Allergology International. 2005;54:387-392

ORIGINAL ARTICLE

Cross-reactive Carbohydrate Determinant Contributes to the False Positive IgE Antibody to Peanut

Komei Ito[1], Masashi Morishita[1], Mihoko Ohshima[2], Tatsuo Sakamoto[2] and Akira Tanaka[3]

ABSTRACT

Background: The importance of peanut allergy has not been well recognized in Japanese society. IgE antibody to peanut can be, however, detected in patients without clinical peanut allergy.

Methods: Clinical characteristics of 14 patients (aged 1–8 years) with peanut allergy were evaluated. IgE antibodies to peanut from patients with and without clinical peanut allergy were compared with those to soybean and other nuts. To examine the role of cross-reactive carbohydrate determinant (CCD) on the clinically false positive detection of peanut IgE, horseradish peroxidase (HRP) and bromelain specific IgE were measured by Uni CAP IgE kit. Inhibition of peanut IgE by HRP was also examined.

Results: The patients repeatedly experienced potentially life-threatening symptoms, including anaphylaxis. Sera from patients with peanut allergy had negative or relatively low IgE antibodies to other nuts. However, clinically false positive peanut IgE showed significant correlation-coefficients with soybean, almond, chestnut, pistachio, macadamia and cashew ($r = 0.61–1.00$). Anti-HRP and anti-bromelain IgE antibodies were detected in the clinically false positive sera, but not in the sera from patients with peanut allergy. Two out of four clinically false positive peanut IgE antibodies were significantly inhibited by HRP.

Conclusions: Social education about the features of peanut allergy is needed in Japan. Anti-CCD IgE antibody was suggested to be one of the mechanisms contributing to the false positive detection of peanut IgE. Detection of anti-HRP or anti-bromelain IgE can be a useful tool to recognize the presence of anti-CCD antibodies.

KEY WORDS

anaphylaxis, cross-reactive carbohydrate determinant, food hypersensitivity, immunoglobulin E, peanut hypersensitivity

INTRODUCTION

Allergies to peanut account for the majority of fatal and near-fatal anaphylactic reactions to foods.[1] In the United States, 3 million people are allergic to peanut or tree nuts, and peanut-induced anaphylaxis causes 50 to 100 deaths per year.[2] In Japan, peanut contributes to 2.4% of immediate type allergic reactions to food.[3] According to the results from this nation-wide study, and because of the severity of allergic symptoms to peanut reported overseas, the Japanese National Ministry of Health, Labor and Welfare designated peanut as one of the five major food allergens required to be specified on the label of food products. However, peanut allergy is not well understood in the community, partially because of the lack of precise information about the prevalence or clinical importance of peanut allergy among the Japanese population.

The first aim of this paper is to report the clinical importance of peanut allergy in Japanese children, and allergic cross-reactivity of peanut to soybean and other nuts.

Measurement of IgE antibody to peanut is a screening test for diagnosis of peanut allergy. However, positive IgE antibody does not always indicate a definitive diagnosis of peanut allergy.[4] The presence of IgE antibody to the carbohydrate (oligosaccharide) moieties in the plant antigen is one of the well-known

[1]Division of Allergology, Aichi Children's Health and Medical Center, [2]Department of Pediatrics, Nagoya University Graduate School of Medicine, Aichi and [3]Sweden Diagnostics KK, Tokyo, Japan.
Correspondence: Dr. Komei Ito, Division of Allergology, Aichi Children's Health and Medical Center, 1–2 Osakada, Morioka, Obu, Aichi 474–8710, JAPAN.
Email: koumei_ito@mx.achmc.pref.aichi.jp
Received 12 August 2004. Accepted for publication 27 December 2004.

