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Cross-reactivity of a new food ingredient, dun pea, with legumes, and risk of anaphylaxis in legume allergic children

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Key words

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Summary

Background. *Legume allergy is the fifth food allergy in Europe. The dun pea (Pisum sativum sativum var. arvense), a pea belonging to the same subspecies as green pea, has been recently introduced as an ingredient in the human food industry. The aims of this study were to evaluate the cross-reactivity between dun pea and other legumes and to search for modification of allergenicity induced by food technologies.* ***Methods.*** *A series of 36 patients with legume and/or peanut allergy was studied. They underwent skin tests to peanut and a panel of legumes including dun pea. Specific IgE to dun pea and cross-reactivity to peanut allergens, particularly to Ara h 1, were evaluated by ELISA. Proteins and allergens of different pea extracts were studied by SDS-PAGE and immunoblots.* ***Results.*** *In France and Belgium, 7.7% of severe food anaphylaxis cases were due to legumes. Patients with isolated legume allergy had positive prick tests to dun pea, whereas patients with isolated peanut allergy had negative prick tests. Cross-reactivity between sIgE to peanut and dun pea was observed, and more frequently than expected (96%) peanut-allergic patients with legume sensitization or allergy had sIgE to Ara h 1. Analysis of dun pea allergens suggested that protein epitopes were presented differently in dun pea seeds, isolate and flour.* ***Conclusions.*** *This study identifies, for the first time, a risk of dun pea allergy in legume-allergic patients and in a subset of peanut-allergic patients.*

Introduction

Legumes are a staple food in many European and Asian countries. Common edible seeds are soybean, lentil, chickpea, green pea, white bean, the spice fenugreek, and lupine seed flour used as an ingredient. Although the peanut belongs to the *Leguminosae* family, it represented a particular case because peanut allergy is more often isolate, without clinical cross-reaction with pre-cited legumes. Legume allergy is the fifth most prevalent food allergy in Spanish children (1). In France, the records from the Allergy Vigilance Network point for the period 2002-2012 (**table 1**) to a prevalence of 6.8% out of 566 children (4th rank after peanut, tree nuts and milk) and 8.3% out of 684 adults (3rd rank after shellfish and tree nuts) (2). Sensitivity to legumes is frequent in Japan for soybean, in India for chick pea (3) and to a lesser degree in the USA (4). There is now genuine concern about sensitivity to legumes with the extensive use of protein ingredients in industrial foods. Given the public health threat related to GMOs, concentrates and isolates of soy proteins are often replaced by lupine flour. The growing incidence of anaphylaxis to lupine proteins, including a specific risk for peanut-allergic patients, has been demonstrated (5-7). In 2006, lupine and derivative products were added to the list of allergenic foods requiring mandatory labelling in the European Union (8). Consequently, food manufacturers have increasingly used another source of legume protein, botanically very close to green pea, dun pea (*Pisum sativum sativum* var. *arvense*). It was originally cultivated for animal food. Currently, dun pea proteins are found in the breadcrumbs used for coating meats, in the ingredients of minced steak, in specialized food for sportsmen and in pharmaceutical protein substitutes. In the absence of mandato-

ry labelling, dun pea is a masked ingredient under the generic term of vegetal protein. The ingredient used is a concentrate or an isolate of the protein delivering a large amount of protein. Moreover, the commercial advertising from the manufacturers of this ingredient guarantees that it is a non-allergenic food. Since several cases of severe anaphylaxis to dun pea have been registered by the French Allergy Vigilance network, the aims of this study were to evaluate the rate of sensitization to dun pea in legume-allergic patients and in peanut-allergic patients, and to search for modification of allergenicity induced by food technologies.

