, SIT was not accompanied by a significant decrease in the severity of allergy to apple compared to placebo. Therefore, oral allergy syndrome (OAS) to apple should not be considered as a main criterion for selecting patients for birch pollen immunotherapy at present.

Does absorption across the buccal mucosa explain early onset of food-induced allergic systemic reactions?

Journal of Allergy and Clinical Immunology 2005

Christina Glattre Dirks, Mona H Pedersen, Michael H Platzer, Carsten Bindslev-Jensen, Per S Skov, Lars K Poulsen

Link: https://pubmed.ncbi.nlm.nih.gov/15940158/

No abstract available

The effects of gastric digestion on codfish allergenicity

Journal of Allergy and Clinical Immunology 2005

Eva Untersmayr, Lars K Poulsen, Michael H Platzer, Mona H Pedersen, George Boltz-Nitulescu, Per Stahl Skov, Erika Jensen-Jarolim

Link: https://pubmed.ncbi.nlm.nih.gov/15696099/

Abstract

Background: In a recent murine study, we showed that impaired gastric digestion supports the induction of fish allergy by protecting the digestion-sensitive major allergen parvalbumin and thus enhancing its sensitizing properties.

Objective: The aim of the present study was to investigate whether impairment of peptic degradation might also play a role in the effector phase of codfish allergy.

Methods: The resistance of cod proteins to digestion by simulated gastric fluid was assessed in vitro . Gastric solutions with pH values ranging from 1.25 to 5.0 were prepared, and the influence of the pH on protein degradation was evaluated by means of SDS-PAGE and IgE immunoblotting. The allergenic potency of digested and undigested cod extract was further characterized in RAST inhibition and basophil histamine release experiments.

Results: The digestion experiments revealed that codfish proteins were degraded within 1 minute under physiologic gastric conditions. An only marginal pH shift from 2.5 to 2.75 abrogated completely the digestion of cod allergens. In RAST inhibition experiments digested cod extracts showed a reduced IgE-binding capability that was dependent on the digestion time. Moreover, peptic fragments expressed a 10,000 times reduced allergenic potency, as evaluated on the basis of histamine release from human basophils.

Conclusion: Codfish allergens have a grossly reduced ability to trigger an intestinal allergic reaction when they are physiologically degraded. Impairment of the physiologic digestion might thus lower the threshold levels of a food allergen in sensitized patients.

Incomplete digestion of codfish represents a risk factor for anaphylaxis in patients with allergy

Journal of Allergy and Clinical Immunology 2007

Eva Untersmayr, Helle Vestergaard, Hans-Jørgen Malling, Louise Bjerremann Jensen, Michael H Platzer, George Boltz-Nitulescu, Otto Scheiner, Per Stahl Skov, Erika Jensen-Jarolim, Lars K Poulsen

Link: https://pubmed.ncbi.nlm.nih.gov/17215033/

Abstract

Background: Fish represents one of the most important allergenic foods causing severe allergic reactions. Nevertheless, it has been shown that gastric digestion significantly reduces its allergenic capacity.

Objective: In this study, we assessed the absorption kinetics of fish proteins and investigated the clinical reactivity of patients with fish allergy to codfish digested at physiological or elevated gastric pH.

Methods: Healthy individuals were openly challenged with codfish and blood samples were evaluated by histamine release for absorbed fish allergens. Patients with allergy were recruited on the basis of previously diagnosed codfish allergy. Fish extracts were digested with gastric enzymes at pH 2.0 and 3.0 and used for histamine release, skin prick tests, and titrated double-blind placebo-controlled food challenges.

Results: Ingestion experiments in subjects without allergy revealed absorption of biologically active fish allergens only 10 minutes after ingestion with maximal serum levels after 1 to 2 hours. Incubation of fish proteins with digestive enzymes at pH 2.0 resulted in a fragmentation of the proteins leading to a reduced biological activity evidenced by a significantly smaller wheal reaction and reduced histamine release. Fish digested at pH 3.0 revealed comparable reactivity patterns as undigested extracts. Moreover, these test materials triggered reactions at 10-fold to 30-fold lower cumulated challenge doses in patients with allergy.

