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Cross-reactivity between peanut and lupin proteins

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ABSTRACT

Peanut-allergic individuals may also react to lupin, which, for this reason, has been included in the EU list of food allergens. Since there is not yet any general consensus on the major allergen/s in lupin, the objective of this investigation was to compare the reactivity of the main lupin proteins towards the IgE of the sera of allergic patients. Both *Lupinus albus* and *Lupinus angustifolius* were investigated. ELISA's, Western blotting and mass spectrometry, including also *de novo* sequencing of the unknown lupin proteins, were used for identifying the IgE-binding proteins. Significant differences in the protein reactivities were observed. In particular, there was a direct relationship between the level of peanut-specific IgE's and the cross-reactivity to lupin proteins; also the reactivity of each serum appeared to be unique. Although numerous lupin proteins bind IgE's, our data suggest a predominant contribution of α-conglutin in the reactivity of both *L. albus* and *L. angustifolius*.

1. Introduction

Lupin has been included in the EU list of allergens to be declared in food labels (Commission Directive 2006/142/EC), and it is potentially life-threatening (NDA Scientific Panel on Dietetic Products, 2005) taking into account that at least 151 cases of lupin allergy have been described worldwide (Jappe & Vieths, 2010).

Allergic reactions to foods containing traces of lupin protein have been documented in patients already allergic to other legumes, such as peanut, soybean, lentils, beans and peas (Faeste, Løvik, Wiker, & Egaas, 2004; Kanny, Guérin, & Moneret-Vautrin, 2000; Leduc, Moneret-Vautrin, & Guérin, 2002; Moneret-Vautrin et al., 1999). A controlled study in peanut allergic patients suggested a cross-reactivity rate of about 30% (Moneret-Vautrin et al., 1999), but higher rates (68%) have also been reported (Leduc et al., 2002).

Although peanut allergic patients are likely to be the major risk group (Gayraud et al., 2009), sensitisation via ingestion has been suggested also in individuals without known peanut allergy (Novembre et al., 1999; Parisot, Aparicio, Moneret-Vautrin, & Guerin, 2001; Quaresma, Viseu, Martins, Tomaz, & Inácio, 2007; Smith, Gillis, & Kette, 2004; Wassenberg & Hofer, 2007).

The clinical symptoms range from mild local reactions to systemic anaphylaxis. The ingested doses of lupin flour triggering clinical reactions range from 265 to 1000 mg (Kanny et al., 2000; Moneret-Vautrin et al., 1999). A detailed study (Peeters et al., 2007) reported that the minimal eliciting dose may be 1 mg or less for subjective symptoms (oral allergy symptoms) and 300 mg for objective symptoms. These doses are much higher than those identified for peanut allergy (Taylor et al., 2010; Wensing et al., 2002).

Lupin allergy is rapidly increasing in France, where the addition of lupin flour to wheat flour was firstly permitted in 1997 (Moneret-Vautrin et al., 1999): this seed was the fourth most frequent cause of severe food-associated anaphylaxis reported to the French Allergy Vigilance Network in 2002 (Moneret-Vautrin, Kanny, & Parisot, 2002). Around 1% of the UK population, including 250,000 pre-school children, suffers from peanut allergy and appears to be at risk since up to half of peanut-allergic population may be pre-sensitised for lupin allergens (Radcliffe, Scadding, & Brown, 2005). The first report on lupin anaphylaxis in UK was in 1999. Three cases of anaphylaxis have been also described in Australia (Smith et al., 2004). There is also some evidence of the allergenic potential of lupin after inhalation (Moreno-Ancillo, Gil-Adrados, Domínguez-Noche, & Cosmes, 2005; Novembre et al., 1999; Prieto et al., 2010), as a cause of occupational asthma and food allergy in exposed workers (Crespo et al., 2001; Parisot et al., 2001).

Abbreviations: IgE, immunoglobulin E; LPE, lupin protein extract; PPE, peanut protein extract; IAM, Iodoacetamide; CHAPS, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate hydrate; IPG, immobilised pH gradient; TBS, Tris buffered saline; HRP, horseradish peroxidase; ECL, enhanced chemiluminescence.

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Despite several studies in the literature, there is no consensus about the presence of one or more major allergens in lupin. The storage globulins α-conglutin (legumin-like, 11 S), β-conglutin (vicilin-like, 7 S), γ-conglutin (7 S), and δ-conglutin (2 S-albumin) seem all to be responsible for the allergenic reactivity.

The generic term lupin refers to at least two major different species, *Lupinus albus* and *Lupinus angustifolius*. Since the latter has been only recently included in human foods, the data on its allergic potential are scarce (Goggin, Mir, Smith, Stuckey, & Smith, 2008). The scope of the present investigation was to compare the potential IgE binding of the main globulins of both lupin species and to identify reactive peptides. The sera from peanut-allergic patients were screened and different approaches were used to identify the IgE-reacting proteins/peptides, such as ELISA assays, Western blotting and mass spectrometry (HPLC-Chip-Ion Trap and HPLC-Chip-qTOF), including also *de novo* sequencing of unknown proteins.

2. Materials and methods

2.1. Samples

Dry lupin seeds from *L. albus cv.* Ares (A) and Multitalia (B); *L. angustifolius cv.* Arabella (C) and Boregine (D) were investigated: A and D were provided by the Fraunhofer-Institut für Verfahrenstechnik und Verpackung (Freising, Germany); B and C by Dr. Annicchiarico, CRA-ISCF (Lodi, Italy). Peanuts of the runner market type (Argentina) were provided by Imko Nut Products (Doetinchem, The Netherlands).

