

REGULAR ARTICLE

Lupin sensitization and clinical allergy in food allergic children in Norway

Helene Lindvik (helene.lindvik@rikshospitalet.no)¹, Lise Holden², Martinus Løvik³, Milada Cvancarova⁴, Ragnhild Halvorsen¹

¹Voksentoppen, Department of Pediatrics, Rikshospitalet-Radiumhospitalet Medical Center, Oslo, Norway

²National Veterinary Institute, Oslo, Norway

³Norwegian Institute of Public Health, Oslo, and Norwegian University of Technology and Sciences, Trondheim, Norway

⁴Department of Biostatistics, Rikshospitalet-Radiumhospitalet Medical Center, Oslo, Norway

Keywords

Allergy, Cross-allergy, Lupin, Peanut, Sensitization

Correspondence

Helene Lindvik, Ullveien 14,
0791 Oslo, Norway.
Tel: + 47 22 13 65 00 |
Fax: + 47 22 13 65 05 |
Email: helene.lindvik@rikshospitalet.no

Received

6 June 2007; revised 21 September 2007;
accepted 1 October 2007.

DOI:10.1111/j.1651-2227.2007.00568.x

Abstract

Aim: The aim of the present pilot study was to investigate to what extent children in Norway sensitized to lupin had clinical lupin allergy, and to compare sensitization to lupin with sensitization to other legumes.

Methods: Thirty-five children with food allergy referred to a national referral hospital were evaluated with skin prick test (SPT) and analysis of serum-specific IgE to lupin, peanut, pea and soy. The children with positive SPTs to lupin were offered oral food challenges with lupin flour.

Results: Fifteen children (43%) had positive SPT and 17 children (49%) had serum-specific IgE to lupin. Ten SPT-positive children underwent oral food challenges and one experienced an allergic reaction to lupin flour. This child was one of six challenged children with IgE antibodies to peanut > 15 kU_A/L. There was a strong relationship between positive SPT to lupin flour and positive SPT to soy and between positive SPT to lupin and specific IgE to soy, pea and peanut.

Conclusions: Children with sensitization to lupin are not likely to have a clinical lupin allergy. Avoidance of lupin on the basis of lupin sensitization or peanut allergy would lead to unnecessarily strict diets. Food challenge is currently necessary to diagnose lupin allergy.

INTRODUCTION

Food allergies are likely to be influenced by regional dietary habits and may be altered by changes in diet. Introducing new foods or food ingredients into a population may result in the development of new food allergies.

Lupin flour is a new food ingredient in Norway and has lately become increasingly used in food production in Europe due to its nutritional and technical properties and low cost. The first case of lupin allergy was reported in 1994 (1). Since then several cases, also from Norway, have been documented (2–4). Lupin allergy may produce a variety of symptoms (5), also anaphylactic reactions (6,7).

The European Food Safety Authority (EFSA) recognized lupin as a food allergen in December 2005 (8) and lupin was recently added to the EU food allergen list (9).

Sensitization to food allergens without allergic symptoms upon ingestion of the food items is common (10). Children allergic to one legume are often sensitized to other legumes because of cross-reactive IgE-antibodies, but only a few have clinical allergy to more than one legume (11). Oral food challenge may be necessary to distinguish those allergic to more than one legume from those just sensitized. The prevalence of lupin allergy and its relationship to lupin sensitization is unknown. It is of importance to differentiate those who are truly allergic to lupin from those sensitized, but without clinical allergy. The frequency of clinically significant peanut-lupin cross-allergy has been reported to be high in comparison with the frequency of cross-allergy between other legumes (5,12).

The aim of the present pilot study was to investigate to what extent children sensitized to lupin had clinical symptoms of lupin allergy and to compare sensitization to lupin with sensitization to other legumes.

MATERIAL AND METHODS

Patients

Thirty-five children, 11 girls and 24 boys, of age 2–17 years (median age 6 years) all referred to Voksentoppen, Department of Pediatrics, Rikshospitalet-Radiumhospitalet Medical Center, primarily for evaluation of known or suspected IgE-mediated food allergy were included in the study over a 3-month period. One of the children was referred because of suspected lupin allergy. Children referred primarily for evaluation of asthma or atopic eczema were not included. Exclusion criteria were other serious chronic illness and acute disease. Written informed consent was obtained from all parents. Parents of five children withdrew their consent when their child was eligible for oral food challenge. The study was performed in collaboration with the National Veterinary Institute and the Norwegian Institute of Public Health and was approved by the Ethical Committee for Southern Norway.