Table 1 Clinical features of peanut allergy

No	Peanut IgE (UA/ml)	Age (Years)	First episode (Years)	Symptoms	Other food allergies	Total IgE (IU/ml)
1	100	6.7	3	Anaphylaxis x2, asthma *	milk, soybean (itch)	689
2	49.6	4.2	2	Anaphylaxis, urticaria	milk	812
3	45.6	3.6		Anaphylaxis x2	milk, walnut (urticaria)	741
4	10.3	6.1		Anaphylaxis	—	1180
5	2.7	4.8	1	Anaphylaxis	milk, eggs	608
6	100	5.6		Urticaria	—	1918
7	85.6	5.0	2	Urticaria, vomiting, diarrhea	—	8316
8	16.1	2.8	3	Urticaria	—	307
9	8.17	2.1	1	Urticaria	eggs, wheat, soybean (urticaria)	215
10	1	2.8		Urticaria, abdominal pain	—	26
11	15.4	1.3	1	Erythema	eggs	583
12	2.01	3.0	2	Erythema, itchy skin	—	217
13	1.08	8.2	3	Erythema	eggs, gelatin, soybean (eczema)	203
14	100	2.3		Eczema after 2 days	milk, eggs, shrimp, chocolate (eczema)	11270

* Induced by prick-to-prick test with peanut butter

Table 2 Clinical features of nut allergy.

No	Age (Years)	Nuts	Symptoms	Walnut IgE (UA/ml)	Peanut IgE (UA/ml)	Peanut allergy
1	3	Walnut	Urticaria	0.35	45.6	+
2	5	Walnut, pine nut	Anaphylaxis	5.52	0.8	-
3	2	Walnut	Cough	0.65	2.2	-
4	3	Walnut	Anaphylaxis	3.36	0.5	-
5	6	Walnut	Urticaria	4.17	14.1	-
6	5	Mixed nuts †	Erythema	4.21	3.4	-

† Almond, cashew, walnut

mechanisms contributing to the clinically false positive detection of IgE antibodies.[5] The common structures of N-linked glycan in plants (fruits, vegetables and pollens) have been well characterized and designated as cross-reactive carbohydrate determinant (CCD).[6] Natural Ara h 1, the major peanut allergen, has a single N-glycosylation site bearing five glycan species in a one to one ratio.[7]

The second aim of this paper is to investigate the role of anti-CCD IgE antibodies in the clinically false positive IgE antibodies to peanut, soybean and other nuts.

METHODS

Fourteen patients (aged 1−8 years, mean ± SD : 4.17 ± 2.0 years) with apparent history of immediate type peanut allergy were recruited to reveal the clinical characteristics of peanut allergy. Six patients with tree nut allergy (aged 2−6 years, 4.63 ± 1.69 years) were also analyzed. Oral challenge tests were not always performed to confirm the diagnosis, because severe anaphylaxis might occur in those with peanut and tree nut allergy.[1]

Sera from the 14 patients with peanut allergy and 8 patients without clinical peanut allergy despite the detection of IgE to peanut (designated as clinically false positive sera) were served for measurement of IgE antibodies to other nuts by UniCAP specific IgE kit (Pharmacia Diagnostics AB, Sweden). The nuts examined were soybean, walnut, almond, cashew nut, chestnut, pistachio, macadamia and pine nut, although some data were lacking due to the limitations of the sera obtained.

Four representative sera from patients with peanut allergy and four clinically false positive sera were examined for IgE antibodies to horseradish peroxidase (HRP)[8] and bromelain (from pineapple stem)[9] by UniCAP specific IgE kit. The UniCAP inhibition test was also performed using peanut ImmunoCAP and HRP (Sigma P6782, St. Louis, MO, USA) as an inhibitor. HRP contains 6 N-linked glycans and bromelain carries only one IgE-binding glycan.[10]

Informed consent was obtained from parents of the subjects to donate their sera for this study.