2.2. Patients

The sera were collected from 34 patients with positive case history of peanut allergy. The sera were provided by 5 hospitals in The Netherlands: University Medical Centre Utrecht (UMCU, Utrecht), Amsterdam Medical Centre (AMC, Amsterdam), Rijnstate Hospital (Arnhem), Gelderse Vallei Hospital (Ede), and Stichting Huisartsenlaboratorium Oost (SHO, Velp). The control sera were collected from 5 non-atopic, non-allergic healthy volunteers. Supplementary Table I summarises the specific IgE levels of each patient determined by the ImmunoCAP (Phadia, Uppsala, Sweden), the allergy symptoms, and the results of their SPTs. The patients were carefully informed of the modalities and scope of the study and, after receiving all the information, they signed the written informed consent.

2.3. Laboratory preparation of total protein extracts from lupin and peanut seeds

Each legume protein was extracted in optimised conditions. Lupin protein extract (LPE) – Defatted lupin flour was extracted with 100 mM Tris–HCl, 0.5 M NaCl, pH 8.0, for 2 h at room temperature (RT), with gentle stirring. The slurry was centrifuged at 6000*g*, 4 °C, for 30 min, and the extracted proteins were stored at −80 °C until use.

Peanut protein extract (PPE) – The peanut proteins were extracted from defatted peanut meal by stirring in 15 mM sodium phosphate buffer, pH 6.2, with a 1:10 (w/v) ratio for 1 h at RT.

2.4. Purification of lupin globulin proteins

α-, β- and γ-Conglutins were purified according to a published method (Dooper, Holden, Faeste, Thompson, & Egaas, 2007), using a preparative HPLC 1200 (Agilent Technologies, Santa Clara, CA). The purity of the column fractions was confirmed by MS/MS analysis. Samples were denatured with 6 M urea, reduced with 1 M 1,4-dithiothreitol (DTT) (50 mol of DTT vs 1 mol of Cys) and alkylated with 1 M iodoacetamide (IAM) (200 mol of IAM vs 1 mol of Cys). The proteins were then digested with sequencing-grade trypsin (0.5 μg/μl, Promega) with a ratio of 1:50 w/w enzyme/protein for 16 h at 37 °C, and the peptides were analysed using HPLC-Chip-Ion Trap (Agilent Technologies) (Sirtori, Resta, Brambilla, Zacherl, & Arnoldi, 2010).

2.5. Indirect ELISA

The 96-well MaxiSorb microplates (Fisher Scientific, The Netherlands) were coated with 500 ng/well (5 μg/ml) of LPE, PPE and purified protein fractions in coating buffer (0.1% w/v BSA in PBS: 1.4 mM KH_2PO_4, 8 mM Na_2HPO_4, 2.7 mM KCl, 130 mM NaCl, pH 7.4), and incubated o/n at 4 °C. The negative control and a blank sample for non-specific secondary antibody binding were included. All subsequent incubations were performed at RT on a microplate shaker. The coating solution was removed and 200 μl/well of blocking buffer (2% w/v BSA in PBS, pH 7.4) was added and incubated for 1 h. The microplates were washed after each incubation step in a microplate washer with 400 μl/well of washing buffer (0.05% v/v Tween-20, 0.05% w/v BSA in PBS pH 7.4). The 1:3 serum dilutions in PBS containing 0.1% BSA were added at 100 μl/well and incubated for 1.5 h. The wells were then incubated with 100 μl of 1:500 monoclonal biotin-conjugated mouse anti-human IgE (BD Biosciences Pharmingen, USA) and 100 μl of 1:10,000 streptavidin-poly-HRP (Sanquin, The Netherlands), in dilution buffer (0.1% w/v BSA in PBS, pH 7.4) both during 1 h. The enzymatic colour development started by the addition of 100 μl/well 3,3′,5,5′-tetramethylbenzidine substrate solution (KPL, Gaithersburg, MD) and the reaction was stopped by adding 100 μl/well of 1 M H_3PO_4. Colour development was measured at 450 nm using a microplate reader (Anthos 2020, Anthos Labtec Instruments, Austria). The measurements were performed in triplicate.

2.6. Inhibition ELISA

All sera were assessed by inhibition ELISA with PPE as inhibitor. Inhibition experiments were performed by coating 96-well MaxiSorb microplates with 5 μg/ml LPE and blocking as described above. Diluted sera (1:2, 1:3, 1:5, and 1:10) were pre-incubated in triplicate with PPE at the final concentrations of 500–0.5 ng/ml o/n at 4 °C. Subsequently, the inhibitor mixtures (including also sera without inhibitor as control) were transferred to coated wells (100 μl/well) and incubated for 1 h. The detection was performed in the same way as for indirect ELISA assay. Percentage inhibition was calculated according to:

$$\%\text{inhibition} = [1 - (OD_{450}\ \text{serum with inhibitor}/OD_{450}\ \text{serum without inhibitor})] \times 100$$

2.7. Bidimensional electrophoresis (2-DE)

LPE or desalted column fractions were diluted in IEF sample buffer (7 M urea, 2 M thiourea, 3% (w/v) 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate hydrate (CHAPS), 1% ampholyte pH 3–10 and pH 4–8); the proteins were reduced with 65 mM DTT and alkylated with 200 mM IAM (Sirtori et al., 2010). Isoelectric focusing was performed on pH 3–10 non-linear IPG strips (Bio-Rad Laboratories Inc., USA), and the second dimension was done on 13% SDS–PAGE; Bio-Safe Coomassie (Bio-Rad) was used for the staining. Gels were scanned in a VersaDoc 3000 Imaging System (Bio-Rad). The