Skin prick tests

Skin prick tests (SPTs) were performed with Lupipan[®], a flour from *Lupinus angustifolius* seeds (Soja Austria, Vienna, Austria), Lopino[®], a processed crude protein preparation (heat-treated by boiling) from *L. albus* seeds (Lupina, Visbek, Germany), peanut 5000 PNU (protein nitrogen

units)/mL, pea 5000 PNU/mL (Allergopharma, Reinbek, Germany) and soy 1:20 W/V (Solu-prick, ALK-Abello Laboratories, Copenhagen, Denmark). The lupin flour preparation for SPT was made by mixing natural lupin flour (Lupipan) with the negative control (see below) directly on the skin, and SPT was performed by pricking through the material. For Lopino® the prick-prick method was used (13). Testing was performed on the volar side of the underarm, in duplicate for children 4 years of age and older. A positive SPT result was defined as a wheal with a diameter (mean of longest and the midpoint orthogonal diameter) ≥ 3 mm larger than the negative control (13). Mean of duplicate tests was calculated. Histamine dihydrochloride (10 mg/mL) (ALK-Abello Laboratories, Copenhagen, Denmark) served as a positive control and Allergopharma's diluent as a negative control. SPT with the lupin flour preparation was performed in the same manner on a number of nonatopic children without any positive results.

Blood samples

Venous blood was drawn in the morning and sera were analyzed for specific IgE to lupin seeds (*L. albus*), peanut, pea and soy at Voksentoppen according to the manufacturer's specifications (ImmunoCAP 100, Phadia AB, Uppsala, Sweden). Single analyses were performed. The cut-off value for a positive result was set at 0.35 kU_A/L.

Oral food challenges

Children with positive SPT to lupin flour (Lupipan®) or the processed lupin preparation (Lopino®) were offered an open oral food challenge. Food challenge was regarded as positive if objective symptoms such as rhinoconjunctivitis, asthma, urticaria, angioedema, exacerbation of atopic dermatitis, vomiting or diarrhoea occurred. Double-blind placebo-controlled food challenges were planned for confirming open oral food challenges when positive. For practical reasons, children with positive specific IgE, but negative SPT to the two lupin preparations, were not offered an oral food challenge with lupin flour. Challenge was performed with pancakes containing 10 g lupin flour each with increasing doses: $\frac{1}{4}$ pancake (2.5 g lupin flour), $\frac{1}{2}$ pancake (5 g lupin flour) and $1\frac{1}{4}$ pancake (12.5 g lupin flour; cumulative dose 20 g) at hourly intervals. The children were observed in the hospital for at least 4 h after the last challenge dose. Outpatients were told to contact the investigator if symptoms occurred after leaving the hospital.

Electrophoresis and IgE immunoblotting

Protein extractions and electrophoresis of lupin flour and Lopino protein extracts were performed as previously described (14). In brief, lupin flour and Lopino® protein extracts were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions using 4–12% Bis-Tris pre-cast gels. The gels were either stained with SimplyBlue Safestain (Invitrogen, Carlsbad, CA, USA), or the separated proteins were electrophoretically transferred from the gel onto a nitrocellulose membrane. The membrane was blocked with

Table 1 Overview of skin prick tests and specific IgE results to legumes in children (n = 35) with known or suspected food allergy

	Lupipan®	Lopino®	Lupin seed	Peanut	Pea	Soy
Positive skin prick test (>3 mm)	15	4	-	22	6	10
Negative skin prick test (<3 mm)	20	31	-	13	29	25
Specific IgE > 0.35 kU _A /L	-	-	17	28	15	20
Specific IgE < 0.35 kU _A /L	-	-	18	7	20	15

tris-buffered saline containing 0.05% Triton X 100 and 5% horse serum and incubated overnight at 4°C with patient serum (diluted 1:20). For detection of IgE, the membrane was first incubated with rabbit anti-human IgE (Dakocytomation, Glostrup, Denmark), followed by incubation with goat anti-rabbit IgG HRP conjugate (Zymed, San Francisco, CA, USA), and binding was finally made visible with 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution (Zymed). For the protein staining 18 µg protein was loaded onto the gel, while 5 µg protein was applied for the immunoblot.