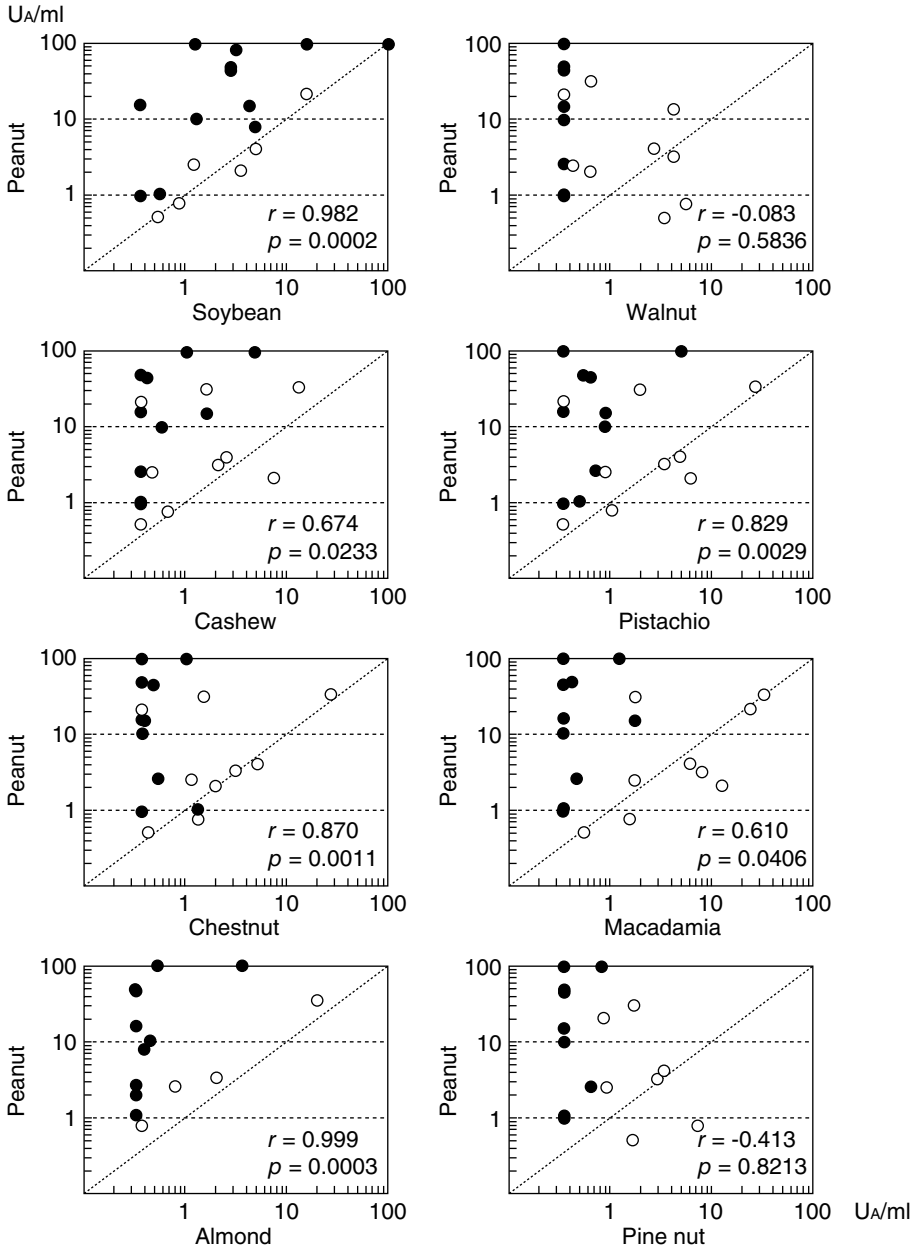

Fig. 1 Correlation of IgE antibodies to peanut and other nuts. Closed circles indicate sera from patients with peanut allergy, and open circles indicate sera from patients without peanut allergy. Pearson's correlation-coefficient was calculated only with the clinically false positive sera.

RESULTS

The clinical symptoms of 14 patients with peanut allergy were mostly severe, including anaphylaxis (n = 5), systemic urticaria (n = 5), erythema (n = 3), asthma and gastrointestinal symptoms (Table 1). The severity of symptoms was compatible to that reported in the United States or European countries.[1] Most of the patients had their first episode at 1−3 years of age. Although the parents were careful to avoid any food contaminated with peanut, some patients repeated the symptoms by eating processed foods such as curry, sandwiches and crackers (contamination from the manufacturing line), or by contact to nutshells.