Conclusion: Our data indicate the paramount importance of gastric digestion for fish allergens because the quantitatively significant absorption and elicitation of symptoms seemed to take place in the intestine.

Clinical implications: Hindered digestion puts patients with fish allergy at risk to develop severe allergic reactions at minute amounts of allergens.

The biological activity of a recombinantly expressed (His)6-tagged peanut allergen (rAra h 1) is unaffected by endotoxin removal

Journal of Immunological Methods 2008

Louise Bjerremann Jensen, Anna Maria Torp, Sven Bode Andersen, Per Stahl Skov, Lars K Poulsen, Edward F Knol, Els van Hoffen

Link: https://pubmed.ncbi.nlm.nih.gov/18377922/

Abstract

The application of recombinant (His)(6)-tagged proteins in cell culture assays is associated with problems due to lipopolysaccharide (LPS) contamination. LPS stimulates cells of the immune system, thereby masking antigen-specific activation of T cells. Due to the affinity of LPS for histidine it is associated with difficulties to remove LPS from recombinant (His)(6)-tagged proteins. Here we describe that the Triton X-114 phase separation method can be used to remove LPS from (His)(6)-tagged proteins and that the recombinant proteins retain their biological activity.

Risk Assessment of Clinical Reactions to Legumes in Peanut-Allergic Children

World Allergy Organ journal 2008

Louise Bjerremann Jensen, Milene Andersen, Per Stahl Skov, Lars K Poulsen, Carsten Bindslev-Jensen

Link: https://pubmed.ncbi.nlm.nih.gov/23282674/

Abstract

Peanut-allergic children might be at risk for reactions to other legumes. However, it is not always possible to perform multiple oral food challenges in children. On the basis of patient case history, in vitro diagnostic tests, and eventually food challenges, we aimed at developing an algorithm for risk assessment of possible clinical reactions to other legumes (soybean, lupine, fresh, and blanched green pea). Seventy-five consecutive patients with a positive oral food challenge to peanut were included in the study. All tests were run as part of the routine allergy examination. A high proportion of patients and/or caretakers refused the administered legume oral food challenges. Obtained diagnoses from histamine release did not correlate significantly to the outcome of the algorithm. Interestingly, threshold from peanut challenges did not correlate with the risk assessment.The algorithm presented in this study can be used when advising peanut-allergic children and their caretakers about what other legumes to avoid in the diet.

Development of a hypoallergenic recombinant parvalbumin for first-in-man subcutaneous immunotherapy of fish allergy

International Archives of Allergy and Immunology 2015

Laurian Zuidmeer-Jongejan, Hans Huber, Ines Swoboda, Neil Rigby, Serge A Versteeg, Bettina M Jensen, Suzanne Quaak, Jaap H Akkerdaas, Lars Blom, Juan Asturias, Carsten Bindslev-Jensen, Maria L Bernardi, Michael Clausen, Rosa Ferrara, Martina Hauer, Jet Heyse, Stephan Kopp, Marek L Kowalski, Anna Lewandowska-Polak, Birgit Linhart, Bernhard Maderegger, Bernard Maillere, Adriano Mari, Alberto Martinez, E N Clare Mills, Angela Neubauer, Claudio Nicoletti, Nikolaos G Papadopoulos, Antonio Portoles, Ville Ranta-Panula, Sara Santos-Magadan, Heidi J Schnoor, Sigurveig T Sigurdardottir, Per Stahl-Skov, George Stavroulakis, Georg Stegfellner, Sonia Vázquez-Cortés, Marianne Witten, Frank Stolz, Lars K Poulsen, Montserrat Fernandez-Rivas, Rudolf Valenta, Ronald van Ree

Link: https://pubmed.ncbi.nlm.nih.gov/25765512/

Abstract

Background: The FAST (food allergy-specific immunotherapy) project aims at developing safe and effective subcutaneous immunotherapy for fish allergy, using recombinant hypoallergenic carp parvalbumin, Cyp c 1.

