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Characterization of IgE-binding epitopes of peanut (*Arachis hypogaea*) PNA lectin allergen cross-reacting with other structurally related legume lectins

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ABSTRACT

Sera from peanut allergic patients contain IgE that specifically interact with the peanut lectin PNA and other closely related legume lectins like LcA from lentil, PsA from pea and PHA from kidney bean. The IgE-binding activity of PNA and legume lectins was assessed by immunoblotting, surface plasmon resonance (SPR) and ELISA measurements, using sera from peanut allergic patients as a IgE source. This IgE-binding cross-reactivity most probably depends on the occurrence of structurally related epitopes that have been identified on the molecular surface of PNA and other legume lectins. These epitopes definitely differ from those responsible for the allergenicity of the major allergens Ara h 1, Ara h 2 and Ara h 3, also recognized by the IgE-containing sera of peanut allergic patients. Peanut lectin PNA and other legume lectins have been characterized as potential allergens for patients allergic to edible legume seeds. However, the clinical significance of the lectin–IgE interaction has to be addressed.

1. Introduction

Allergy to peanut and other legume seeds has now become a major concern of public health not only in children and adolescents, but also in adults with a worrying increase of Quincke œdema or anaphylactic shocks during the last 5 years, especially in industrial countries (Sampson, 2004). The major (glyco)protein allergens responsible for peanut allergy have been identified and characterized. They belong to the different families of vicilins (Ara h 1) (Burks et al., 1991), conglutins (Ara h 2, Ara h 6, and Ara h 7) (Burks et al., 1992), and glycinins (Ara h 3 and Ara h 4) (Rabjohn et al., 1999). Similar allergens also occur in the closely related edible legumes (pea, soybean, kidney bean, and lupine) (de Leon et al., 2003; Goetz et al., 2005; Magni et al., 2005) and other tree nuts (Pecan nut, Brazil nut, and Pistachio) (Wang et al., 2002, 2003; Robotham et al., 2005) that apparently give rise to IgE-binding cross-reactions with the peanut allergens (Barre et al., 2008). However, seed proteins consist of an extremely complex mixture of individual proteins with particular properties (enzyme activity, sugar- or lipid-binding activity) besides participating in the protein bodies as seed storage proteins (Shewry et al., 1995). Among these proteins, lectins are known for a long time as a widespread group of plant proteins with interesting properties like their ability to trigger some non-specific lymphoblastic transformation in relation to their carbohydrate-binding properties (Stavy et al., 1971). Besides their occurrence in seeds, lectins have been characterized in other plant organes, namely in tubers, bulbs or fruits (Van Damme et al., 1998).

Although lectins are abundantly represented in, e.g. edible fruits (banana and tomato), bulbs (onion, garlic, and shallot), tubers (potato) or in dietary legume seeds (soybean, peanut, kidney bean, and pea), their potential allergenicity has been only scarcely investigated and thereby appears as poorly documented. However, several reports exist on the potential allergenicity of Con A from the Jack bean (*Canavalia ensiformis*) seeds (Gollapudi and Kind, 1975; Siraganian and Siraganian, 1975; Mitchell and Clarke, 1979) and other plant lectins (Barnett et al., 1983; Zavazal and Krauz, 1985; Shibasaki et al., 1992; Burks et al., 1994; Chung and Champagne, 1999; Haas et al., 1999; Gruber et al., 2005). A few cases of type I allergy to elderberry (*Sambucus nigra*) have been recently attributed to a ribosomal activating protein (RIP) of the pollen (Förster-Waldl et al., 2003). In fact, the amino acid sequence stretches available for this so-called RIP suggest that this protein most probably corresponds to a lectin of the ricin B-chain family that also occurs in the elderberry fruits (Van Damme et al., 1997). However, rather conflicting results were reported on the allergenicity of the peanut (*Arachis hypogea*) lectin PNA. In a previous study on 12 patients with atopic dermatitis and a positive food