Three patients were also allergic to soybean, but the symptoms were mild and consisted of atypical immediate reactions (eczema, urticaria). One patient had an allergy to walnut (urticaria), but IgE antibody to walnut was negative.

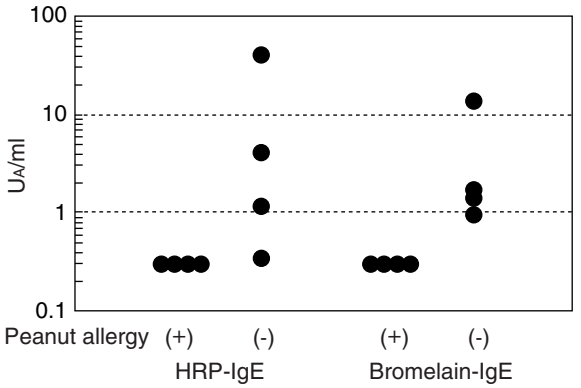

Fig. 2 IgE antibodies to HRP and bromelain in the sera from patients with and without peanut allergy.

Six patients had allergies to other nuts, including walnut (n = 5), pine nut (n = 1) and mixed nuts (almond, cashew and walnut, n = 1), which caused anaphylaxis (n = 2), urticaria (n = 2) and cough (n = 1). Only one patient was cross-reactive to peanut (Table 2). Although most of the patients avoided peanut and tree nuts, clinical cross-reactivity between peanut and other tree nuts was not common.

Correlations of IgE antibodies between peanut and other nuts are shown in Figure 1. Most of the sera from patients with peanut allergy had negative IgE to walnut, chestnut, macadamia, almond and pine nut. Although IgE antibody to soybean was positive in most of the patients with peanut allergy, IgE antibody to peanut was higher than that to soybean. These data suggested that IgE antibody from patients with peanut allergy tend to bind to the peanut-specific epitope which did not cross-react to other nuts.

On the other hand, clinically false positive sera showed significant correlation-coefficients between IgE antibodies to peanut and soybean (r = 0.98, p = 0.0024), almond (r = 1.00, p = 0.0003), chestnut (r = 0.87, p = 0.0011), pistachio (r = 0.83, p = 0.0029), macadamia (r = 0.61, p = 0.0406) and cashew (r = 0.67, p = 0.0233). These data suggested that false positive peanut IgE recognized the common structure between peanut and these nuts. No significant correlation was observed between clinically false positive IgE to peanut and walnut or pine nut (Fig. 1).

Anti-CCD IgE antibodies were measured to reveal one of the mechanisms of the clinically false positive IgE to peanut. Anti-HRP and anti-bromelain IgE were detected in 4 representative sera from patients without peanut allergy. However, the 4 representative sera from patients with peanut allergy showed negative IgE to HRP and bromelain (Fig. 2).

IgE inhibition tests showed that pre-incubation of the sera with HRP significantly abrogated IgE binding to peanut in 2 of the 4 sera with false positive peanut IgE in a dose-dependent manner. Another serum showed partial (40%) inhibition, and the other was not inhibited. These findings suggested that CCD might contribute to the clinically false positive peanut IgE in some patients. On the other hand, no inhibition was observed in the sera from any of the 4 patients with peanut allergy (Fig. 3).

DISCUSSION

In the United States and Europe, many authors have emphasized the importance of peanut allergy because of the life-threatening symptoms and increasing number of affected patients.[1,2] The prevalence of peanut allergy in the Japanese population is not clear. Here we report on 14 children with peanut allergy in our clinic. This number was compatible to that of buckwheat, fish or shrimp allergy in our clinic (data not shown). The severity of allergic symptoms of each patient was similar to that reported previously, and the patients repeatedly experienced potentially life-threatening symptoms.

It is known that almost 30% of patients with peanut allergy also respond to other tree nuts.[11] Our findings also suggested that 28.6% (4 out of 14) patients with peanut allergy reacted to soybean or walnut. However, the reactions to soybean observed in the 3 cases were not typical immediate-type responses, such as worsening of eczema.

