

Skin test, RAST and clinical reactions to peanut allergens in children

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Summary

One-hundred-and-four children were skin-tested with four peanut-allergen preparations, a commercial extract, extracts of raw and roast peanuts prepared by NH_4HCO_3 extraction, and a wheatgerm lectin-reactive glycoprotein obtained by affinity chromatography. The presence of symptoms after ingestion of peanut or peanut products was also recorded. The roast allergen extract provided the greatest specificity with eight symptomatic children having a positive skin test and only one positive skin-test reaction in an asymptomatic child in the group of 104 children tested. Despite differences in the incidence of skin-test reactions there was a strong correlation between raw, roast and commercial RAST suggesting common allergens were being identified by circulating IgE. Clinical sensitivity was observed particularly in younger children with 75% of the children being under 4 years of age. A positive roast skin test or a RAST test adds confirmation to the clinical history of allergic reactions to peanuts.

Introduction

Peanuts are one of the more common food substances which elicit allergic reactions in children. In a group of children extensively investigated for food allergies only milk, eggs and peanuts were identified as giving immediate reactions (Bock *et al.*, 1978). In another study 70% of asthmatic children were positive on skin-prick testing, however, only 56% of the sixteen children who underwent formal allergen challenge developed symptoms (Bock *et al.*, 1977). In addition to skin testing, determination of specific circulating IgE and RAST has been suggested as a diagnostic aid in peanut allergy. In the study of Bock *et al.* (1978), fourteen children with confirmed reactions to peanut

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protein, were all positive on skin-prick testing with a commercial extract. Gillespie, Nakajima and Gleich (1976) reported seventeen adults and one child with a history of sensitivity to nuts and peanuts and five of these had a raised peanut RAST, however the association of RAST results and the history of symptoms on peanut ingestion was unclear. A partially purified allergen isolated from raw peanuts was shown to give positive skin-prick tests in two out of three peanut-sensitive adults and raised specific IgE binding in all subjects as compared with non-allergic controls (Sachs, Jones & Yunginger, 1981). In the present study we describe the features of twelve children with clinical sensitivity to peanuts and describe skin-prick tests and RAST with extracts from raw and roast peanuts and a peanut glycoprotein reactive with wheatgerm lectin.

Methods

Preparation of allergens

Raw, shelled peanuts of the Virginia Bunch variety were supplied by the Peanut Marketing Board, Kingaroy, Queensland. To facilitate manual removal of the skins, peanuts were heated gently (45°C for 5 min). Skinned peanuts were finely ground and defatted by Soxhlet extraction with petroleum ether 40–60°C to produce defatted peanut flour. Crude peanut extract (RAW) was obtained by stirring the flour for 4 hr with 0.1 M ammonium bicarbonate solution (pH 8.0) 1:9 w/v. Insoluble debris was removed by centrifugation at 5°C (20 000 rpm for 30 min). The supernatant was filtered through glass wool and lyophilized. Roast peanut extract (ROAST) was obtained by similar treatment of peanuts which had been heated, prior to defatting, to simulate commercial roasting (145°C for 1 hr).

The wheatgerm lectin-reactive glycoprotein (PURE) was obtained by affinity chromatography of a solution of crude peanut extract (20 mg/ml) in Tris/HCl buffer (pH 8.0) 0.5 M, on a column of wheatgerm lectin–Sepharose 6MB. The bound material was eluted by a solution of *N*-acetyl glucosamine (20 mg/ml) in the Tris buffer. The eluate with absorbance at 280 nm was combined and lyophilized.

Prick-test solutions were prepared by dissolving the appropriate extract (1 mg/ml) in 50% v/v glycerol–water containing phenol 0.5% w/v as preservative. Commercial peanut allergen (1:10 w/v in 50% glycerol) [COMM] was purchased from Hollister-Stier Laboratories.

Skin-prick tests

Skin-prick tests were done on the forearm. Reactions were read at 10 min and a wheal larger than 2 mm was taken as positive. All patients were tested with a phenol/saline control and histamine (1 mg/ml) solutions. In a previous study 96% of a group of asthmatic children who reacted to one or more of a group of fourteen inhalant allergens were identified by three allergens: *D. pteronyssinus*, cat fur and rye grass (Bencard, Brentford) (Van Asperen *et al.*, 1980). In the present study all children were skin-tested with these three antigens.

RAST test

Allergen RAST discs were prepared by coupling, via the cyanogen bromide activation procedure of Ceska, Eriksson and Varga (1972) the extracts (100 µg/disc) to 6 mm diameter discs punched from Whatman grade-42 filter paper. Phadebas peanut

allergen discs (Pharmacia Diagnostics Ab, Uppsala) were used, for comparative purposes. RAST was carried out according to the Phadebas procedure.