Table 1 *- 96 cases of severe anaphylaxis to legumes registered between 2002-2012 by the French Allergy Vigilance Network (diagnosis established on an anaphylactic reaction (grade 2, 3 and 4) and further work-up showing positive prick test and specific IgE).*

Legumes	**Children ≤ 16 years**	**Adults > 16 years**
Soybean	15	21
Lupine flour	7	34
Lentil	8	1
Green pea	3	0
Dun pea	2	0
Chickpea	1	0
Fenugreek	1	1
White bean	1	0
Broad bean	1	0
Lucerne (alfalfa)	0	1
Total / total cases of FA	**39**/566 6.8%	**57**/684 8.3%

FA: food allergy

Material and Methods

Patients

The study was approved by the local ethical committee and written informed consent was obtained from the parents of each subject, which allowed the use of the samples for research purposes (authorization No. AC-2008-449 of French Ministry of Research).
Thirty-six patients were recruited. The clinical criteria of selection were isolated or associated clinical allergy to legumes or peanut. Twenty-nine patients had prick tests (PT) to a legume panel including dun pea. The seven remaining patients were not tested because of current consumption of legumes without any clinical reaction.

Group 1 included 6 patients with isolated legume allergy. In this group, peanut allergy was excluded because of a negative history, negative PT and the absence of specific IgE (sIgE) to Ara h 2, 3, 6 and 7 (9).
Group 2 included 30 patients with peanut allergy: (i) Subgroup 2a: 13 patients with isolated peanut allergy and not sensitized to legume or with current consumption of legumes without any clinical reaction, (ii) Subgroup 2b: 8 patients sensitized to legume (on avoidance diets for legumes, without any previous clinical reaction), and (iii) Subgroup 2c: 9 patients with both peanut and legume allergies.

Skin testing

PTs were performed in accordance with previously published methodology (10). PT was considered positive if the mean wheal diameter was at least 2.5 mm larger than the diameter of the negative control. The positive control was codeine phosphate 9% (ALK-Abello, France). The timing of recording was 15 minutes.
Fresh raw legumes were tested: green pea, chickpea, lentil, soybean, white bean, broad bean, and roasted peanut. Dun pea was tested using protein isolate, Pisane® M9 (Cosucra, Belgium) and lupine (*Lupinus albus*) as a flour (Sotexpro, France).

Pea extracts

Biological analyses were performed with dun pea seeds, dun pea flour (Sotexpro, France), dun pea isolate (Pisane® M9) and green pea. Peas were homogenized in a phosphate buffered saline, pH 7.4 (Sigma, MO, USA) with Ultra-turrax. After centrifugation, the protein concentration in supernatants (= pea extracts) was determined by Bradford assay.

Specific IgE measurements and inhibitions

Specific IgE to peanut were measured using commercial ImmunoCAP® (Thermo Fisher Scientific, Uppsala, Sweden). Specific IgE antibodies to dun pea were measured by coating 2.5 µg of biotinylated dun pea isolate extract to streptavidin ImmunoCAP®. All sIgE measurements were performed on the ImmunoCAP100 instrument, following the manufacturer's instructions (Thermo Fisher Scientific). Specific IgE > 0.35 kU/L were considered positive.
Since Ara h 1 shares a 50% homology with the green pea allergen Pis s 1, sIgE to rAra h 1 were measured by enzyme-linked immunosorbent assay (ELISA). Recombinant Ara h 1 (9) was coated to microplate wells (MaxiSorp®, Nunc). After blocking, the diluted serum 1:100 was incubated for two hours. The presence of IgE was revealed by addition of horseradish-peroxidase (HRP) labeled goat anti-human IgE (KPL, MN, USA) and substrate UltraTMB (Sigma). Specific IgE were extrapolated compared to a standard curve using purified IgE (Millipore, CA, USA) and final results

were expressed in kU/L. Values were means of three wells. Specific IgE > 1.0 kU/L were considered positive.
For ELISA inhibition, the immuno-assay was performed as explained above except that dun pea isolate extract was coated to microplate wells, and the diluted sera pre-incubated overnight with peanut extract. Results are expressed as inhibition percentage.

SDS-PAGE, Immunoblot and immunoblot inhibition assays

Proteins of pea extracts (13 µg) were separated by SDS-PAGE and revealed by Coomassie blue staining or transferred to polyvinylidene difluoride (PVDF) membrane (0.45 µm, GE Healthcare, Buckinghamshire, UK) for immunoblotting. After blocking, membranes were incubated with patient's serum diluted 1:5 in TBST buffer (100 mM Tris pH 7.5, 154 mM NaCl, 0.1% (v/v) Tween 20) containing 5% (w/v) defatted milk (TBSTM). Membranes were then washed with TBST buffer and incubated with (HRP) labeled polyclonal anti-human IgE (dilution 1:1000 in TBSTM). After washing, IgE-reactive bands were revealed by chemiluminescence (ECL Advance, GE Healthcare, Buckinghamshire, UK). Two Negatives controls immunoblots were carried out: first one with the anti-human IgE antibody alone, and second with a serum of a non-allergic patient. They were performed for all extracts (data not shown).
Immunoblot inhibition assays were carried out using the same method, except that the sera were pre-incubated overnight at 4°C with 650 µg proteins (50x excess) of dun pea or dun pea flour extracts.

