

Case report

Anaphylactic reaction caused by neoallergens in heated pecan nut

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An atopic girl experienced an anaphylactic reaction after eating cookies containing pecan nuts. Investigations revealed that she had developed IgE antibodies exclusively specific against allergenic determinants present in aged or heated pecan nuts, but not in fresh pecans. These neoallergenic determinants were located on protein(s) with a molecular weight of approximately 15 kDa. Neoallergens appearing during heating or storing of foods may be important in some anaphylactic reactions.

Key words: food anaphylaxis; neoallergens;
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Most food allergens are native proteins whose allergenic properties are either destroyed or unaffected by heating (6). However, the occurrence of neoallergens which appear as a result of domestic handling, such as cooking, is possible. Interestingly, Küstner of the classical Prausnitz-Küstner test was allergic to cooked fish, but not to raw fish (3).

We present the case of a child sensitized to heated pecan nuts with IgE antibodies directed exclusively to neoallergens.

Case report

Our patient was an 8-year-old girl with previous oral reactions to pineapple and tomato and a maternal family history of atopic eczema. Within 15–20 min after eating a cookie (Karen's Creole Candies: "PRALINES", A Creole Confection, the New Orleans School of Cooking and Louisiana General Store, New Orleans, LA, USA), she vomited and had an outbreak of hives. She was taken to the emergency clinic, where generalized urticaria and wheezing were diagnosed. Her blood pressure was 100/72 mmHg. For unknown reasons, she did not receive epinephrine; nevertheless, the symptoms subsided after treatment with intravenous methylprednisolone and theophylline.

The list of ingredients revealed that the cookies

contained pecan nuts, refined sugar, brown sugar, butter, and milk. Since the reaction, the patient has eaten, without any symptoms, all of these ingredients except pecan nuts.

Material and methods

Serum levels of IgE and IgE antibody were determined with an enzyme immunoassay (Pharmacia CAP System FEIA, and Phadezym RAST, Pharmacia, Uppsala, Sweden) following the instructions of the manufacturer. Fresh, heated, and stored pecan nuts were extracted 1/10 w/v in 0.15 M saline containing 0.02% NaN₃ and coupled to CNBr-activated filter paper disks (1) at a volume of 10 µl extract per disk. The disks were negative (< 0.35 PRU/ml) when tested against normal serum and serum with high (1000 kU/l) IgE concentration. SDS-PAGE, followed by immunoblotting, was performed essentially as already described (2), using a low-molecular-weight protein marker (LMW Electrophoresis Calibration Kit, Pharmacia, Uppsala, Sweden).

Results

The patient was skin prick tested with the following materials moistened in physiologic saline: the cookie; pecan nuts from the cookie; cotton seed; peanut;

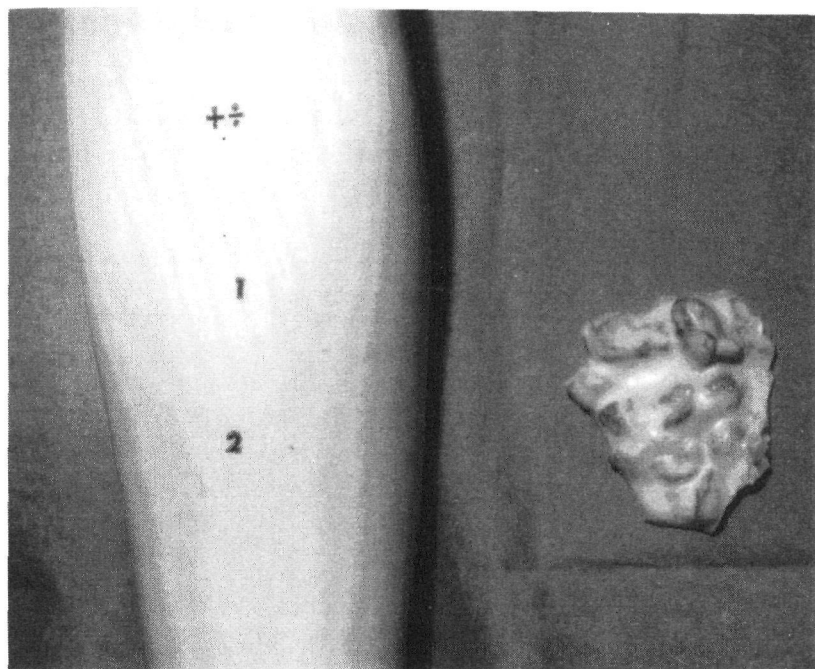


Fig. 1. Skin prick tests were positive to extract in saline of cookie (no. 1) and of pecan nuts from cookie (no. 2). Five nonallergic patients tested did not react. Controls: histamine dihydrochloride 10 mg/ml (+ left) and physiologic saline (÷ right). On right, piece of cookie is shown.