Subjects

Children aged 7 months to 14 years who had immediate hypersensitivity skin-prick tests requested as part of evaluation of possible allergic disease were in addition tested with the peanut allergens. Their mothers were asked if the child ate peanuts or peanut-containing foods and if these caused any symptoms. Fifty-seven percent of the children were more than 5 years old.

Results

One-hundred-and-four children were skin-tested with the four peanut-allergen preparations and asked whether peanut or peanut-containing foods caused any symptoms. The results of the skin tests are shown in Table 1. Sixty children reacted to one or more of the inhalant allergens and of these forty-one (68%) children reacted to one or more of the peanut allergens. Forty-four children did not react to the inhalant allergens and two of these (5%) reacted to one or more of the peanut-allergen preparations. In this group of 104 children eight had symptoms on ingestion of peanuts or peanut products and all eight were positive on the ROAST skin test. The symptoms occurred within minutes of ingestion of peanuts or peanut butter and consisted of facial urticaria and/or angioedema in all cases.

With the later addition of a further four cases not included in the 104 children, we identified twelve children with a history of immediate reactions to peanut or peanut products. The clinical features and skin-test results of these children are shown in Table 2. The age of the children ranged from 5 months to 14 years with nine children being less than 4 years. In all cases symptoms of urticaria and/or angioedema were reported within minutes of peanut ingestion. Eleven of the twelve children had a history of wheezing; however, in only one case was wheeze reported to be a feature of the immediate reaction. Despite the high level of sensitivity to the PURE allergen in the total population only seven of the twelve symptomatic children reacted to this allergen on skin-testing. Seven of the twelve children also reacted to egg with urticaria and/or angioedema.

RAST using RAW and ROAST allergens was done on twelve sera from children with a history of symptoms and on six sera from children who were prick-test-positive to one or more of the peanut allergens but who tolerated peanuts without symptoms.

Table 1. Number of children positive ($n=60$) or negative ($n=44$) to inhalant allergens, reacting to prick tests with peanut allergens

Skin-test reactions						
Inhalant allergens	<i>n</i>	Peanut allergens				
		Any positive	PURE	RAW	ROAST	COMM
Positive	60	41	36	24	8	17
Negative	44	2	0	1	1	2

Table 2. Clinical signs and prick-test responses to peanut allergens in twelve children with clinical allergy to peanuts

Symptoms	
Urticaria/angioedema	12
Rhinorrhoea	3
Choking	1
Vomiting	1
Wheezing	1
Egg sensitivity	7
Positive skin tests	
PURE	7
RAW	11
ROAST	12
COMM	11

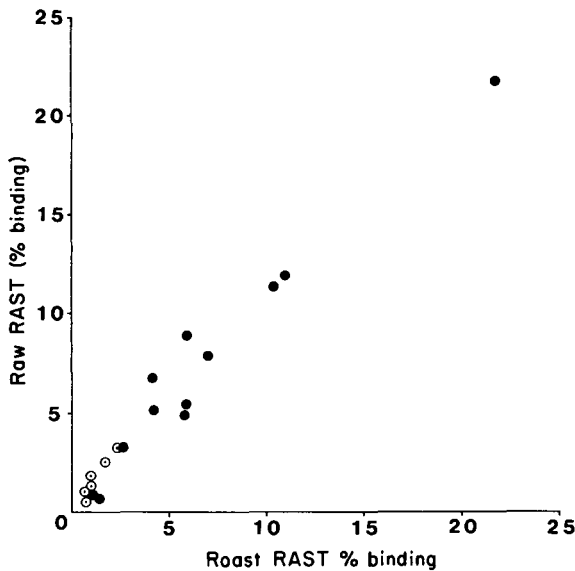


Fig. 1. RAW and ROAST RAST results. ○, asymptomatic skin-test-positive; ●, symptomatic skin-test-positive.

The results are shown in Fig. 1 where the % binding of the RAW RAST is plotted against the ROAST. Results from the two tests were strongly correlated, having a correlation coefficient of 0.98 ($n = 18$). In ten samples where the commercial RAST was also done the correlation coefficient of COMM and RAW RAST was 0.94. Binding values were higher in the symptomatic children than the skin-test-positive but asymptomatic children. None of the asymptomatic, but nine of the twelve symptomatic children, had RAST binding levels above 4% for both RAW and ROAST allergens.