RESULTS

Skin prick tests and serum-specific IgE

Fifteen of 35 children (43%) had positive SPT to Lupipan®, four of these had also positive SPT to Lopino® (Tables 1 and 2). Seventeen children (49%) had specific IgE to lupin seeds ranging from 0.4 to 20.2 kU_A/L, of whom five had negative SPT to Lupipan® or Lopino®.

All children sensitized to lupin were also sensitized to one or more of the other legumes tested for. Of the 15 children with positive SPT to lupin nine had positive SPT to peanut, five to pea and eight to soy. Regarding the 17 children with positive specific IgE to lupin, all had positive specific IgE to peanut, 13 had positive specific IgE to pea and 16 had positive specific IgE to soy.

Of the 22 children with positive SPT to peanut nine had positive SPT to Lupipan®, four to pea and nine to soy. Twenty-eight children had positive specific IgE to peanut, of these 17 children had positive specific IgE to lupin seed, 15 to pea and 20 to soy. Sixteen children had specific IgE to peanuts > 15 kU_A/L, eight of whom were also SPT positive to lupin flour.

Oral food challenges

Ten of the 15 children with positive SPT to lupin flour underwent oral food challenges. The parents of five children rejected food challenge and withdrew their consent from that part of the study. One child (patient 3) experienced an allergic reaction to lupin flour during challenge. This boy had a positive SPT to lupin flour (7 mm), a negative SPT to Lopino®, positive specific IgE to lupin flour (6.0 kU_A/L) and to peanut (>100 kU_A/L) (Table 2). Five minutes after ingestion of 2.5 g lupin flour he complained of itching in the throat and difficulty in swallowing. Urticaria, facial

Table 2 Skin prick tests (mm) and serum specific IgE (kU_A/L) to legumes in sensitized children*

Patient	Gender	Age (year)	SPT Lupipan® (mm)	SPT Lopino® (mm)	SPT soy (mm)	SPT pea (mm)	SPT peanut (mm)	Specific IgE lupin seed (kU _A /L)	Specific IgE soy (kU _A /L)	Specific IgE pea (kU _A /L)	Specific IgE peanut (kU _A /L)	Challenge symptoms†
1	m	3.5	14	5	3.5	0	4.5	3.1	<0.35	<0.35	41.3	n.d‡
2	m	17	11	4	4.5	3.5	6.5	16.8	21.8	19.1	>100	no
3	m	12	7	2	3	3	7	6	7	5.8	>100	yes
4	m	5.5	5.5	2.5	5	3.5	7.5	6.1	5.6	4.6	54.2	no
5	m	2.5	5	2	2.5	1.5	0	2.5	0.9	3.1	0.5	no
6	m	6.5	5	3.5	5	11	5	6.2	6.8	11.6	16.4	no
7	f	7	5	0	0	0	1	<0.35	<0.35	<0.35	2.1	n.d‡
8	f	5.5	5	3	1	2	0.5	3.1	2.6	1.8	3.1	n.d‡
9	m	16	4.5	0	1	1.5	2.5	<0.35	0.4	<0.35	2.5	no
10	m	4	4.5	0.5	2	0	5.5	<0.35	2.3	<0.35	4.6	n.d‡
11	m	8	4.5	0	2.5	4	0	2	1.3	0.7	19.7	no
12	f	4	4.5	0.5	3	1	7	20.2	25.8	19.4	>100	no
13	m	2.5	4	0	0	0	11	1.4	1.3	1.4	>100	n.d‡
14	m	9.5	3	0	3	1	5	1.4	1	6.1	12	no
15	f	14.5	3	0	3.5	1	0	1.8	1.1	0.4	3.7	no
16	f	6	2.5	0	1.5	2	0	<0.35	1	<0.35	2.6	no
17	m	2	2	0	0	0	11	<0.35	0.4	0.4	16.5	
18	m	4	2	0	3	0	7.5	0.6	0.6	<0.35	89	
19	m	3	2	0	2	0	0	7.8	4.6	2.7	23.8	
20	f	5	2	0	1.5	0	3.5	<0.35	<0.35	0.35	<0.35	
21	f	9.5	2	0	0	1	6.5	<0.35	<0.35	<0.35	1.2	
22	m	7	1.5	0	2.5	1	4	<0.35	<0.35	<0.35	0.7	
23	m	6	1	0	2.5	1.5	5	<0.35	<0.35	0.4	0.7	
24	m	6	1	0	3.5	0	6	<0.35	<0.35	<0.35	0.6	
25	m	7	1	0.5	0.5	1.5	6	1.4	0.5	<0.35	64.8	
26	f	3	1	0	0	0	5.5	0.8	0.8	0.8	21.2	
27	m	5	0	0	0	0	4.5	<0.35	<0.35	<0.35	<0.35	
28	m	7	0	0	0.5	3.5	1	<0.35	<0.35	<0.35	<0.35	
29	m	4	0	0	0	0	8	<0.35	<0.35	<0.35	45.2	
30	m	10.5	0	0	0	0	7	<0.35	<0.35	<0.35	53.1	
31	f	6.5	0	0	0	0	5.5	0.4	0.4	<0.35	25.2	