Results

Cross-reactivity of dun pea with other legumes

Thirty-six patients were recruited to investigate clinical and biological cross-reactivity between dun pea and other legumes. Group 1 (isolated legume allergy) included allergy to lentil (4), dun pea (3), green pea (3), soybean (2), broad bean (2), lupine (1), chickpea (1) (**table 2**). PTs were positive for at least four legumes. PT to dun pea was positive in 6/6 cases (6.5-23 mm; mean: 12.4 mm). Specific IgE to dun pea were present in 5/6 sera (0.5-83 kU/L).

Table 2 *- Group 1: Patients with isolated legume allergy.*

						PT to legume (mm)								sIgE		
ID	**Sex**	**Age (years)**	**Legumes**	**Symptoms**	**Time between first reaction and tests**	**dun pea**	**green pea**	**chick pea**	**lentil**	**lupine**	**soybean**	**white bean**	**broad bean**	**sIgE to dun pea (kU/L)**	**Inhibition of dun pea by peanut (%)**	**sIgE to Ara h 1 (kU/L)**
HH	F	1	soybean	AD	4 months	7.7	8	0	5.5	2	2	0	3	2.4	2	2.3
RY	M	8	green pea	LAO	3 years (positive LT grade 3)	12	5	2.5	4	0	0	0	3.5	2.9	6	5.7
			dun pea	Localized U, Cj	1 year (positive LT grade 4)											
SK	M	8	lentil	U, AO, Ab P	1 year	6.5	7.5	3	3	0	0	2.5	2	0.5	7	< 1.0
HE	M	18	green pea	U	16 years	23	7	7	12	17	4.5	2.5	7	83.0	9	68.5
			soybean	U, LAO	16 years (negative OC to 7 g)											
			lentil	U	16 years											
			chick pea	U	16 years											
			broad bean	U, C	8 years											
PC	F	8	green pea	AD	7 years (positive OC to 60 g)	12	11	6	0	1.5	2	0	8	46.8	10	30.7
			lentil	AD	6 years (positive LT grade 3)											
			broad bean	LAO, U	4 years (positive LT grade 2											
BM	F	6	dun pea	LAO	3 years	13.5	6	0	9	0	0	0	5.5	< 0.35	nd	4
			lentil	AO	3 years											

AD: atopic dermatitis, LAO: laryngeal angioedema, U: urticaria, Cj: conjunctivitis, AO: angioedema, AbP: abdominal pain, C: cough, LT: labial test, OC: oral challenge

Table 3 *- Peanut-allergic subjects with isolated peanut allergy (Subgroup 2a) or with sensitization to legumes (Subgroup 2b).*

