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C. Richard[1], S. Jacquenet[1], D.A. Moneret-Vautrin[2]

A plausible allergy to peanut revealed only by Immunoblot

[1]Genclis SAS, Vandoeuvre-Lès-Nancy, France
[2]Lorraine-University, Nancy. Durkheim Hospital, Epinal, France. AllergyVigilance Network, Vandoeuvre-Lès-Nancy, France

Corresponding author
Christelle Richard
Genclis, 15 rue du bois de la Champelle
54500 Vandoeuvre-Lès-Nancy, France
Tel: +33 03 83 67 82 11
Fax: +33 03 83 67 89 99
E-mail: c.richard@genclis.com

Peanut allergy is currently linked to sensitization to major allergens. Ara h 2 is the leading one and is the best predictor of allergy, since a level > 0.23 kU/L is observed in 93% of cases, with a specificity of 97% (1). A level of Ara h 2-specific IgEs > 0.55 kU/L has an absolute specificity and sensitivity of 93% of cases in adults (2). In young children aged less than 15 months, sensitivity and specificity have been calculated at 73% and 95% (3). Most peanut allergens are glycosylated. Sensitization to carbohydrate determinants (CDD) is frequent in pollinic patients and cases of hymenoptera anaphylaxis (4,5). Such anti-CCD sIgE could explain positive ImmunoCAP to peanut, in peanut tolerant patients (6,7). It has been assumed that sensitization to CCDs is not clinically relevant (6-8). For this reason, we present a rare case of plausible peanut allergy characterized by a mono-sensitization to CCDs.

A 49 years old female presented in 2012 with serious systemic reaction including abdominal pain, vomiting, erythema, vertigo and sudation, 2 hours after ingesting approximately 4 g of Curly® (containing 59% maize flour and 30% peanut flour), together with 2 glasses of rum-based cocktail and wine. She had a rather specific past history, since she was not atopic. Prick tests to 13 common aeroallergens were negative. However, she had semi-delayed anaphylaxis to mammal meats linked to sensitization to alpha-galactose that had been diagnosed in 2010. Recovery was obtained by avoidance diet, excluding mammal meats, pork and beef kidney and milk proteins with alcohol (9,10). Specific IgEs gradually decreased (**table 1**). She also experienced anaphylaxis to wasp venom in 2011, linked to sensitization to Ves v 5. She had concurrently anti-CCD IgEs (**table 1**). She is treated by specific immunotherapy at present.

Prick tests to natural roasted peanut, to peanut commercial extract (Stallergenes), to maize flour and to soy were negative on a skin normally reacting to 9% codeine phosphate. Total IgEs were 149 kU/L. No IgEs were detected to peanut and recombinant allergens: rAra h 1, rAra h 2, rAra h 3, rAra h 8, rAra h 9 (ImmunoCAP system, Thermo Fisher). Furthermore, sIgE to rAra h 6 and rAra h 7 were determined by ELISA test performed in the Genclis lab, and were negative. A basophil activation test to peanut extract (made from peanut flour, Byrd Mill) was then performed by flow cytometry identifying CD63 and was positive with a stimulation index > 2 for three concentrations of peanut extract.

The patient gave her informed consent for an open oral challenge with Curly. She tolerated 14.3 g of the product (peanut protein equivalent 3.3 g). She refused our offer to repeat the test with alcohol.

Protein extracts of peanut and Curly® were prepared. Specific IgE to CCDs were screened using FEIA CAP system (bromelain and HRP), and DPC to ascorbate oxydase. Only CAP to bromelain was positive (4.84 kU/L). Three inhibition tests were performed using 100 to 1000 µg/mL of protein as inhibitors (**figure 1**). Specific IgE to bromelain were inhibited: 30% by