Ara h 1, the major peanut allergen, belongs to the cupin superfamily, named vicilin.[12] Although homologous proteins exist in the other legume families,[12] patients with peanut allergy had no or relatively lower IgE antibodies to other tree nuts or soybean. These findings suggested the presence of peanut-specific epitopes that are preferentially recognized by IgE antibodies of patients with peanut allergy.

Shin *et al.* reported that three Ara h 1 molecules assemble to form a highly stable trimeric complex,[13] and IgE-binding epitopes are clustered near the regions of monomer-monomer contact.[14] According to this trimeric structure, Ara h 1 may possess resistance to protease digestion, and the increased number of IgE-binding epitopes in one molecule may induce a strong release of chemical mediators from mast cells. Cross-reactivity of this trimeric Ara h 1 between homologous proteins of other tree nuts may be of interest.

On the other hand, IgE antibody to peanut can be detected from atopic patients without clinical symptoms to peanut. The presence of IgE antibody to the carbohydrate moiety (CCD) is a well-known mechanism to explain the detection of clinically false positive IgE to plant allergens.[6] Figure 4 shows the structures of N-linked glycans of HRP, bromelain and Ara h 1. Common structures of glycans have been characterized from a variety of plant allergens.[15] The presence of β(1, 2)-Xylose and α(1, 3)-Fucose residues linked to the core $Man_nGlcNAc_2$ backbone is known to contribute to the IgE binding.[16] Anti-CCD IgE anti-

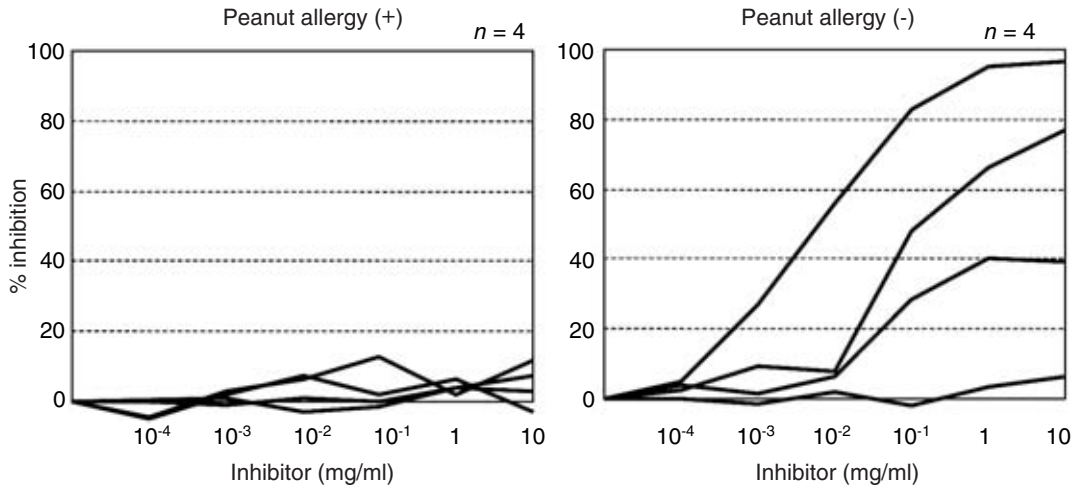

Fig. 3 Peanut IgE inhibition tests with HRP, a representative CCD antigen. Individual sera from 4 patients with and without peanut allergy were pre-incubated with the indicated concentrations of HRP, and peanut IgE antibodies were detected by UniCAP. Percent inhibition of peanut IgE titers are shown.

