INDUCTION OF IgE ANTIBODY RESPONSE BY THE GREEN SEED EXTRACT OF *VIGNA SINENSIS* IN MICE

T. Raghava Rao, D.N. Rao*, B. Veerendra Kumar, P. Aparanji, K. Srinivas and Rao R Athota

*Department of Biochemistry, AIIMS, New Delhi-110029, Department of Biochemistry, Andhra University, Visakhapatnam - 530 003. Andhra Pradesh

ABSTRACT

Sensitization to ingested foods is a known fact and several food allergens have been characterized. It has been observed in our survey that the people complained of allergic symptoms after consumption of the vegetable *Vigna sinensis*. In this study, the experiments were carried to investigate the IgE antibody response against the green seed extract of *vigna sinensis* in mice and found that the primary, secondary and tertiary immunization with or without adjuvant by different doses induced a significant production of IgE antibodies. The presence of IgE antibodies in the mice sera were determined by passive cutaneous anaphylaxis and enzyme linked immunosorbent assay. It was also confirmed that these allergens were found to be heat resistant and shared a common epitope(s) with the other legume foods, as evidenced by the cross-reactive studies.

KEYWORDS

Vigna sinensis, Food allergy, PCA, ELISA

INTRODUCTION

Allergic reactions to foods are recognized as an abnormal immunological response and the food allergy may be a sole or a contributing cause, producing symptoms of the immediate or delayed type, acute or chronic, mild or severe(1). Sensitivity to foods may manifest itself in the respiratory system as rhinitis, bronchial asthma(2), in the alimentary system as manifestations of gastroenteritis such as nausea, diarrhea(3) and in the cutaneous system as dermatitis, urticaria(4) and anaphylaxis(5). Apart from these, food sensitivity may be an occasional or rare cause of many manifestations. Rare manifestations of food allergy include migraine(6) other types of severe headache, epilepsy(7) and arthritis(8).

A few foods have often violent allergic reactions in sensitive individuals and some other foods have been implicated in definite symptoms with clear confirming immunological findings. The chances of developing food sensitivity to a food protein depend on several

factors, e.g. the extent to which the food in question is a staple item of the diet, atopic hereditary background of the subject, permeability of the gut, an antecedent episode of gastroenteritis, nasopharyngeal incoordination, and other yet unknown influences(9).

Among different types of sensitizing foods, milk(10) and egg(11) are known to cause severe allergic symptoms. Baldo and Blands, considered that the cereal grains were the most common food allergens, e.g. corn and wheat being the most frequent causes of chronic food allergy(12,13). Other food allergens of importance are seafood(14), fruits and vegetables(15). Some of these foods have been well documented to produce hypersensitivity reactions and their major allergens have been studied(16). Although vegetables are the important sources of known allergens, there is no sufficient information about allergy induced by vegetables as compared with other allergens. The diversity of allergenic materials and the relative ease of avoiding the ingestion of many vegetable foods have limited the extent to isolate and characterize these allergens.

MATERIALS AND METHODS

Animals

Balb/c (H-2^d) inbred mice (8 to 12 weeks old) were

Author for correspondence:

Prof. Rao R. Athota
Dept. of Biochemistry
Andhra University
Visakhapatnam - 530 003
Andhra Pradesh
Email: raoathota@yahoo.com

purchased from the Laboratory Animal Information Service Center (LAISC), National Institute of Nutrition, Hyderabad, India. Male albino Wistar rats weighing about 200 g were also procured from LAISC for their use in passive cutaneous anaphylaxis (PCA) to determine the IgE titers.

Preparation of *Vigna sinensis* seed extract

Vigna sinensis vegetables were obtained from the local market and seeds were collected and were thoroughly cleaned and prepared a 40% (w/v) extract in PBS (pH 7.4, phosphate 0.05 M and NaCl 0.15 M). The resulting homogenate was filtered and centrifuged at 5,000 rpm for 30 minutes. The pelleted material was discarded and the clear supernatant represented the extract, which was used as starting material for the identification and characterization of allergens.

Immunization protocol

Mice were immunized intraperitoneally (i.p) with different doses of *Vigna sinensis* seed extract with or without adjuvant alum in 0.5 ml/mouse on days 0, 8, 28, and 56 and were bled 14 days after primary dose and 7 days after secondary and tertiary immunizations. The sera obtained after secondary antibody response with 1.0 µg dose was considered as positive sera for determination of PCA titers and ELISA.

Measurement of IgE antibody

The IgE antibody response was measured by passive cutaneous anaphylaxis(17). In brief, the anti-sera of mice were passively transferred intradermally onto the shaved dorsal side of Wistar male rats in aliquots of 50 µl each in 2 - or 4 - fold serial dilutions. After 24 hrs the rats were challenged intravenously (penal vein) with a corresponding 5 mg of *Vigna sinensis* extract (for cross-reactivity studies different antigen extract were employed) in the presence of 0.5% Evans blue dye. The appearance of blue spots measuring greater than 5 mm was taken as a positive response and the reciprocal of highest dilution of anti-sera, which gave blue spots, was considered as an IgE antibody titer.