*Four children participating in this study (two boys and two girls) were not sensitized to any of the legumes tested and are not included in the table.

†Ten children were selected for oral food challenge on the basis of results from SPT to lupin flour, one child with a negative SPT to lupin flour also underwent oral food challenge.

‡n.d.: not done. Children with positive SPT to lupin flour who were not challenged with lupin flour.

angioedema and cough were observed. He was treated with an antihistamine. This child had been referred to Voksentopen with suspected lupin allergy after having experienced a serious allergic reaction one year earlier when eating a roll containing lupin flour. It was, however, not possible for logistic reasons to repeat the challenge in a double-blind fashion.

Thus, no child out of nine unselected children sensitized to lupin developed symptoms during food challenge, while the suspected clinical allergy of one child was verified.

Electrophoresis and IgE Immunoblotting

To investigate the protein composition in the two lupin preparations used for SPTs, the extracts were analyzed by SDS-PAGE. The protein staining of lupin flour and Lopino extracts revealed a complex pattern of components with molecular weights from 4 to 64 kDa (Fig. 1a). A decrease in high-molecular weight bands above 49 kDa and between 16 and 38 kDa were observed in the Lopino® extract, compared to in the lupin flour extract. Additionally, differences in band

intensities of common bands in the two extracts were seen. Immunoblotting with serum from the lupin-allergic patient revealed IgE-binding components with molecular weights from 18 to 60 kDa (Fig. 1b). In the lupin flour extract, several IgE-binding bands were recognized, particularly at approximately 49, 56 and 60 kDa. In the Lopino® extract, only one IgE-binding component at 56 kDa was strongly stained.

DISCUSSION

In the present study the occurrence of symptomatic lupin allergy in SPT positive, unselected food allergic subjects was not found in nine subjects. Additionally, a lupin-sensitized case was referred because of suspected lupin allergy developed symptoms when challenged orally. Thus, in this small patient cohort we have demonstrated a marked discrepancy between the number of children sensitized to lupin and the number of children demonstrating allergic symptoms in contrast to two previously published studies (5,12).