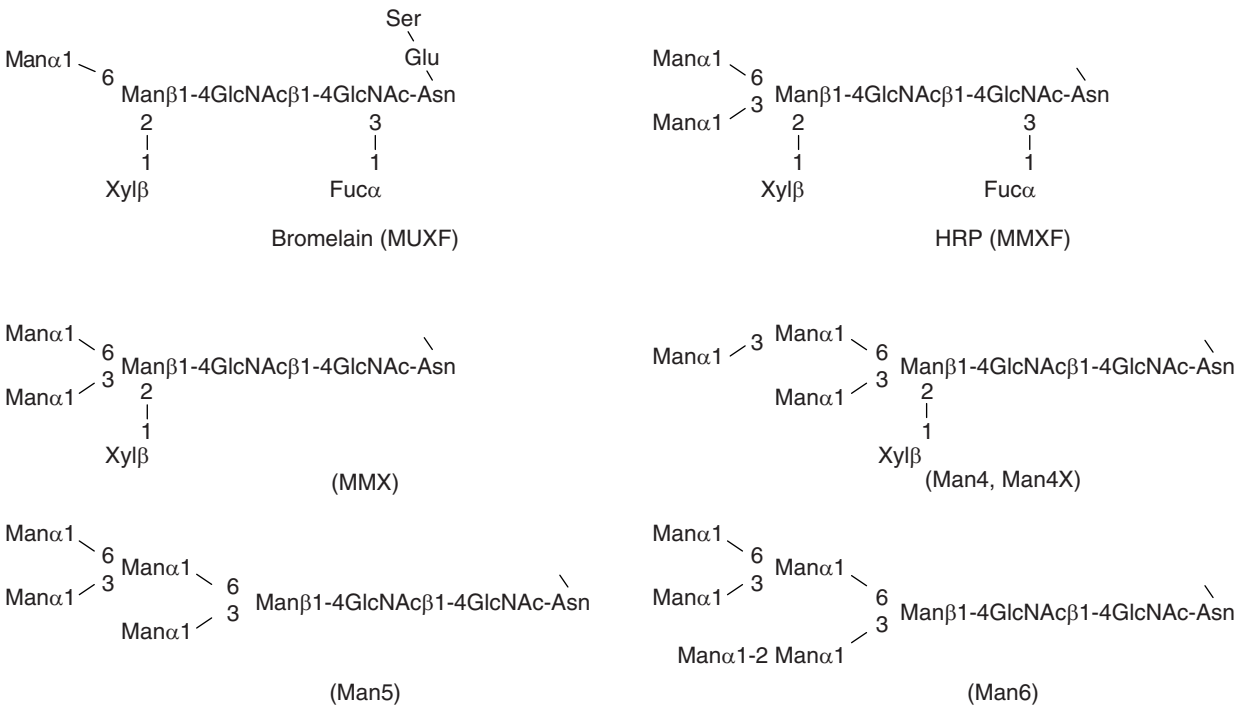

Fig. 4 Structures of the N-linked glycans known as cross-reactive carbohydrate determinant (CCD). Bromelain from pineapple stem has one xylose-containing N-linked glycan (MUXF). Horseradish peroxidase (HRP) has 6 xylose and fucose-containing glycans (MMXF). Ara h 1 has one glycosylation site bearing either one of the 5 oligosaccharides (MMX, Man4X, Man4, Man5, Man6).

bodies have poor biologic activity in terms of weak basophil histamine release and negative skin prick tests,[4] because CCD on a monoglycosylated allergen can not cross-link high-affinity IgE receptors on mast cells and basophils. Van der Veen *et al*. have reported that positive IgE antibodies to peanut without clinical symptoms were the consequence of anti-CCD IgE that cross-reacted to peanut and grass-pollen aller-

gens.[17]

Our findings also support the idea that anti-CCD IgE antibodies contribute to the clinically false positive IgE antibodies to peanut and tree nuts in some patients. In general, anti-CCD IgE antibody binds to multiple plant allergens including pollens, vegetables, fruits and nuts. Care should be taken to consider the presence of anti-CCD IgE in the sera with positive IgE antibodies to multiple allergens, especially in the Japanese clinical practice where detection of IgE antibodies is more commonly used than skin prick tests or oral food challenge for the screening of food allergy.

Detection of anti-HRP or anti-bromelain IgE by UniCAP is a practical tool for the detection of anti-CCD IgE. Furthermore, the RAST inhibition test may be required to confirm the presence of CCD in the allergen. In the present study, one false positive serum showed negative inhibition of peanut IgE with HRP, suggesting that CCD is not always the feature of clinically false positive IgE.