pea; and flours of wheat, rye, barley, and oats. She reacted only to the cookie, and to pecan nuts from the cookie, with a wheal of 5 × 5 mm (Fig. 1). Histamine dihydrochloride 10 mg/ml gave a similar reaction.

Her serum IgE was 630 kU/l, and the Pharmacia CAP System test to pecan was negative in a sample taken just after the reaction. The test was negative

Table 1. Results (in PRU/ml) of serum of patient tested in RAST against various cookies containing pecan nuts

Brand name of cookie	PRU/ml
Pralines*	70
Pecan Supremes	25
Pecan Sandies	23
Chocolate Chunk Pecan	27
Turtles Milk Chocolate Pecan	26

* Cookie causing clinical reaction.

Table 2. Results (in PRU/ml) of serum of patient tested in RAST against extracts of pecan nuts of various origin

Origin	Whole nuts/kernels	PRU/ml	Comments
1. Health food store	Kernels	90	
2. USA	Whole nuts	<0.35	
3. South Africa	Kernels	40	
4. South Africa	Whole nuts	<0.35	
5. Same as no. 4	Whole nuts	59	Roasted in laboratory
6. Australia	Whole nuts	4.1	
7. Same as no. 6	Whole nuts	48	Roasted in laboratory
8. USA	Whole nuts	11	
9. Same as no. 8	Whole nuts	70	Roasted in laboratory

in repeated samples drawn weeks and months afterwards.

RAST allergen disks produced with extracts of various brands of pecan cookies (Table 1) and pecan nuts of various origins (Table 2) revealed that the patient's serum invariably reacted weakly or not at all to whole fresh nuts but strongly to cookies with pecan and to pecan kernels, especially if they had first been heated ("roasted") in the laboratory.

