

Poster Group 2

Food Allergy I

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Effect of pH on thermal stability and IgE binding ability of whiff parvalbumin

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Background: The major allergen of most fish, beta-parvalbumin, is a calcium-binding sarcoplasmic protein which is described as cross-reactive. In Spain, where fish consumption is high, allergy against whiff is frequent. We aimed to determine the thermal stability of whiff (*Lepidorhombus whiffiagonis*) parvalbumin by circular dichroism spectroscopy (CD) and IgE ELISA inhibition experiments.

Methods: Parvalbumin was purified from a protein extract of whiff by a combination of chromatographical methods. Molecular mass was determined by ESI-QTOF and the partial amino acid sequence was obtained by NanoLC-MSMS-based peptide mapping. The effect of pH on thermal stability on native or calcium-depleted protein was determined by CD. Further, the IgE binding ability of the treated allergen was determined by ELISA inhibition assays with four patients' sera allergic to fish parvalbumins.

Results: Thirty milligrams whiff beta-parvalbumin were extracted from 100 g of fish. Mass spectroscopic analysis of the purified protein revealed the presence of only one peak at 11.6 kDa. The CD analysis of the calcium-bound protein revealed a remarkable thermal stability and refolding capacity. According to these observations the heat-treated protein was able to inhibit IgE binding of four patients' sera to the native protein by 100%. Calcium depletion induced a loss of the broad minimum at 222 nm but the IgE binding could still be inhibited by 100%. Additionally, the native protein under alkaline conditions showed a melting point at around 55°C and could inhibit the IgE binding to the native protein in a range of 60 to 100%.

Conclusion: Confirmed by ELISA inhibition assays, the calcium-bound whiff parvalbumin revealed a remarkable thermal stability. A decrease of the alpha helical content of the calcium-depleted protein did not reduce the IgE binding ability.

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Allergy to colostrum. Our unsuccessful investigation

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Introduction: Colostrum, a complex mixture of bioactives that promotes neonate growth, is the first milk produced by mammals for their young ones.

Case report: We present a 37 year-old man, without allergic respiratory symptoms who developed urticaria and facial angioedema minutes after drinking boiled colostrum produced by one of his cows. He denied previous intake of milk from that cow. Moreover he referred no problems drinking milk or derived products (including colostrum) from other cows. The patient denied any treatment given to the cow and he also denied suffering any drug allergy.

Objective: The aim of this study was to study the suggestive allergy reaction suffered by the patient after taking colostrum.

Material and methods: Patient was skin prick tested with a battery of common inhalant (pollens present in our area) and food allergens, including latex, Anisakis, milk and milk-derived proteins. Colostrum from two animals was provided. Two different fractions (soluble and seric proteins) were obtained from different colostrum (fresh and boiled). Protein profile of the extracts was evaluated by SDS-PAGE. Prick-Prick tests with different colostrum (fresh and boiled) were conducted. Specific IgE to milk and derived proteins was measured by CAP and direct ELISA. Immunoblot experiments with colostrum were also conducted.

Results: The skin prick test with aeroallergens and foods was negative in all cases. Skin tests with colostrum (prick-prick, and with our home-made extracts) were positive but only for the extracts made of the colostrum of the specific cow. More than 10 controls were negative. The measurement of specific IgE (CAP and direct ELISA) against milk and milk-derived, as well as against colostrum proteins was negative. SDS-PAGE experiments showed multiple bands in a molecular weight range of 10 and 100 kDa. No bands were detected by IgE immunoblotting experiments.

Conclusions:

- We present a patient who developed

generalised urticaria and angioedema, immediately after the intake of colostrum of a specific cow.

- The positive skin prick test to the colostrum point out an IgE mediated hypersensitivity to a/some protein/s contained in that colostrum but not in others.
- Nevertheless the clinical history, a specific IgE against colostrum was detected in our patient serum.
- In spite of our study we have not been able to identify the responsible of that reaction suffered by our patient.

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Egg allergy and Hodgkin's lymphoma. Case report

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We report a case of a 17 years old woman. She referred recurrent oral allergy syndrome since she was eleven. At 14 years old she began recurrent eyelid angioedema. After an episode of glottis oedema related to cake ingestion, she decided to avoid cakes and pastry. She was referred to our allergy department because she maintained recurrent angioedema. When asked, boiled egg tolerance was reported. Her personal history included Hodgkin's lymphoma at 11 years old, in remission after 6 months of chemotherapy, and grasses allergic rhinoconjunctivitis since 14 years old. The immunoallergic study revealed: normal cell counts, serum immunoglobulins, complement fractions, lung function and chest X-ray. The paranasal sinus X-ray showed maxillary sinusitis. It was performed SPT with a battery of common aeroallergens (ALK Abelló, Madrid, Spain), food commercial extracts (Leti, Madrid, Spain) and fresh egg. The table presents the cutaneous and serum specific IgE reactivity.

