

A Case of Peanut Cross-Allergy to Lupine Flour in a Hot Dog Bread

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Key Words

Allergy · Hidden allergen · IgE · Lupine · Norwegian
National Register for Severe Allergic Reactions to Food ·
Peanut

Abstract

Background: In a case monitored by the Norwegian National Register for Severe Allergic Reactions to Food, a patient with peanut allergy experienced an allergic reaction after eating a particular brand of hot dog bread. The aim of this study was to identify the eliciting allergen. **Methods:** Extracts from the hot dog bread and reference material from peanut, lupine and lupine-fortified food products were analysed by immunochemical methods with patient serum and a new polyclonal anti-lupine antibody. **Results:** Evidence could be provided that the hot dog bread contained proteins from lupine but not from peanut. **Conclusion:** Crossed peanut-lupine allergy can have clinical significance. A peanut-allergic patient reacted against hidden lupine protein in a hot dog bread. Presented with our results, the producer confirmed the use of lupine flour and changed the ingredient list.

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Introduction

Peanut is one of the ‘big eight’ major food allergens and is seen as a major health concern because of the high frequency of fatal anaphylactic reactions [1]. Cross-allergic clinical reactions to other members of the legume family, like soy, peas, beans and lentils, occur in about 5% of peanut-allergic patients [2], but were found to be 68% with lupine, an ancient cultivated plant that has recently been introduced into processed foods [3, 4]. ‘Sweet lupine’ strains, like *Lupinus albus*, *Lupinus luteus*, *Lupinus angustifolius*, and *Lupinus mutabilis*, are used as protein sources in food, such as bread, cookies or pasta. In several European countries, like France, the Netherlands, Italy, Spain and Germany, lupine-fortified food products are increasingly distributed, especially in bakeries’ goods which can contain up to 10% of lupine flour, and in dietary and health foods [5]. In Norway, lupine products are unknown so far, but in an actual case from the National Register for Severe Allergic Reactions to Food [6], it was discovered that a patient with peanut allergy reacted against an unfamiliar allergen in a particular brand of hot dog bread. A sample purchased at random at a local store was analysed with patient serum and a new polyclonal anti-lupine antibody.

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Material and Methods

Patient's History

A 24-year-old woman had experienced acute swelling of the lips, urticaria and rhinoconjunctivitis on four separate occasions when eating a certain brand of hot dog bread. She was found to be skin prick test positive (+++) to cat, birch and grass pollen, and negative to *Alternaria*, *Cladosporium*, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, dog, *Artemisia vulgaris* and hazel pollen.

Identification of Specific IgE in the Patient Serum

Patient serum and pooled control serum were analysed with the Pharmacia UniCAP® System (12 food allergens). Additionally, the sera (diluted 1:20) were tested on a dot blot matrix with 150 different allergens (protein extracts from Allergon AS, Sweden). Among these, especially soy, yeast, milk, barley, rye, sesame, oat, wheat, and peanut were of interest in this case.

Ingredients of the Hot Dog Bread

According to the ingredient list, the hot dog bread contained wheat flour, water, yeast, sugar, margarine, soy protein, milk powder, fibre, salt, emulsifiers E471 (mono- and diglycerides of fatty acids), E472e (mono- and diacetyl tartaric acid esters of mono- and diglycerides of fatty acids), E322 (lecithins) and E412 (guar gum), acidic modulators E330 (citric acid), E262 (sodium acetates), E260 (acetic acid), E920 (L-cysteine), and flour additive E300 (ascorbic acid).

Production of Polyclonal Anti-Lupine Antibodies

Lopino®, a crude protein preparation from *L. albus* seeds, was homogenised in 100 mM Tris-glycine/1 mM dithioerythritol (DTE, Aldrich), pH 8.7 (w/v 1:8), extracted overnight (45 °C) and centrifuged (20 min, 15,000 g, 4 °C). Proteins in the supernatant were precipitated (65% ammonium sulphate), centrifuged (15 min, 10,000 g, 4 °C), re-suspended (50 mM Tris/1 mM DTE pH 8.0), dialysed against water, freeze-dried and dissolved in PBS. A chinchilla rabbit was injected subcutaneously with 1.5 ml of a diluted (1:1 Freund's complete adjuvant) 200 µg/ml protein solution. After a second (Freund's incomplete adjuvant, week 4), and a third dose (50 µl, week 5), the rabbit was finally bled in week 9, and the serum centrifuged for further use.