					Peanut			PT to legume (mm)									sIgE		
	ID	Sex	Age (years)	Sensitization / Avoidance of legumes	PT to peanut (mm)	sIgE to peanut (kU/L)	Positive DBP-CFC to peanut: ED (g)	dun pea	green pea	chick pea	lentil	lupine	soybean	white bean	broad bean	sIgE to dun pea (kU/L)	Inhibition of dun pea by peanut (%)	sIgE to Ara h 1 (kU/L)
2a	VE	F	15	no	5	45.6	3.5	0	0	0	0	0	0	0	0	0.7	34	22.9
	BS	F	9	no	10	53.1	0.965	nd	nd	nd	nd	nd	nd	nd	nd	< 0.35	nd	< 1.0
	BR	M	7	no	15	13.1	0.215	0	0	0	0	0	1.5	0	0	< 0.35	nd	< 1.0
	BE	F	7	no	5	nd	0.5	nd	nd	nd	nd	nd	nd	nd	nd	< 0.35	nd	< 1.0
	BF	F	11	no	12	2.47	0.965	nd	nd	nd	nd	nd	nd	nd	nd	< 0.35	nd	4.3
	BL	M	5	no	10	11.5	0.065	nd	nd	nd	nd	nd	nd	nd	nd	< 0.35	nd	20.6
	BJ	F	8	no	16	2.5	05	nd	nd	nd	nd	nd	nd	nd	nd	< 0.35	nd	< 1.0
	BC	F	5	no	11.5	nd	0.5	0	0	0	0	0	0	0	0	< 0.35	nd	282
	CP	F	6	no	18	< 0.35	7	0	2	0	0	0	0	0	0	< 0.35	nd	< 1.0
	GM	F	10	no	5	96.1	0.4	0	0	0	0	0	0	0	0	< 0.35	nd	35.5
	MA	M	5	no	10	nd	7	0	0	0	0	0	0	0	0	< 0.35	nd	< 1.0
	PL	F	12	no	9	0.78	3.6	nd	nd	nd	nd	nd	nd	nd	nd	< 0.35	nd	< 1.0
	WN	M	8	no	14.5	36.3	0.5	nd	nd	nd	nd	nd	nd	nd	nd	< 0.35	nd	60
2b	CM	M	6	yes	17.5	> 100	0.215	7	3.5	0	2	9	2	2	0	7.2	3	159
	MR	M	11	yes	15	> 100	nd	5	15	0	5.5	6	0	0	3	3.8	55	146
	DR	M	11	yes	9	90.2	0.044	+	+	+	+	0	+	-	7.5	6.0	60	59.7
	BJ	F	3	yes	12.5	> 100	0.065	0	0	0	0	9.5	0	0	0	< 0.35	nd	53
	BA	M	8	yes	12.5	73.3	0.044	0	0	0	3	0	0	0	0	< 0.35	nd	22.9
	FH	M	13	yes	18.5	50.6	0.5	6	2	1.5	0	2.5	2	3	0	< 0.35	nd	36.8
	LE	M	9	yes	11.5	34.6	0.044	nd	nd	nd	nd	12	nd	nd	nd	< 0.35	nd	37.2
	MC	F	10	yes	17	> 100	0.014	0	0	0	0	6	2	0	0	< 0.35	nd	566

S: sensitization, E: eviction, DBPCFC: double-blind placebo controlled food challenge, ED: eliciting dose

Patient HE had allergy to all legumes since infancy. The recent episode of urticaria and angioedema was linked to dun pea. PT was impressive: 23 mm and sIgE were 83 kU/L.

Subgroup 2a (isolated peanut allergy) had negative PTs to all legumes including dun pea in 6/6 cases (**table 3**). They were not performed in seven cases since patients ate all legumes without any reaction. Specific IgE to dun pea were present in only one case out of 13 and at a low level (0.7 kU/L).

Subgroup 2b (peanut allergy and sensitization to legumes) was sensitized to between one and five legumes (**table 3**). PT to dun pea was positive in 4/7 patients. Specific IgE to dun pea were present in 3/8 sera.

Subgroup 2c (peanut and legume allergy) had allergy to green pea (4), dun pea (3), lentil (3), soybean (2) and lupine (1) (**table 4**). This group had positive PTs to between one and five legumes. PT to dun pea was positive in 9/9 cases (2 cases at 17 mm, mean: 8.8 mm). PTs to green pea and lentil were positive less often, in 2 and 4 cases, respectively. Specific IgE to dun pea were positive in 9/9 cases (0.8 - 68.8 kU/L).

Out of 15 cases of allergy to legumes (group 1 and subgroup 2c), 9 had peanut allergy (subgroup 2c). Conversely, out of 30 cases of peanut allergy, at least 9 also had legume allergy. However, 8 patients were on avoidance diets because of legume sensitization and we cannot be certain of tolerance in the event of ingestion.