The IgE antibodies in the sera were also detected by ELISA. Briefly, the 96 well microtiter plates (Nunc, Denmark) were coated with 100 µl of *Vigna sinensis* extract at a concentration of 100 ng/ml in carbonate buffer (pH. 9.6) and incubated overnight at 4°C. After incubation, the wells were washed

thrice with PBS containing 0.05% Tween-20 (PBS-T). 3% skimmed milk powder (Anikspray, Hindustan Lever Limited, India) in PBS was used to block the nonspecific binding sites for 10-12 hrs at room temperature. After washing the plates, the wells were further incubated with 100µl of diluted sera (1:400 in PBS) in triplicates for one hr at 37°C. The unbound serum constituents were washed off and the level of IgE were measured by incubating with 100 µl of horseradish peroxidase (HRPO) conjugate of goat anti mouse IgE (Nordiac immuno chemicals, Netherlands) at a dilution of 1:1000 for one hr at 37°C. Followed by final washing with PBS-T, 100 µl of freshly prepared substrate solution (10 ml of 1.5M citrate phosphate buffer, pH5.0) containing 4.0 mg of orthophenylene diamine (OPD) and 10 µl H₂O₂ was added per well. The reaction was stopped after 5 min by adding 50µl of 8N H₂SO₄. The color developed was read at 492 nm using ELISA reader (Bio-Rad model-550). The data expressed was the mean of the optical density (OD) of the triplicates after subtracting the absorbance from the pre-immune sera.

RESULTS AND DISCUSSION

As seen in Fig 1 and 2 the primary, secondary and tertiary IgE antibody responses were induced in mice following intra-peritoneal administration of varying doses of *Vigna sinensis* extract in presence or absence of the adjuvant aluminium hydroxide. The IgE antibody production was first detected 14 days after immunization and the titers remained elevated for more than 30 weeks after tertiary booster immunization with 1.0 µg of extract. It is known that the nature of the dose of allergen is an important factor in specific antibody production against *Vigna sinensis* extract i.e. for each booster dose tested there is a clear indication of enhanced IgE antibody response. Adjuvant independent induction of IgE response against *Vigna sinensis* shows the possible presence of potent allergens as reported earlier(19). Moreover, the IgE antibody levels estimated by ELISA (Fig.3) showed a linear correlation with PCA titer. These results were similar to that of various allergens as reported by Ito *et al*(20).

Despite the variety of food proteins in the typical diet, only a few are likely to cause allergic sensitization in susceptible individuals. Individuals allergic to specific food are often advised to avoid other foods also that are closely related, because of the existence of multiple allergenic proteins. The

case study reports of the allergic patients, who visit the Government TB and Allergic Hospital at Visakhapatnam for treatment of allergic diseases, showed that they had allergic symptoms after consumption of certain group of foods, mostly legume foods. Legume foods are better known for their cross-reactivity(21). Interestingly, as seen in Table 1, *Vigna sinensis* specific IgE antibodies were found to cross-react with different food extracts of legume family but not with food extracts of other families. Previously, it was reported that cross-reactivity of allergens exist in the same food groups (22). However, investigations of this phenomenon in the area of food hypersensitivity has been very limited. The cross reactive nature of *Vigna sinensis* specific IgE antibody with legume foods extract implies the possible presence of shared antigenic determinants in the legume family foods. Furthermore, attempts were made to identify the

allergens in this study with raw seed extract, since, both boiled and unboiled extracts exhibited elevated levels of IgE antibody response. Food allergens are known to be heat-and acid-stable(23) and generally survive the digestive process also(24,25). The resistance of the *Vigna sinensis* proteins to heat, proteolytic enzymes and acid are partially responsible for their ability to reach the intestinal mucosa in an immunogenic form and can stimulate the allergic response after absorption into the blood circulation.

Our data suggest the possible presence of potent allergenic proteins in *Vigna sinensis* which are heat stable and highly cross reactive with legume foods.

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Figure 1.

PCA titers in Wistar rats against *Vigna sinensis* extract. The bars represent the mean titers in each group (n=4 mice in each group) passively transferred with mice anti *V. sinensis* sera of primary, secondary, and tertiary immune response after immunization with varying dose of antigen with alum adjuvant.

Figure 2.

PCA titers in Wistar rats against *Vigna sinensis* extract. The bars represent the mean titers in each group (n=4 mice in each group) passively transferred with mice anti *V. sinensis* sera of primary, secondary, and tertiary immune response after immunization with varying dose of antigen with alum adjuvant.

Figure 3.

IgE antibody levels against *Vigna sinensis* extract in mice immunized with 1.0 µg/dose of antigen. The bars represent the mean IgE antibody titers in mice sera (n = 4 mice in each group) after primary, secondary and tertiary immune responses.

TABLE 1

**CROSS REACTIVITY OF *VIGNA SINENSIS* SPECIFIC IGE ANTIBODY RAISED IN MICE
WITH OTHER DIETARY SOURCES SHOWN BY PASSIVE CUTANEOUS ANAPHYLAXIS
RESPONSE IN WISTAR RATS**

Antigen for PCA	Name	Family	Reaction
Dolicus lab lab	Beans	Leguminosae	(+)ve
Phacilus aurious	Green gram	Leguminosae	(+)ve
Phaciolus mungo	Black gram	Leguminosae	(+)ve
Elusine coracana	Finger millet	Graminae	(-)ve
Solanum molangenum	Egg plant	Solanaceae	(-)ve
Alium sativum	Garlic	Liliaceae	(-)ve

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