Abbreviations: Con A, concanavalin A from Jack bean (*Canavalia ensiformis*); DbL, *Dolichos biflorus* (horse gram) lectin; IgG, immunoglobulin G; IgE, immunoglobulin E; LcA, *Lens culinaris* (lentil) agglutinin; LoLI, *Lathyrus ochrus* (yellow flowered pea) isolectin I; PHA-E, *Phaseolus vulgaris* (kidney bean) erythroagglutinin; PNA, peanut (*Arachis hypogea*) agglutinin); PsA, *Pisum sativum* (pea) agglutinin; SBA, soybean (*Glycine max*) agglutinin.

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challenge to peanut, PNA reacted in only 50% of the patients and was thus considered as a minor legume allergen (Burks et al., 1994). More recently, a substantial increase in the IgE-binding level of sera was observed in six out of seven patients allergic to peanut (85%) whereas no binding occurred with sera from non-allergic individuals (Gruber et al., 2005). In contradiction with the previous results these data suggest that PNA behaves as a major peanut allergen. According to the high sequential/structural conservation of legume lectins (Van Damme et al., 1998), IgE specific for PNA should also cross-react with other closely related lectins from other legume seeds, e.g. SBL from soybean (*Glycine max*) or PsA from pea (*Pisum sativum*).

Here, we report on immunoblotting, surface plasmon resonance measurements and ELISA using sera from patients allergic to peanut, which confirm the IgE-binding ability of legume lectins and their cross-reactivity with PNA. Exposed IgE-binding epitopic regions were identified on the molecular surface of dimeric (PsA from pea, LcA from lentil) and tetrameric (PNA) legume lectins.

Table 1
Listing of patients allergic to peanut.

Subject	Code	Sex/age (years)	Allergic history[a]	Symptom[b]
J.C.	P1	M/6	PA, TNA, EA	
L.A.	P2	M/4	PA, TNA, MUA, EA, MA, FA, P	PR, E
G.C.	P3	F/15	PA	A
F.E.	P4	F/8	PA, P	E
D.M.	P5	F/6	PA, EA	PR
M.J.	P6	F/6	PA, LA (soybean), EA, P	E
P.A.	P7	M/10	PA, EA, MA, P	A
P.P.	P8	M/11	PA, P	A, PR
O.E.	P9	F/4	PA, TNA, LA (lentil), EA, P	A, PR
G.J.	P10	F/9	PA, TNA, EA, P	E
M.G.	P11	F/19	PA	
G.T.	P12	M/8	PA, EA, P	A, RC
D.R.	P13	M/10	PA, GA, EA, MA, P	A
C.B.	P14	M/4	PA, P	
	P15			
	P16			

[a] PA, peanut allergy; TNA, tree nut allergy; LA, legume allergy; GA, gluten allergy; MUA, mustard allergy; EA, egg allergy; MA, milk allergy; FA, fish allergy; P, pollinosis.
[b] A, asthma; E, eczema; PR, pollinic rhinitis; RC, rhinoconjunctivitis.

2. Materials and methods

2.1. Chemicals

Sugars were purchased from Sigma and rabbit anti-human IgE was obtained from Dako. Sensor chips (CM 5), HBS (10 mM Hepes, 150 mM NaCl, containing 0.05% surfactant P20 and 3.0 mM EDTA, pH 7.4) and all the chemicals required to activate the carboxymethyl-dextran and immobilize the glycoproteins (100 mM *N*-hydroxysuccinimide, 400 mM *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride and 1 M ethanolamine hydrochloride adjusted to pH 8.5 with NaOH) were from *BIA*core AB (Uppsala, Sweden).