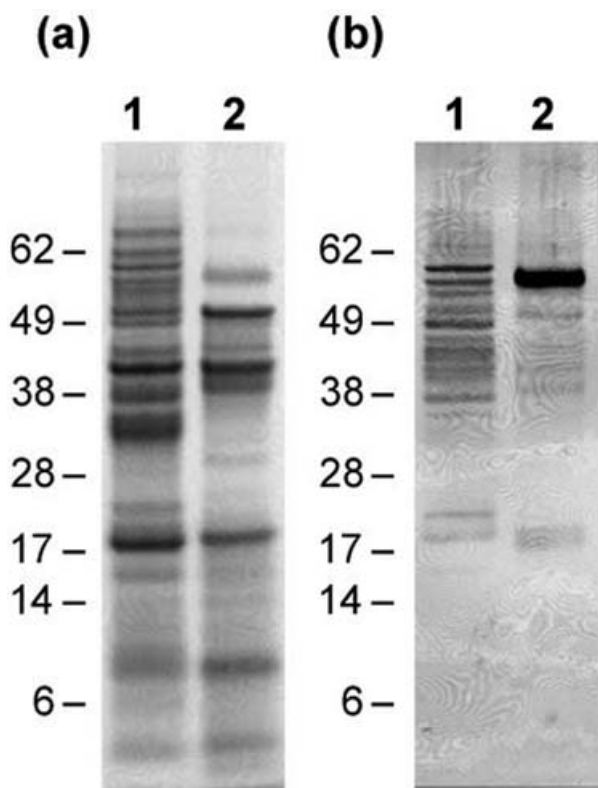


Figure 1 Lupin flour (lane 1) and Lopino[®] (lane 2) extracts separated by SDS-PAGE. Protein staining (A) and IgE immunoblot obtained with serum from a lupin-allergic patient (patient 3, see Table 2) (B). Protein sizes (kDa) are indicated on the left side of the gel and blot.

All children participating in our study were referred because of food allergies and they therefore constituted a selected group with high likeliness of having legume sensitization and allergy. For practical reasons the children were selected for open food challenge on the basis of results from skin prick testing to lupin, and not from lupin-specific IgE. Five children with lupin-specific IgE, without positive SPT did not undergo oral food challenge, and we may therefore have missed one or more allergic individuals. However, our results are valid in relation to clinical allergy and skin prick testing.

All the children sensitized to lupin were sensitized to other legumes as well. Thus, sensitization to lupin could be due to cross-sensitization to other legumes, and if so none of the included children were primarily sensitized to lupin. Positive SPTs and detectable serum IgE to pea or soy, but not to peanut, were seen more often in the children sensitized to lupin. At the present time, there are few data in the literature on lupin-pea and lupin-soy cross-allergic reactions.

Peanut challenges were not performed. Consequently, it is not known which peanut-sensitized children were actually symptomatic on exposure to peanuts. The high incidence of peanut sensitization probably reflects the selected group of children. Sixteen children had IgE antibodies specific to peanut >15 kU_A/L and were thus likely (>95%) to be peanut challenge positive according to some stud-

ies (15). Eight of the peanut-sensitized children had positive SPT to lupin flour. This is in agreement with a study of peanut-allergic children in France, where it was found that 11 of 24 (44%) had a positive SPT to lupin (5). We challenged five unselected children with IgE antibodies to peanut >15 kU_A/L with lupin flour and none experienced an allergic reaction, only the sixth child with IgE antibodies to peanut >15 kU_A/L referred because of suspected clinical allergy to lupin reacted to challenge. This is in contrast to a French study where it was found that seven of eight peanut-allergic children, of whom seven had positive SPT to lupin, showed an allergic reaction to lupin (5). This discrepancy, if not a random effect due to small numbers, may either be due to the fact that lupin flour has been used to a lesser extent or for a shorter period of time in Norway than in France. In addition, the skin test allergen preparations were different.

We performed SPTs with two different lupin preparations. The main differences between them were the species of origin and the degree of protein processing. Also in other studies different lupin preparations and various SPT methods have been used (1,4). Only four out of 15 of the children with positive SPT to lupin flour, had a positive SPT to Lopino[®]. These four children also had positive specific IgE to lupin. Furthermore, the difference in allergen preparations and the performance of clinical tests may also explain the discrepancies between results obtained in other clinical studies with lupin (5,12). Our observations underline the fact that the choice of allergen preparations used in SPTs may have a considerable effect on the outcome of the test, and demonstrate the need for standardized allergen preparations and testing procedures.