Discussion

We evaluated skin-prick and RAST-testing to peanut extracts in immediate reactions to peanut allergens in children. Positive skin-prick tests to peanut allergens were strongly associated with atopy as defined by a positive skin test to one or more of three inhalant allergens. This finding indicates that the skin-test reactions are not due to a non-specific irritant effect of the preparations. Sensitization to peanut allergens as determined by a positive skin-prick test appears to be extremely common in atopic children, with 68% of atopic children reacting to one or more peanut-allergen preparations. Sensitization, however, was not necessarily related to clinical symptoms. The ROAST allergen preparation provided the greatest specificity with reference to symptoms. No patients were identified who had symptoms and a negative ROAST test while in a group of 104 children surveyed only one child had a positive ROAST test and no symptoms. Heiner and Neucere (1975) had previously demonstrated that roasting of peanuts did not destroy RAST activity, suggesting that clinically relevant allergens are heat stable. Crossed radioimmuno-electrophoresis on the present RAW and ROAST fraction demonstrated that sixteen allergenic peaks were detected with the RAW preparation and this had decreased to seven on the ROAST preparation (Barnett *et al.*, 1983). The present observations provide further evidence that the clinically important peanut allergens are heat stable.

The wheatgerm lectin-reactive material separated here by affinity chromatography was first described by Horst *et al.* (1980) and because of its specific lectin interaction appears to be a glycoprotein. SDS electrophoresis of the material prepared for this study revealed three components (Barnett *et al.*, unpublished). Skin-testing with this material indicated a high rate of sensitization (60%) in atopic children. This reaction appears to be a specific IgE-mediated reaction rather than a non-specific irritant effect as none of the forty-four skin-test-negative children showed a positive prick test. However, only 58% of the symptomatic children reacted with this material suggesting that important allergens have been removed during the purification process. This finding of an increased proportion of patients with detectable sensitivity, but decreased specificity for clinical reactions after purification, indicates the difficulties of identifying relevant allergens by skin- or RAST-testing alone without reference to clinical symptoms.

The RAST proved reasonably accurate in distinguishing between asymptomatic skin-test-positive individuals and those with a history of symptoms. There was a strong correlation between the RAW, ROAST and COMM RAST suggesting that common allergens are being measured. RAST binding above 4% was only observed in symptomatic individuals. We compared RAST levels in symptomatic individuals with those who are asymptomatic but skin-test-positive, in contrast to previous studies which have utilized non-atopic controls (Gillespie *et al.*, 1976; Sachs *et al.*, 1981). One explanation of the high correlation between the RAW and ROAST extracts on RAST but the different incidence of sensitization detected by skin tests is that additional allergens in the RAW preparation are identified by skin tests and a number of these allergens are destroyed by heating. In contrast, the allergens recognized by circulating IgE are heat stable.

In this study a history of symptoms was not confirmed by re-challenge. The symptoms described were in all cases of an immediate nature and occurred within minutes of exposure to peanuts. There is less likely to be confusion about the role of foods in this situation than with symptoms which are said to come on hours after food

ingestion. In all cases in the present study urticaria/angioedema was a feature of the reaction whilst in the study of Bock *et al.* (1978), urticaria was noted in nine children whilst three had symptoms of nausea and vomiting in the absence of urticaria and angioedema. It was of interest that although twelve subjects suffered from asthma only one of the twelve reported wheeze as a symptom of the clinical reaction. This experience is different from that of Bock *et al.* (1978), who noted wheeze in six of twelve children after peanut challenge. In the present study, clinical sensitivity was observed particularly in younger children with nine of the twelve being under 4 years of age, despite a greater proportion of children over 5 in the whole population tested. We have found only one case of clinical symptoms in more than three-hundred children skin-tested with peanut allergens who has been over 6 years of age. These findings suggest that sensitization to peanuts is particularly likely to occur in early childhood, and with time sensitivity may be lost in a situation analogous to that observed with milk proteins. Peanut sensitivity occurred in a group of children who had a more generalized tendency to food allergic reactions as evidenced by the high incidence of clinical reactions to egg.

It is of interest that in two cases immediate reactions to peanut occurred with the first exposure in breast-fed infants. While inadvertent exposure to cow's milk proteins may be possible, exposure at this age to peanut allergens unknown to the mother is unlikely, particularly as both hydrolysed peanut protein and peanut oil which are less readily identifiable appear to be non-allergenic (Nordlee *et al.*, 1981). This observation raises the possibility that sensitization may occur via the breast milk as has been suggested for milk and egg proteins (Warner, 1980).

The clinical story of immediate reactions after ingestion of peanuts is readily identified on the history and the positive RAST and skin test adds confirmation. We have not investigated the possibility of delayed reactions to peanuts which are not recognized by the patient but such reactions have been described after food challenge in younger children to soya-bean protein (Bock *et al.*, 1978). Whether such symptoms occur from peanuts remains to be proven.

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