Table 4 *- Peanut-allergic subjects with legume allergy (Subgroup 2c).*

						Peanut			PT to legume (mm)									sIgE		
ID	Sex	Age (years)	Legume allergy	Symptoms	Time between first reaction and tests	PT to peanut (mm)	IgE to peanut (kU/L)	Positive DBPCFC to peanut: ED (g)	dun pea	green pea	chick pea	lentil	lupine	soybean	white bean	broad bean	DBPCFC to dun pea: ED	sIgE to dun pea (kU/L)	Inhibition of dun pea by peanut (%)	sIgE to Ara h 1 (kU/L)
DM	F	8	dun pea	A*	1 year	11	14.9	0.215	17	0	5	14	14	0	0	6.5	nd	25.2	3	33.4
VV	M	10	dun pea	U	1 year (positive OC to 0.125 g)	9	51.8	nd	7.5	8	1.5	7.5	0	0	0	4	positive 0.215 g	14.8	10	18.8
BM	M	5	dun pea	Localized U*	1 year	20	> 100	nd	4	0	1.5	1	9	2	0	1	nd	0.8	18	1.3
MM	M	4	soybean green pea	U U	2 years 2 years	4	94.4	nd	5	2	0	0	0	0	0	0	nd	1.61	41	980
ML	M	8	green pea lentil soybean	AO, W, V AO, W, V AO, W, V	1 year 1 year 1 year	8.5	> 100	nd	17	4	0	12	4	0	2	5	negative 7 g	68.8	44	415
BC	F	10	lentil	AO	1 year	11.5	> 100	0.215	3	0	3	2	0	0	0	2	nd	2.21	58	209
KQ	M	10	green pea lentil			18	nd	0.027	11	0	+	0	14	8	0	0	nd	7.7	68	171
MB	M	6	green pea chick pea lentil	U U A, RC	2 years 2 years 1 year	5	> 100	0.265	6	0	0	5.5	3	0	0	0	nd	16.2	69	64
PF	F	15	lupine	E, AbP	1 year (positive OC to 7.9 g)	13	> 100	nd	9	1	0	0	3	1	1	0	nd	1.66	83	940

A: asthma, *ingestion of sausage including dun pea, U: urticaria, AO: angioedema, W: wheezing, V: vomiting, RC: rhinoconjunctivitis, E: erythema, Ab P: abdominal pain

PT to dun pea

Overall, 23 patients were sensitized to legumes (groups 1, 2b and 2c). Although dun pea and green pea were both variants of *Pisum sativum sativum*, PTs to dun pea and green pea did not yield the same results. Hence, PT to dun pea was positive in 19/23 cases, while PT to green pea was positive in only 11/23 cases. The specificity of PT to dun pea could be ascertained since it was always negative when there was no sensitization to other legumes (group 2a) (**table 3**). Moreover, sensitivity was very high: PTs were positive in the 6 patients with history of dun pea allergy (3 cases in group 1 and 3 cases in group 2c). It should be noted that in 5 green pea allergic-patients, PTs to dun pea were positive in all patients, though PTs to green pea were negative in some cases (patient PC in group 1 and patients MM, ML, KQ and MB in group 2c).

Nineteen patients had positive PTs to dun pea and/or green pea. The wheal diameter of PTs to dun pea (9.1 ± 5.6 mm) was significantly higher than those of green pea (4.3 ± 4.3 mm) (p = 0.006). These observations could be related to the fact that dun

pea isolate contains more proteins (90%) than green pea (6%). However, there was no correlation between both PTs ($r^2 = 0.060$ and $p = 0.313$), suggesting different allergenic profiles.

Dun pea-sIgE

sIgE to dun pea were detected in 18/36 patients (50%), with levels ranging from 0.5-83 kU/L (median 4.9 kU/L). The concordance of PT and sIgE was analyzed in 28 cases. Double positivity was observed in 17 cases and double negativity in 8 cases (total concordance in 89%). Specific IgE to dun pea were detected in one case with negative PT (subgroup 2a, patient VE) and were not detected in 2 cases with positive PT (group 1, patient BM and subgroup 2b, patient FH).

Cross-reactivity between peanut and dun pea

To determine whether there was any cross-reactivity between dun pea and peanut, ELISA inhibition was performed. When patients were allergic to peanut, sensitized or allergic to legumes, an inhibition was observed in 9/13 cases (34%-83% inhibition) (**table 3** and **4**). These patients had sIgE to Ara h 1 in 22/23 cases (96%). The same sIgE were detected in only 6/13 cases (46%) in subgroup 2a with isolated peanut allergy. In group 1 with isolated legume allergy, sIgE to Ara h 1 were detected in 5/6 patients (**table 2**).
Specific IgE to Ara h 1 were evaluated in peanut-allergic patients according to their lack of sensitization to legumes (subgroup 2a) or the presence of sensitization or allergy to legumes (subgroup 2b and 2c). No sIgE to Ara h 1 were detected in the first subgroup in six out of 13 patients. Specific IgE to Ara h 1 were detected in all patients (17/17) with sensitization or allergy to legumes ($p = 0.003$).