Objectives: Preclinical characterization and good manufacturing practice (GMP) production of mutant Cyp (mCyp) c 1.

Methods: Escherichia coli-produced mCyp c 1 was purified using standard chromatographic techniques. Physicochemical properties were investigated by gel electrophoresis, size exclusion chromatography, circular dichroism spectroscopy, reverse-phase high-performance liquid chromatography and mass spectrometry. Allergenicity was assessed by ImmunoCAP inhibition and basophil histamine release assay, immunogenicity by immunization of laboratory animals and stimulation of patients' peripheral blood mononuclear cells (PBMCs). Reference molecules were purified wild-type Cyp c 1 (natural and/or recombinant). GMP-compliant alum-adsorbed mCyp c 1 was tested for acute toxicity in mice and rabbits and for repeated-dose toxicity in mice. Accelerated and real-time protocols were used to evaluate stability of mCyp c 1 as drug substance and drug product.

Results: Purified mCyp c 1 behaves as a folded and stable molecule. Using sera of 26 double-blind placebo-controlled food-challenge-proven fish-allergic patients, reduction in allergenic activity ranged from 10- to 5,000-fold (1,000-fold on average), but with retained immunogenicity (immunization in mice/rabbits) and potency to stimulate human PBMCs. Toxicity studies revealed no toxic effects and real-time stability studies on the Al(OH)3-adsorbed drug product demonstrated at least 20 months of stability.

Conclusion: The GMP drug product developed for treatment of fish allergy has the characteristics targeted for in FAST: i.e. hypoallergenicity with retained immunogenicity. These results have warranted first-in-man immunotherapy studies to evaluate the safety of this innovative vaccine.

Is a positive intracutaneous test induced by penicillin mediated by histamine? A cutaneous microdialysis study in penicillin-allergic patients

Clinical and Translational Allergy 2017

Line K Tannert, Sidsel Falkencrone, Charlotte G Mortz, Carsten Bindslev-Jensen, Per Stahl Skov

Link: https://pubmed.ncbi.nlm.nih.gov/29177030/

Abstract

Background: Diagnostic workup of penicillin allergy comprises skin testing with penicillins, and patients are deemed allergic if skin test is positive. However, the literature suggests that skin test-positive patients may be challenge-negative, indicating that the skin test may be falsely positive.

Objective: To investigate real-time histamine release from a positive intracutaneous test induced by penicillin in patients with positive and negative challenges to penicillin.

Methods: Skin microdialysis was performed in 21 penicillin-allergic patients with positive skin test, 13 non-allergic volunteers serving as negative controls, and 7 grass pollen-allergic patients serving as positive controls. Histamine was measured by microdialysis after skin test with penicillin/grass/NaCl. Penicillin challenge was subsequently performed in 12 of the patients.

Results: Only 10/21 patients (47.6%) were skin test positive at microdialysis. During microdialysis 13 single intracutaneous tests were positive and histamine was detected in 4/13 occurring in four challenge positive patients. Thirteen/21 patients (61.9%) were deemed allergic to penicillin; eight had positive skin test. Two patients with positive skin test were challenge negative. In grass pollen allergic patients, 7/7 had a positive intracutaneous test to grass and all released histamine in the wheals. All 13 negative controls had negative intracutaneous test to penicillin and no histamine release.

Conclusion: Histamine was only detected in the minority of positive intracutaneous tests with penicillin in penicillin-allergic patients. Other mediators may be involved.

Keywords: Histamine release; Penicillin challenge; Penicillin intracutaneous test; Penicillin-allergy; Skin microdialysis.

Clinical relevance of sensitization to hydrolyzed wheat protein in wheat-sensitized subjects

Journal of Allergy and Clinical Immunology 2018

Morten J Christensen, Per Stahl Skov, Lars K Poulsen, Carsten Bindslev-Jensen, Charlotte G Mortz

Link: https://pubmed.ncbi.nlm.nih.gov/29031598/

No abstract available

High-dimensional immune profiles correlate with phenotypes of peanut allergy during food-allergic reactions

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Abstract

Background: Food challenges carry a burden of safety, effort and resources. Clinical reactivity and presentation, such as thresholds and symptoms, are considered challenging to predict ex vivo.