2.2. Isolation and purification of lectins

Except for PNA, DbL and PHA-E, which were purchased from Sigma, other legume lectins were isolated from dry seeds and purified by affinity chromatography. SBA was isolated by affinity chromatography on *N*-caproylgalactosamine-Sepharose and subsequent elution with 100 mM galactose, according to Allen and Neuberger (1975). LcA, PsA and LoLI were specifically retained on Sephadex G100 and eluted by adding 100 mM glucose to the Tris-buffered saline (pH 7.6) elution buffer, as previously described (Rougé and Sousa-Cavada, 1984). The purity of the lectin preparations was checked by SDS-PAGE in 12.5% acrylamide gels followed by Coomassie blue staining (Laemmli, 1970).

2.3. Sera from allergic patients

Sera from 16 patients allergic to peanut (Table 1), which had been previously challenged by skin prick tests and oral provocation tests with the offending food, were used as an IgE source for immunoblotting, SRP analyses and ELISA measurements.

2.4. Immunoblotting

One millilitre of each lectin sample used at three concentrations of 50 ng ml^{-1}, 100 ng ml^{-1} and 200 ng ml^{-1} in 10 mM PBS (pH 7.4) containing 20 mM Gal and Man, was spotted onto a Hybond ECL nitrocellulose membrane (Amersham) and the membrane was air-dried for 1 h at room temperature. After a pre-incubation in PBS containing 20 mM Gal and Man, 3% (w/v) BSA and 0.1% (v/v) Tween for 1 h, the membrane was soaked in the patient IgE-containing sera diluted 1:10 in the same buffer and incubated for 2 h in a moist chamber. After three washings of 10 min each with the same buffer, the membrane was soaked in rabbit HRP-labelled anti-human IgE diluted 1/5000 in the buffer and incubated for 1 h under gentle stirring. Following three washings of 10 min each with buffer, the immunolabelled spots were detected using the ECL Plus detection (Amersham) after 1 min exposure in cassette. All the handlings were carried out at room temperature. In order to prevent a possible sugar-binding mediated interaction of the immobilized lectins with the glycoproteins from human sera or the rabbit anti-IgE antibodies that could lead to false positive reactions, the nitrocellulose membrane bearing the immobilized lectins was pre-incubated with PBS containing both Man and Gal at 20 mM concentration. The following incubation steps with the IgE-containing sera and the anti-IgE rabbit antibodies were similarly performed in the presence of both sugars. In these conditions, the revealed interactions do not depend on the carbohydrate-binding properties of the lectins (binding of lectins to the glycan moiety of IgE or other serum proteins) but only depend on a specific lectin–IgE interaction.

2.5. Surface plasmon resonance analysis

The specific interaction of IgE-containing sera from peanut allergic patients with immobilized lectins was performed by surface plasmon resonance (SPR) using a biosensor *BIA*core X (*BIA*core AB, Uppsala, Sweden). The sera diluted 1:50 in HBS (pH 7.4) containing 20 mM Man and Gal, were injected for 5 min onto the lectin-bound surface of the sensor chip at a flow rate of 5 mL min^{-1}. The change of the SPR response (expressed as resonance units or RU) was monitored at 25 °C for 9.30 min. The same glycoprotein sensor chip surfaces were used repeatedly after removing the remaining immobilized lectin washing with 10 mM HCl for 2 min. For immobilization on the sensor chip, lectins were used at a concentration of 500 μg mL^{-1} in 5 mM sodium acetate buffer, pH 4.0. According to the change of SPR response as a result of the immobilization of the lectins on the carboxymethylated dextran layer covering the sensor chip, an estimated surface concentration of 5–10 ng mm^{-2} of dextran was calculated depending on the immobilized lectins.

The interaction of anti-IgE antibodies with the IgE previously bound to the immobilized lectins was monitored by injecting rabbit anti-human IgE serum diluted 1:10 in HBS (pH 7.4) containing 20 mM Gal and Man, at the beginning of the dissociation phase of the IgE/lectin complex during 5 min at a flow rate of 5 mL min^{-1}, and the change of the SPR response (RU) was monitored at 25 °C during 9.30 min. All SPR measurements were performed in duplicate.