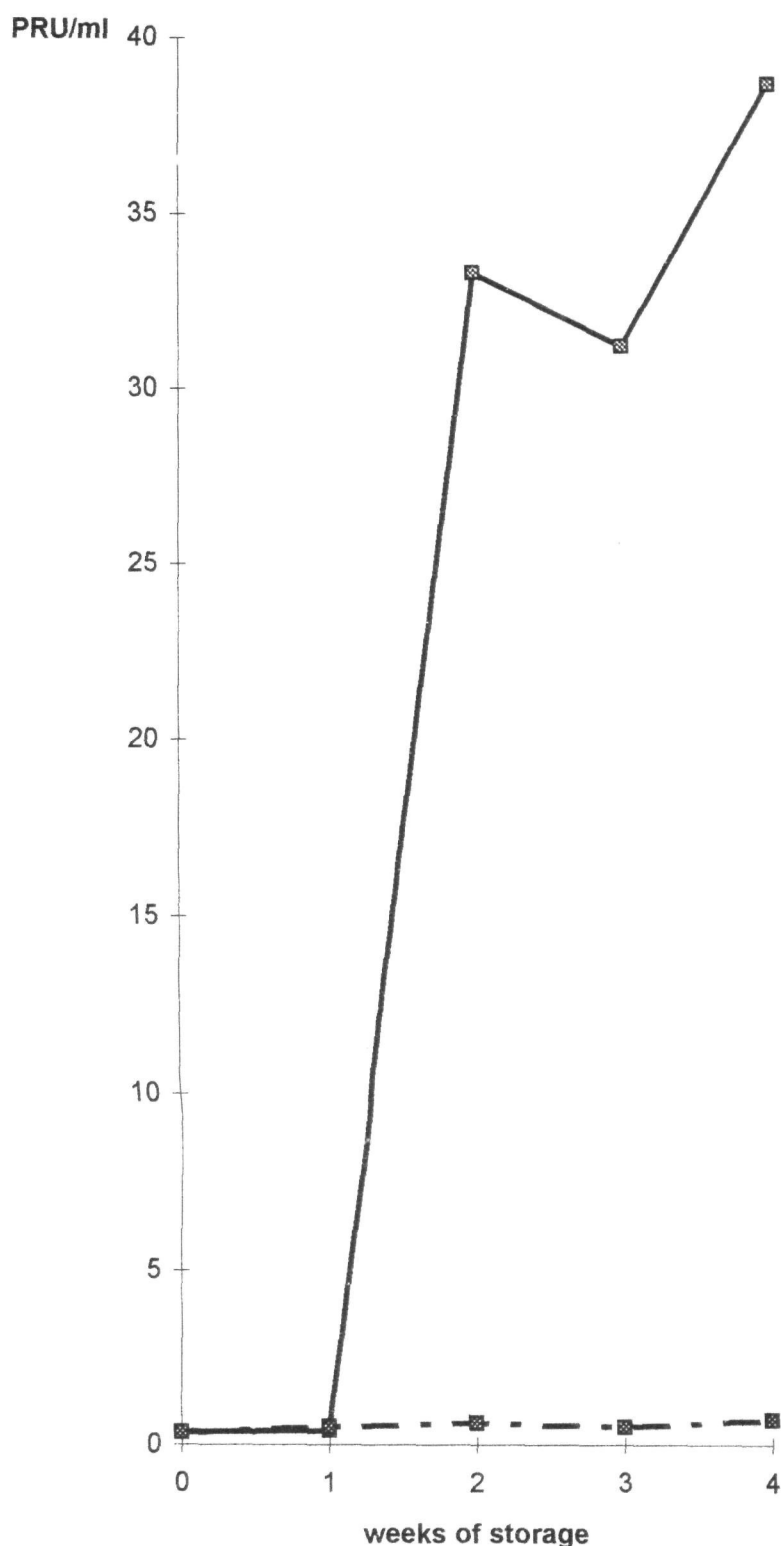


Fig. 2. Whole pecan nuts were stored at 30°C for 0–4 weeks, cracked, extracted 1/10 w/v in 0.15 M saline, and coupled to RAST disks. Serum from patient (solid line) drawn 7 months after anaphylaxis reacted strongly in RAST only to pecan stored for 2 weeks (33 PRU/ml) or more, while another pecan-positive serum (dashed line) showed only very small increase (from 0.6 PRU/ml at 0 weeks to 1.4 PRU/ml at 4 weeks). Control sera were negative.