In conclusion, peanut allergy causes severe, sometimes life-threatening reactions in Japanese children. Social education about the importance of peanut allergy is needed. However, detection of IgE antibody to peanut does not always indicate a definitive diagnosis of peanut allergy, and the presence of anti-CCD IgE antibody should be considered in a case of multiple positive IgE antibodies to plant allergens, including peanut.

REFERENCES

1. Sampson HA. Peanut Allergy. *N. Engl. J. Med.* 2002;**346**: 1294-1299.
2. Leung DYM, Sampson HA, Yunginger JW *et al.* Effect of anti-IgE therapy in patients with peanut allergy. *N. Engl. J. Med.* 2003;**348**:986-993.
3. Imai T, Iikura Y. [The national survey of immediate type of food allergy.] *Jpn. J. Allergol.* 2003;**52**:1006-1013(in Japanese).
4. Van Ree R, Aalberse RC. Specific IgE without clinical allergy. *J. Allergy Clin. Immunol.* 1999;**103**:1000-1001.
5. Mari A, Iacovacci P, Afferni C *et al.* Specific IgE to cross-reactive carbohydrate determinants strongly affect the in vitro diagnosis of allergic disease. *J. Allergy Clin. Immunol.* 1999;**103**:1005-1011.
6. Aalberse RC, van Ree R. Crossreactive carbohydrate Determinants. *Clin. Rev. Allergy Immunol.* 1997;**15**:375-387.
7. Kolarich D, Altmann F. N-glycan analysis by matrix-assisted laser desorption/ionization mass spectrometry of electrophoretically separated nonmammalian proteins : Application to peanut allergen Ara h 1 and olive pollen allergen Ole e 1. *Anal. Biochem.* 2000;**285**:64-75.
8. Kurosaka A, Yano A, Kuroda Y *et al.* The structure of a neural specific carbohydrate epitope of horseradish peroxidase recognized by anti-horseradish peroxidase antiserum. *J. Biol. Chem.* 1991;**266**:4168-4172.
9. Bouwstra JB, Spoelstra EC, de Waard P *et al.* Conformational studies on the N-linked carbohydrate chain of bromelain. *Eur. J. Biochem.* 1990;**190**:113-122.
10. Foetisch K, Westphal S, Lauer I *et al.* Biological activity of IgE specific for cross-reactive carbohydrate determinants. *J. Allergy Clin. Immunol.* 2003;**111**:889-896.
11. Sicherer SH, Burks AW, Sampson HA. Clinical features of acute allergic reactions to peanut and tree nuts in children. *Pediatrics* 1998;**102**:131.
12. Breiteneder H, Radauer C. A classification of plant food allergens. *J. Allergy Clin. Immunol.* 2004;**113**:821-830.
13. Shin DS, Compadre CM, Maleki SJ *et al.* Biochemical and structural analysis of the IgE binding sites on Ara h 1, an abundant and highly allergenic peanut protein. *J. Biol. Chem.* 1998;**273**:13753-13759.
14. Maleki SJ, Kopper RA, Shin DS *et al.* Structure of the major peanut allergen Ara h 1 may protect IgE-binding epitopes from degradation. *J. Immunol.* 2000;**164**:5844-5849.
15. Fotisch K, Vieths S. N- and O-linked oligosaccharides of allergenic glycoproteins. *Glycoconj. J.* 2001;**18**:373-390.
16. van Ree R, Cabanes-Macheteau M, Akkerdaas J *et al.* β(1,2)-Xylose and α(1,3)-Fucose residues have a strong contribution in IgE binding to plant glycoallergens. *J. Biol. Chem.* 2000;**275**:11451-11458.
17. Van der Veen MJ, van Ree R, Aalberse RC *et al.* Poor biologic activity of cross-reactive IgE directed to carbohydrate determinants of glycoproteins. *J. Allergy Clin. Immunol.* 1997;**100**:327-334.