Identification of Allergens

Food extracts (100 mM Tris-glycine extraction buffer pH 8.7 (w/v 1:5), 45 °C overnight), purified lupine, peanut protein, and a protein marker (ProSieve 10 – 190 kD, Cambrex) were separated on a 12% sodium dodecyl sulphate-polyacrylamide gel (SDS-PAGE) (Mini Protean 3 cell, Bio-Rad 165-3301), and stained with 0.1% Coomassie brilliant blue R-250. Western blot analysis (Mini Trans-Blott cell, Bio-Rad 170-3935, nitrocellulose membrane, blocking with 1% BSA, 50 mM TBS pH 8.3), were performed with either polyclonal anti-lupine serum, anti-peanut serum (both diluted 1:1,000,000) or patient serum (diluted 1:5) in the first incubation (4 °C, overnight). In the second step, either polyclonal goat anti-rabbit antibodies (Zy-Max™ 81-6120: IgG (H + L), HRP-conjugate 1.5 mg/ml, 1:5,000, 2 h, room temperature) or polyclonal rabbit anti-human IgE (HRP-conjugate; Dako P0295, diluted 1:1,000) was used. After adding tetramethyl benzidine solution (Zymed, 00-2019), positive signals developed within about 2 or 10 min, respectively.

Results

Analysis of the patient serum showed an anti-peanut IgE concentration of 8.70 kUA/l (class 3). The only positives on the extended test matrix were peanut and sugar beet.

The proteins in the food extracts were analysed by correlation to a protein weight marker and to purified lupine (80, 50, 40, 33, 25, 18, 15 kD) and peanut proteins (80, 45, 43, 40, 19 kD) (fig. 1a). Western blot analysis of the same extracts with polyclonal anti-lupine serum revealed signals at 65, 60, 50, 40, 38 and 33 kD in the hot dog bread from this case, while nothing was identified in a hot dog bread of a different brand or in four different kinds of bakery bread. From the positive controls, purified lupine protein and Lopino® produced the strongest signals (80, 65, 50, 40, 38, 33, 25, 15 kD), whereas Lupipan®, Lupisan®, lupine-sausage and lupine-pastry showed bands of lesser sharpness and intensity at 65, 50, 40, 33, 25, and 15 kD. In the peanut extract, two very weak bands (43, 40 kD) were detected, the control β-casein was negative (fig. 1b).

The analysis with the polyclonal anti-peanut serum revealed strong signals with the peanut extract at 75, 45, 43, 40, and 19 kD, and weak unspecific binding to two bands with high protein amounts in purified lupine protein and Lopino (fig. 1c).

The patient serum reacted with the peanut extract (80, 65, 33, 19, 17 kD), and with proteins in the of case hot dog bread (38, 25 kD), the purified lupine preparation (80, 65 kD) and the two lupine flours Lupipan® (65 kD) and Lupisan® (65, 25, 23, 15 kD) (fig. 2). The control serum gave no reaction.

Discussion

Hidden allergens in processed foods represent a severe health risk for patients with food allergies [7]. Food alert systems and allergy registers have recently been installed in some countries [8]. Since dietary vigilance is the only precaution possible, complete and correct labelling is important and therefore an actual issue with the Food Authorities. In Europe, mandatory labelling of twelve important allergens, independently of their actual amount in the food, is in preparation [9]. Among these is peanut which causes a high rate of fatal incidents [10], while lupine, the only legume reported to have a high cross-reactive rate of clinical significance to peanut, has been omitted [11, 12]. Since lupine was officially admitted as a food ingredient in France in 1997, its use in bakery goods, pas-

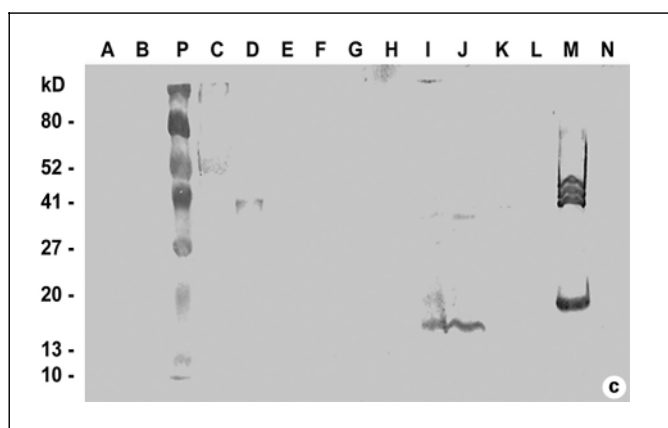
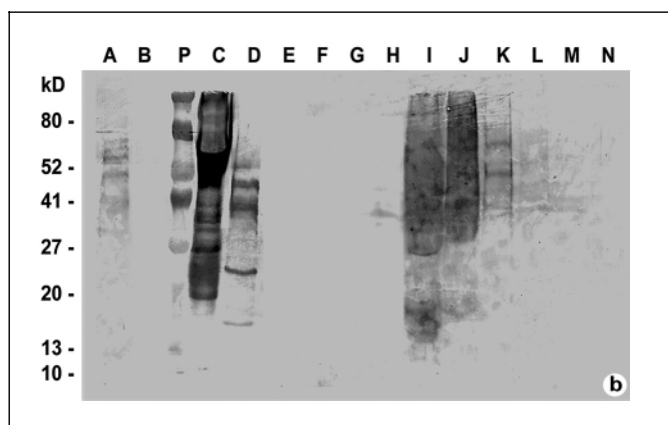
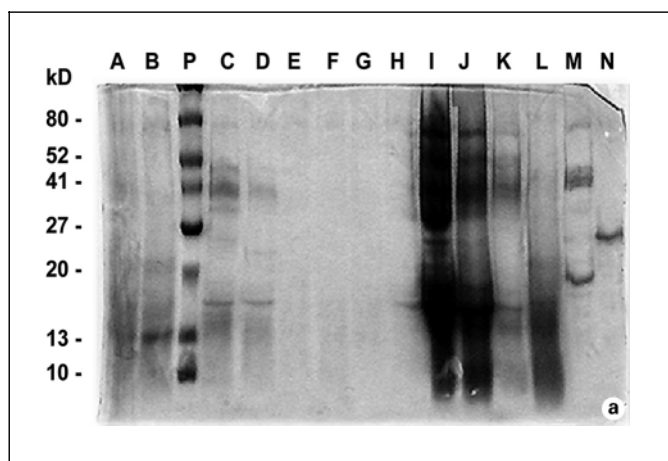