Allergenicity in different dun pea extracts

SDS-PAGE separation followed by protein staining of the different pea extracts (green pea, dun pea seed, dun pea flour and dun pea isolate) revealed complex electrophoretic patterns, including components ranging from around 100 to 9 kDa (**figure 1A**). Some proteins, 70 kDa, 50 kDa, 38 kDa, 28 kDa, 21 kDa, 17kDa, 14 kDa and 9 kDa, were present in the four extracts. Interestingly, the profile of dun pea isolate was closer to green pea than dun pea seed. Although the electrophoretic profiles of dun pea seed and green pea were different (**figure 1A**), immunoblot of patient HE with both extracts (**figure 1B**) revealed a similar allergenic profile, showing that dun pea seed and green pea share common allergens.
An immunoblot with dun pea seed, flour and isolate was performed with serum from patient HE (**figure 2A**). Proteins of 28 kDa, 17 kDa and 14 kDa were strongly recognized by the IgE in the three extracts. Proteins of 70 kDa, 50 kDa and 38 kDa were strongly recognized in the seed and the isolate, but weakly recognized in the flour. Although present in all three extracts (**figure 1A**), the 9 kDa proteins were recognized only in the seed extract.
Cross-inhibitions were performed between seed and flour extracts (**figure 2B**). They showed that 50 kDa and 28 kDa proteins of seed were better inhibited by dun pea flour extract, than by dun pea seed extract. Moreover, all proteins recognized by the IgE in flour extract were inhibited by seed and flour extracts. Finally, the 9kDa proteins more present in seed extract than in flour extract (**figure 1A**) were still recognized by IgE in the presence of inhibitor flour extract (**figure 2B$_2$**). Taken together, these observations suggest that the epitopes were presented differently in both preparations.

Figure 1 *- A. Protein staining of green pea (GS), dun pea (DS), dun pea flour (DF) and dun pea isolate (DI) separated by SDS-PAGE. B. Immunoblot of patient HE with dun pea (DS) and green pea (GS). M: marker of molecular weight.*

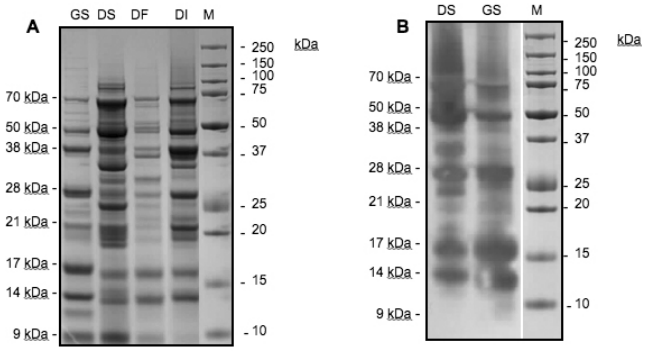
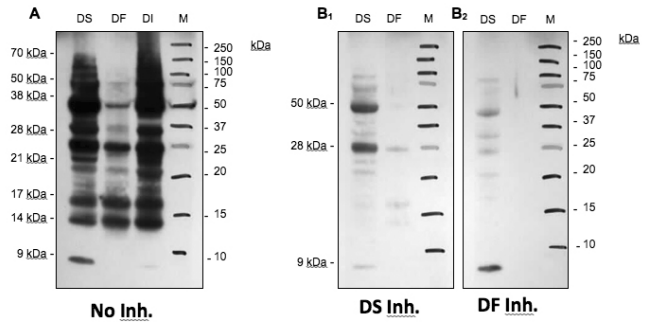


Figure 2 *- A. Immunoblot of patient HE with dun pea (DS), dun pea flour (DF) and dun pea isolate (DI). B. Immunoblot of patient HE inhibited with dun pea (B1) or inhibited with dun pea flour (B2).*



Discussion

Legumes have abundant storage proteins, including the superfamilies of cupins and prolamines (11), explaining the frequent *in vitro* cross-reactivity (12-14).