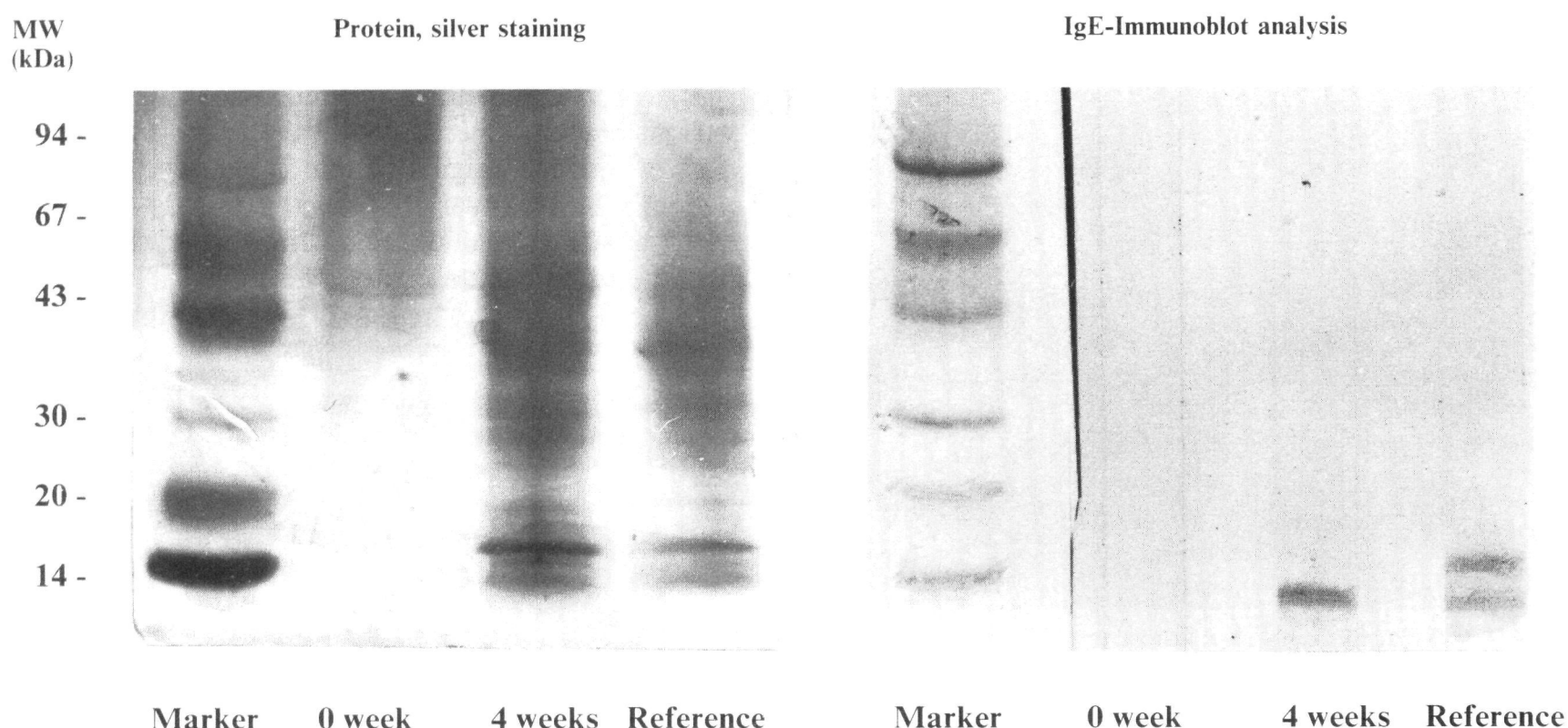


Fig. 3. SDS-PAGE and immunoblotting of extracts described in Fig. 2 revealed that protein pattern (left) cleared up on storing and that two IgE-binding bands of approximately 15 kDa appeared. Bands were generally weak because of insufficient allergenic material. Marker = molecular weight markers. Reference = extract no. 1 in Table 2.

Samples drawn at 1–13 months after the clinical reaction and tested against the updated Pharmacia CAP System pecan (Rf 201) showed positive results, varying randomly between 62 and 94 kU/l. Aging, in the form of storage at 30°C, of pecan nuts revealed that the allergenic determinants in question appeared within a couple of weeks (Fig. 2). Heating or aging of pecan RAST disks did not create any detectable neoallergens. These neoallergenic determinants were located on protein(s) with molecular weight of approximately 15 kDa (Fig. 3).

Discussion

Our patient developed IgE antibodies exclusively to allergenic determinants not present in fresh pecan nuts but appearing during aging and heating of the nuts. The pecan nuts and kernels sold in grocery stores are in this sense not fresh enough since they contain the neoallergen(s). Her IgE response was very high, of the order of 60–90 PRU/ml (RAST class 5). Initial Pharmacia CAP System analyses failed to detect IgE antibodies to pecan. The ImmunoCAPs were produced from fresh pecan nuts. The neoallergens to which our patient reacts are now included in the Pharmacia CAP System pecan (information from Pharmacia Diagnostics).

Heating is generally believed to decrease the allergenicity of food proteins. However, it is possible that, instead, new, probably conformational aller-

genic determinants are created. Their conformational nature is shown by the finding that they did not develop when the allergenic proteins were covalently coupled to a solid phase.

It is rare that an individual is sensitized only to such neoallergens. We have found in the literature mentions of only two patients reacting exclusively to heated food, but not to raw food. One reference concerned fish (3) and the other shrimp (4).

It is remarkable how suddenly during storage these neoallergens appeared, and also that the patient was so strongly sensitized to these neoallergens, but not to any allergen in fresh pecan nuts.

The neoallergens have about the same molecular weight as many major allergens. Perhaps this is the reason for their high immunogenicity.

The possible presence of neoallergens in food should be remembered, especially in cases of "idiopathic anaphylaxis". Some of these cases are certainly caused by hidden exposure to food(s) to which the patients are already known to be allergic (5), but others are possibly caused by neoallergens. Therefore, patients should be tested with extracts of food(s) possibly involved, prepared in a similar way to those which were ingested, in addition to extracts of fresh raw material. It would be of value if commercially available diagnostic reagents for *in vivo* or *in vitro* diagnosis of food allergy contained information about the state, whether native or cooked, of the raw material used.

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