Table 1 *- Laboratory results (specific IgE)*

Year	Beef meat (ImmunoCAP)	Pork meat (ImmunoCAP)	Alpha-galactose (Home made ImmunoCAP)	Ves v 5 (ImmunoCAP)	Bromelain (ImmunoCAP)	Ascorbate oxidase (DPC)
2010	16.3 kU/L	28.7 kU/L	209 kU/L	14.0 kU/L	4.84 kU/L	Negative
2011	8.3 kU/L	8 kU/L	54.3 kU/L	2.28 kU/L		
2012			31.7 kU/L			
2013			20.0 kU/L			

bromelain 1000 µg/mL, 32% by peanut extract at 500 µg/mL (51% at 1000 µg/mL) and 57% by Curly® extract at 100 µg/mL (91% at 500 µg/mL). Immunoblot assays were performed with peanut, Curly® extracts and bromelain (**figure 2**). 70 kDa proteins were not recognized by specific IgE since they bound to anti-human IgE. Different proteins were recognized by the IgE in peanut and Curly® extract. Bromelain (22.8 kDa) was recognized by IgE. Inhibition tests were performed using peanut and Curly® extracts. All the specific proteins recognized by IgE in peanut and Curly® extract, as well as bromelain, were inhibited by peanut and Curly® extract. So, the patient's sIgE recognizing CCD of bromelain were inhibited by CCD present in peanut and Curly® extract. This might indicate that CCDs were clinically relevant in this case. However, we cannot exclude sensitization to buried protein epitopes, not available to IgE binding in the commercial extract as well in native peanut flour and for prick test. The fact that the oral challenge was negative to 3.3 g of peanut proteins does not exclude allergy in our opinion, since the oral challenge was not associated to alcohol. Alcohol is a well-known risk factor for food anaphylaxis, since it promotes intestinal hyperpermeability and moreover brings more carbohydrate residues (8,11). Unfortunately, the patient declined an oral challenge with peanut and alcohol. To conclude, in this case of allergy to peanut, the protein epitopes of seven recombinant peanut allergens were not involved. Cross-sensitization to CCDs between bromelain, peanut and Curly® was demonstrated. CCDs were only slightly clinically relevant since peanut allergy was not confirmed by oral challenge with Curly® under basal conditions and the reaction was only elicited when alcohol was associated with Curly®. Such cases are rare, since only 2/78 peanut-allergic patients may be linked to the presence of anti-CCD IgE (12).

Figure 1 *- Specific IgE inhibition to bromelain (k202) performed by FEIA using a Curly® extract (circles), peanut extract (triangles) and bromelain (diamonds) as inhibitors.*

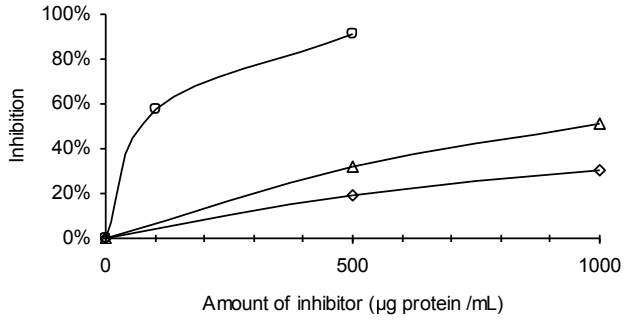
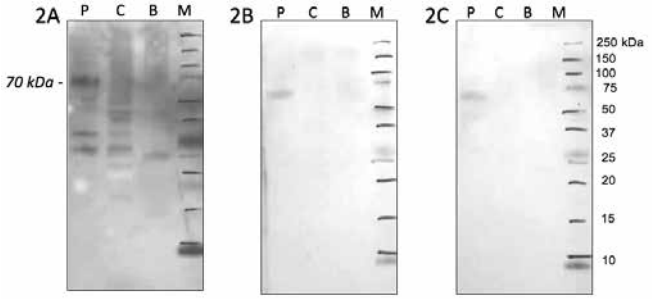


Figure 2 *- **2A:** Immunoblot with peanut extract (lane P), Curly® extract (lane C) and bromelain (lane B). **2B** - Immunoblot inhibited by peanut extract. **2C** - Immunoblot inhibited by Curly® extract.*



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