Like green pea, dun pea is a variety of *Pisum sativum sativum*. Allergens of green pea are Pis s 1 (50 kDa vicilin), Pis s 2 (64 kDa convicilin sharing a 65% homology with the former), Pis s 5 (profilin), Pis s 6 (17 kDa PR10-protein), Pis s albumin (26 kDa) and an agglutinin (30 kDa). Pis s 1 and Pis s 2 are major allergens (15). Other possible allergens are 2S albumins, sharing homologies between lentil and green pea (16).

Pis s 1 shares homology with Len c 1 (90%) from lentil, Lup a 1 (52%) from lupine, Gly m 5 (52%) from soybean, Ara h 1 (50%) from peanut. For Pis s 2, the homologies are respectively 68%, 44%, 45%, 45% (17). Besides, Ara h 1 shares homology with Lup a 1 (60% homology), Len c 1 (58% homology), Gly m 5 (57% homology).

Despite the fact that protein profiles are different between dun pea and green pea, the allergenic profiles are very similar (**figures 1A and 1B**). We can thus postulate that dun pea contains an allergen homolog to Pis s 1. Even if there is only 50% homology between Pis s 1 and Ara h 1, attention is drawn to the 96% of positive ELISA to Ara h 1 in our series of patients sensitized or allergic to legumes. This incidence is much higher than that observed in large peanut-allergic populations (9,18). Furthermore, in patients allergic to peanut, sIgE to Ara h 1 was detected in all patients (17/17) if they are were sensitized or allergic to legumes, whilst they were detected only in 7/13 patients who were not sensitized to legumes. These data, together with the results from inhibition of sIgE to dun pea by peanut suggest a higher risk of sensitization or clinical reactions to legumes and to dun pea, in a subgroup of peanut-allergic patients sensitized to Ara h 1.

However, up to now the clinical risk of cross-reacting foods, namely legumes, depends on complex factors (19) and cannot be evaluated by this small series. According to Sicherer, established allergy to more than one legume could indicate higher risk for multiple allergies (19). Out of our 36 patients, eight fulfilled this condition (group 1 and subgroup 2c).

Little information is available concerning the influence of food technologies on legume allergenicity (20). Moreover, detailed information about the technological processes for industrial foods is rarely obtained from the food industries. Flour is obtained by physical processing: dehulling, micro-crushing and extrusion. We have no additional data for the isolate.

Immunoblots of serum from patient HE with the same amount of protein amount for each extract illustrate the difference of IgE reactivity with seed, flour and isolate extracts. Hence, (i) allergenic profiles were different between flour and isolate (**figure 2A**), (ii) IgE recognized the 9 kDa proteins in dun pea seed but not in flour and isolate (**figure 2A**), (iii) the absence of inhibition of the 9 kDa proteins of seed by flour (**figure 2B$_2$**) confirmed that the 9 kDa proteins present in flour and isolate were no longer able to bind the sIgE, and finally (iv) seed and flour differentially inhibited IgE binding to 28 kDa and 50 kDa proteins in seed (**figure 2B$_2$**). These observations raise the hypothesis that manufacturing processes may be different for the two types of ingredients, thus modifying the allergenicity of native proteins.

Legumes are staple foods worldwide and attention is drawn to the prevalence of legume allergy. Owing to their nutritional properties, they are used increasingly as protein ingredients, and the recent introduction of dun pea flour or dun pea isolate by food ingredient producers has been considered a safe alternative to the use of soybean or lupine proteins. However, the quantity of dun pea proteins included in a 20% enriched steak mince is 17 g, instead of 12 g ingested in a routine portion of green peas. Misleading allegations claim that dun pea products are not allergenic. Since their presence on the labelling may be only notified as "vegetable proteins", consumers, health services and regulatory authorities cannot currently identify the allergic risk of dun pea, and widely to all peas. This study documents the *in vitro* cross-reactivity of dun pea with other legumes and peanut, and highlights some cases of clinical reactions to dun pea in patients allergic to legumes (1) or peanut (3). Further studies should clarify the extent of the risk of pea used as an ingredient.

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Conflict of interest

CR and SJ are employed by Genclis SAS.

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