Aims: To identify changes of peripheral immune signatures during oral food challenges (OFC) that correlate with the clinical outcome in patients with peanut allergy (PA).

Methods: Children with a positive (OFC^+ , n = 16) or a negative (OFC^- , n = 10) OFC-outcome were included (controls, n = 7). Single-cell mass cytometry/unsupervised analysis allowed unbiased immunophenotyping during OFC.

Results: Peripheral immune profiles correlated with OFC outcome. OFC^+ -profiles revealed mainly decreased Th2 cells, memory Treg and activated NK cells, which had an increased homing marker expression signifying immune cell migration into effector tissues along with symptom onset. OFC^- -profiles had also signs of ongoing inflammation, but with a signature of a controlled response, lacking homing marker expression and featuring a concomitant increase of Th2-shifted $CD4^+$ T cells and Treg cells. Low versus high threshold reactivity-groups had differential frequencies of intermediate monocytes and myeloid dendritic cells at baseline. Low threshold was associated with increased $CD8^+$ T cells and reduced memory cells (central memory [CM] $CD4^+$ [Th2] T cells, CM $CD8^+$ T cells, Treg). Immune signatures also discriminated patients with preferential skin versus gastrointestinal symptoms, whereby skin signs correlated with increased expression of CCR4, a molecule enabling skin trafficking, on various immune cell types.

Conclusion: We showed that peripheral immune signatures reflected dynamics of clinical outcome during OFC with peanut. Those immune alterations hold promise as a basis for predictive OFC biomarker discovery to monitor disease outcome and therapy of PA.

Delayed reaction in alpha-gal allergy is reflected in serum levels after ingestion of pork kidney, and absorption is dependent on food processing

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Detection of Sensitization Profiles with Cellular In Vitro Tests in Wheat Allergy Dependent on Augmentation Factors (WALDA)

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Abstract

Wheat allergy dependent on augmentation factors (WALDA) is the most common gluten allergy in adults. IgE-mediated sensitizations are directed towards ω5-gliadin but also to other wheat allergens. The value of the different in vitro cellular tests, namely the basophil activation test (BAT) and the active (aBHRA) and passive basophil histamine-release assays (pBHRA), in the detection of sensitization profiles beyond ω5-gliadin has not been compared. Therefore, 13 patients with challenge-confirmed, ω5-gliadin-positive WALDA and 11 healthy controls were enrolled. Specific IgE (sIgE), skin prick tests, BATs, aBHRA, and pBHRA were performed with allergen test solutions derived from wheat and other cereals, and results were analyzed and compared. This study reveals a distinct and highly individual reactivity of ω5-gliadin-positive WALDA patients to a range of wheat allergens beyond ω5-gliadin in cellular in vitro tests and SPT. In the BAT, for all tested allergens (gluten, high-molecular-weight glutenin subunits, α-amylase/trypsin inhibitors (ATIs), alcohol-free wheat beer, hydrolyzed wheat proteins (HWPs), rye gluten and secalins), basophil activation in patients was significantly higher than in controls ($p = 0.004$-$p < 0.001$). Similarly, significant histamine release was detected in the aBHRA for all test substances, exceeding the cut-off of 10 ng/mL in all tested allergens in 50% of patients. The dependency of tests on sIgE levels against ω5-gliadin differed; in the pBHRA, histamine release to any test substances could only be detected in patients with sIgE against ω5-gliadin ≥ 7.7 kU/L, whereas aBHRA also showed high reactivity in less sensitized patients. In most patients, reactivity to HWPs, ATIs, and rye allergens was observed. Additionally, alcohol-free wheat beer was first described as a promising test substance in ω5-gliadin-positive WALDA. Thus, BAT and aBHRA are valuable tools for the identification of sensitization profiles in WALDA.