To further investigate the difference observed between lupin flour and Lopino[®], the two protein preparations were analyzed by electrophoresis. Although the staining patterns appeared to be quite similar, some differences in the number and intensity of protein bands were seen. This suggests that processing had altered the lupin proteins (e.g. by agglutination or degradation), or there may be differences between the two species of lupin. An immunoblot with IgE from the lupin-allergic patient was performed. This patient had positive lupin flour SPT, but the Lopino[®] SPT was negative. Despite this fact, IgE to both lupin flour and Lopino[®] were detected in the patient's serum. However, the IgE-binding patterns differed considerably, and while the patient's IgE recognized only one dominant band in the Lopino[®] extract, the corresponding band in the lupin flour extract was only weakly recognized. This finding confirms the data from the electrophoresis, and indicates differences in epitope structure or availability between the two preparations.

In conclusion, our study indicates that children with allergic sensitization to lupin are still not very likely to have clinical lupine allergy. Furthermore, we demonstrated that the choice of allergen preparations for use in SPTs may have a considerable effect on the outcome of the test, which emphasizes the need for standardized allergen preparations and testing procedures. We studied a small number

of children and thus definite conclusions should not be drawn. However, in food-allergic children in Norway, clinical lupin allergy seems to be uncommon even in the presence of sensitization to lupin. In order to evaluate changes in the prevalence of clinical lupin allergy associated with dietary changes, further studies are needed. If all lupin-sensitized and peanut-allergic children were advised to avoid lupin, many would have an unnecessarily restrictive diet. At present food challenge is necessary to diagnose lupin allergy in sensitized patients.

ACKNOWLEDGEMENTS

We thank the children and their parents for participating in the study, Torill Tørres and Eli Schröder at Voksentoppen for technical assistance, and Ann Eli Mavrakis for preparing the lupin flour containing pancakes.

References

1. Hefle SL, Lemanske RF, Bush RK. Adverse reaction to lupin-fortified pasta. *J Allergy Clin Immunol* 1994; 94: 167–72.
2. Smith WB, Gillis D, Kette FE. Lupin: a new hidden food allergen. *Med J Aust* 2004; 181: 219–20.
3. Fæste CK, Løvik M, Wiker HG, Egaas E. A case of peanut cross-allergy to lupin flour in a hot dog bread. *Int Arch Allergy Immunol* 2004; 135: 36–9.
4. Moreno-Ancillo A, Gil-Adrados AC, Dominguez-Noche C, Cosmes PM. Lupin inhalation induced asthma in a child. *Pediatr Allergy Immunol* 2005; 16: 542–4.
5. Moneret-Vautrin DA, Guerin L, Kanny G, Flabbee J, Fremont S, Morisset M. Cross-allergenicity of peanut and lupin: the risk of lupin allergy in patients allergic to peanut. *J Allergy Clin Immunol* 1999; 104: 883–8.
6. Matheu V, Barrio M, Sierra Z, Gracia-Bara MT, Tornero P, Baeza ML. Lupine-induced anaphylaxis. *Ann Allergy Asthma Immunol* 1999; 83: 406–8.
7. Radcliffe M, Scadding G, Brown HM. Lupin flour anaphylaxis. *Lancet* 2005; 365: 1360.
8. Becker W, Branca F, Brasseur D, Bresson J-L, Flynn A, Jackson AA. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the evaluation of Lupin for labelling purposes. *EFSA J* 2005; 302: 1–11.
9. European Commission. Commission Directive 2006/241/XE. *Off J Eur Union* 2006; L368: 110.
10. Sampson HA. Food allergy. Part 2: diagnosis and management. *J Allergy Clin Immunol* 1999; 103: 981–9.
11. Sicherer SH. Clinical implications of cross-reactive food allergens. *J Allergy Clin Immunol* 2001; 108: 881–90.
12. Leduc V, Moneret-Vautrin DA, Guerin L. Allergenicity of lupin flour. *Allerg Immunol* 2002; 34: 213–7.
13. Dreborg S. Skin tests used in type I allergy testing. Position paper prepared by the Sub-Committee on skin tests of the European Academy of allergology and clinical immunology. *Allergy* 1989; 44 Suppl 10: 13–59.
14. Holden L, Fæste CK, Egaas E. Quantitative sandwich ELISA for determination of Lupine (*Lupinus* spp.) in foods. *J Agric Food Chem* 2005; 53: 5866–71.
15. Sampson HA, Ho DG. Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. *J Allergy Clin Immunol* 1997; 100: 444–51.