Extracts	Cutaneous	Cutaneous	Serum specific IgE (KUI/L)
	specific IgE: prick (mm)	specific IgE: prick- prick (mm)	
Histamine	10	–	–
Grasses	10	–	–
Lolium perenne	–	–	21.9 (class 4)
Cat	8	–	1.3 (class 2)
Whole egg	6	–	–
Egg white	12	–	0.9 (class 2)
Yolk egg	3	–	<0.35
Ovalbumine	6	–	0.99 (class 2)
Ovomucoid	–	–	<0.35
Wheat	–	–	<0.35
Soybean	–	–	<0.35
Cow's milk	Negative	–	–
Flours	Negative	–	–
Boiled egg white	–	–	–
Boiled yolk egg	–	Negative	–
Raw egg white	–	Negative	–
Raw yolk egg	–	16	–
		6	

Discussion: This case documents allergy to raw egg, namely to ovalbumine, that is a thermolabile egg protein. This could be present in food as an occult allergen, what justifies the clinical recurrent angioedema besides egg avoidance. Egg-allergy-related symptoms began soon after the diagnosis of Hodgkin's lymphoma. We hypothesizes that Hodgkin's disease and/or its treatment could had induced immunological suppression that predisposed to egg sensitisation. This case of late and persistent egg allergy is probably related to Hodgkin's disease, in this patient.

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The allergenic potential of wine fined with milk, egg or fish proteins

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Objective: International legislation requires labelling of wines in which potentially allergenic food proteins (casein, egg white, lysozyme, isinglass) have been used in their production. We investigated the allergenic potential of wines fined using such proteins. **Methods:** Wines fined with known concentrations of milk, egg or isinglass (fish

protein) were obtained or produced. Milk and egg protein detection was performed by ELISA. The presence of genetic material from fish was evaluated by PCR. Skin prick tests (SPTs) were performed with the wines in patients with confirmed allergy to milk ($n = 4$), egg ($n = 4$) or fish ($n = 5$) respectively. Basophil activation provoked by wine extracts was determined with Basotest in heparinized blood of selected individuals. Double blind placebo food challenges (DBPCFC) were performed in two cases.

Results: Milk proteins were detectable in all milk-fined wines ($n = 7$). In contrast we could not detect egg proteins in an egg-fined wine or fish genetic material in 3 fish-fined wines. SPTs were positive in 100%, 25 and 40% patients of milk, egg or fish containing wines respectively. Basophil activation was not correlated with wine type, processing aid or subject group. No allergic symptoms were induced after blinded consumption of wine in a milk and a fish allergic patient.

Conclusion: Milk, egg white, or fish proteins used to fine wines, are detectable in a considerable number of cases in skin prick tests; however neither history or challenge reveal clinical reactions. Therefore, wine-finishing proteins seem to represent a low risk in allergic consumers. Nevertheless, further investigation is planned to ascertain whether there is any need for labelling wine processed with allergenic proteins.

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IgE-mediated Allergy to raw cabbage but not to cooked. Cross reactivity clinical study

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Background: IgE mediated allergy to cabbage is the most common and important vegetable allergy in our country.

Objective: To study the clinical features of cabbage allergy-cross reactivity among raw and cooked extracts of Brassica oleracea varieties (cabbage, cauliflower, broccoli) mustard, plant foods and pollens.

Methods: Six allergic to raw cabbage patients participated in this study. Skin prick tests (SPTs) to food allergens, pollens and especially with raw and cooked prepared Brassica oleracea members and mustard were performed. Total IgE and specific IgE to cabbage was determined by CAP method.

Results: Four of the six patients referred anaphylactic reaction whenever eating *only* raw cabbage. On the contrary all patients tolerated cooked cabbage, cooked cauliflower, cooked broccoli, and mustard. Two of the six patients suffered only from oral allergy syndrome with raw cabbage and

tolerated all the aforementioned vegetables. All the patients were atopic (5/6 with Allergic Rhinitis) and had positive SPTs to most common aeroallergens (6/6 had positive SPTs to mugwort pollen). SPTs with raw extracts of cabbage, cauliflower, broccoli, were strong positive whereas all SPTs were negative with the same cooked extracts in all patients. Specific IgE to cabbage was positive in all patients. Four of six patients had also positive SPTs to mustard but no one referred any allergic reaction consuming mustard.

Conclusion: Allergic patients to cabbage are atopic and polysensitized to aeroallergens (mugwort) and food allergens (peanut, tree nuts, and peach). The most impressive finding is that the responsible allergen of cabbage (possibly a lipid transfer protein Bra o 3) seems to be destroyed with the heating (cooking). This became obvious by the negative SPTs to cooked Brassica oleracea, and by the ability of the patients to consume cooked cabbage, broccoli, and cauliflower without any allergic reaction. The cabbage is the only member of the Brassica oleracea family that is consumed raw as salad. On the contrary cauliflower and broccoli are consumed only cooked in our country, so the culprit allergen is possibly destroyed and this is maybe the reason why IgE mediated allergic reactions to these vegetables are infrequently referred in the bibliography. Although cross reactivity with mustard could be suggested by the positive SPTs, this sensitisation was not clinical important because all the patients consumed mustard without any allergic reaction.

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Differences among tomato sensitised subjects according to their pattern of co-sensitisation to fruits

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Background: Cross-reactivity between tomato and pollens or other fruits has been described. Several studies have demonstrated the implication of different families of panallergens as responsible for this phenomenon. Patients sensitised to cross-reactive allergens may show positive skin prick test to a number of different extracts. **Aim:** To study the differences among tomato sensitised subjects, according their pattern of co-sensitisation to other fruits.