Fig. 1. A = Hot dog bread consumed by case; B = different brand hot dog bread; P = pre-stained protein marker; C = purified lupine protein; D = Lopino®; E = wheat bread; F = corn-blended bread; G = dark rye bread; H = mixed bread containing milk; I = Lupipan®; J = Lupisan®; K = sausage with lupine protein; L = pastry with lupine; M = purified peanut protein, β -casein. **a** Coomassie-stained SDS-PAGE. **b** Western blot, polyclonal anti-lupine serum. **c** Western blot, polyclonal anti-peanut serum.

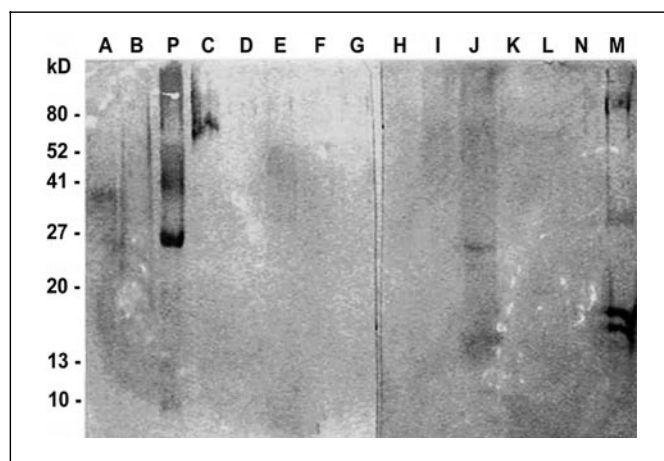


Fig. 2. Western blot with patient serum (proteins: see fig. 1).

ta, beverages and sweets has extensively increased in many countries, especially in South and Central Europe. A number of severe food allergy reactions due to lupine have been reported, which demonstrates that foods containing lupine represent an acute danger for peanut allergic patients [13, 14].

In the case presented here, a patient reacted against an unknown allergen in a hot dog bread. The analysis of the patient's serum showed a high anti-peanut IgE level. As expected, the serum reacted on Western blots to purified peanut protein, but additionally, proteins in two lupine flour references and in the hot dog bread were recognised, with the same molecular weights as described before [15, 16]. Presumably, the proteins with 38 and 15 kD were responsible for the cross-reaction with the patient's anti-peanut IgE. A cross-reaction to soy proteins was not observed. The polyclonal anti-lupine serum produced by us detected three main protein bands at 65, 50 and 40 kD in all extracts containing lupine. Cross-reaction against peanut proteins was negligible at high dilutions. The polyclonal anti-peanut serum did not react to the lupine products, the hot dog breads or the bakery breads. It was concluded that the particular hot dog bread in this case contained lupine protein against which the peanut-allergic patient had reacted. Presented with our results, the producer confirmed the use of lupine flour and has recently included lupine in the ingredient list.

This is the first time that lupine protein has been observed in a food product in Norway. However, the actual frequency of lupine on the Norwegian market is unknown. It can be assumed that lupine is imported as a

protein-rich food additive which has excellent baking properties and is normally well tolerated [17, 18]. Lupine is appreciated as a sustainable protein source and has been introduced in dietary and health foods [19]. Nonetheless, its extended use in processed food products should be documented carefully and accompanied by information to the customers about the potential risks for persons with peanut allergy.

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