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IgE-MEDIATED HYPERSENSITIVITY TO TAUGEH (SPROUTED SMALL GREEN BEANS)

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This report describes a case of allergy toward taugeh (sprouted small green beans), an important constituent of eggroll. With use of the skin prick test, RAST, and histamine release test, IgE antibodies against taugeh extract could be demonstrated. A partial cross-reactivity was observed between IgE-binding components from taugeh and peanuts. Peanut allergic patients could also experience adverse reactions after ingestion of taugeh.

Introduction

PERMEABILITY OF THE GUT to food allergens is thought to be a critical factor in food allergy. Food specific IgE antibodies present on mast cells in the intestinal mucosa can be responsible for an increased uptake of food antigens.¹ The most common foods which can cause IgE-mediated hypersensitivity reactions are nuts and peanuts, eggs, milk, fish and shellfish, and cereals.² The RAST (radioallergosorbent test)³ has become an important tool in investigation of the possible involvement of IgE antibodies in adverse reactions to food. The present study describes a case of allergy towards taugeh, a food thus far not known to be involved in allergic reactions. Taugeh is an important component of egg-roll, a typical dish in Chinese restaurants. With use of the RAST and histamine release test (HRT) we have demonstrated the occurrence of IgE antibodies against taugeh.

Materials and Methods

Subjects

PvE, female, born in 1962, had suffered since the age of five from perennial rhinoconjunctivitis caused by an IgE-mediated allergy to house dust mite and grass pollen. From 1974 to 1978 she had received hyposensitization therapy with grass pollen and house dust mite extract, which resulted in improvement of her symptoms. At age 17 she experienced urticaria and angioe-

dema after the ingestion of food that contained peanuts, hazelnuts, onion and tomatoes. The skin prick test with these foods was positive. Specific IgE against peanut and hazelnut could be demonstrated in serum PvE. At age 18, attacks of exercise-induced urticaria and angioedema started to occur. In 1982 she took a meal that did not contain foods she was known to be allergic to. Within 15 minutes generalized urticaria and facial angioedema occurred. Patient PvE was skin tested with all known ingredients of this meal. Only taugeh gave a positive skin prick test.

Sera with specific IgE antibody against peanuts were selected from samples submitted for diagnostic IgE antibody determinations.

Allergens

Fresh taugeh (sprouted small green beans), small green beans and peanuts were obtained from a local grocery. Seventy grams of taugeh, 10 grams of small green beans or 20 grams of peanuts were homogenized separately with 100 ml of acetone for one minute at room temperature. The acetone was filtered off by suction and the residue was shaken for one hour with 100 ml of acetone at room temperature. After filtering off the acetone, the one hour acetone extraction was repeated twice. The remaining material was air dried at room temperature. The yield (expressed as percentage of the starting material, w/w) was 3% for taugeh, 86% for green beans and 50% for peanuts, respectively.

Estimation of Total IgE and Allergen-Specific IgE

Total IgE was determined by a competitive binding assay (Phadebas[®] IgE Test, Pharmacia) according to the manufacturer's instructions. Allergen-specific IgE was determined by RAST (radioallergosorbent test) using

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agarose beads as allergen-support⁴ or, when indicated, with use of allergen discs from Pharmacia. Allergens were coupled to CNBr-activated Sepharose 4B (Pharmacia) according to the manufacturer's instructions. For the preparation of taugh- and green-bean-Sepharose, 100 mg of acetone-treated taugh or green bean material were extracted with one to two ml of coupling buffer (0.1 M NaHCO₃, 0.5 M NaCl, pH 8.5) for one hour at room temperature. After centrifugation, protein in the supernatant was coupled to 100 mg of CNBr-activated Sepharose 4B. After four cycles of acid/base washings⁴ the allergen-Sepharose preparations were washed once with RAST-buffer (0.82% NaCl, 10 mM phosphate, 0.1% NaN₃, 0.2% Tween 20, 0.3% BSA,* pH 7.4), and finally resuspended in RAST-buffer. One or three mg of taugh-Sepharose or 0.6 mg green bean-Sepharose were used per test. In the RAST, 50 μ l of patient serum were incubated for at least four hours at room temperature with the indicated amount of allergen-Sepharose or allergen-disc. After three washes with physiological saline, 0.2% Tween 20, and one wash with RAST buffer, radioiodinated rabbit antihuman IgE antibodies (Pharmacia) were added.

After an overnight incubation the agarose beads or the discs were washed four times with physiological saline, 0.2% Tween 20. The percentage of bound radioactivity was counted in a LKB-Wallac gamma counter.

RAST Inhibition

Acetone-treated taugh and peanut were extracted for one to two hours in RAST-buffer at room temperature. After centrifugation 100 μ l of supernatant or 100 μ l of tenfold dilutions in RAST-buffer of the supernatant were incubated for at least four hours with 50 μ l of serum PvE. After addition of 1 mg of taugh-Sepharose, the mixture was incubated overnight at room temperature. After three washes with physiological saline, 0.2% Tween 20, and one wash with RAST-buffer, radioiodinated anti-IgE antibodies (Pharmacia) were added. After another overnight incubation at room temperature and four washes with physiological saline, 0.2% Tween 20, the percentage of bound radioactivity was counted in a LKB-Wallac gamma counter.

Histamine Release from Washed Leukocytes

The isolation of human leukocytes and the incubation of leukocytes with allergen were performed according to Siraganian.⁵ In brief, 10 ml of blood was mixed with 1 ml 0.1 M EDTA, pH 7.4 and 2.5 ml physiological saline, that contained 6% clinical dextran (Sigma) and 3% D-glucose. After one hour sedimentation, the yellow buffy-coat layer was taken off. After washing away the plasma, the leukocytes from the buffy-coat layer were incubated with allergen for 45 minutes at 37°C.

In some experiments the leukocytes were incubated

in buffer that contained 50% deuteriumoxide to potentiate the histamine release reaction.⁶ For use in the histamine release test 50 mg of acetone-treated taugh or peanut material were mixed for one hour at room temperature with 1 ml of TACM (TACM: 25 mM Tris, 120 mM NaCl, 5 mM KCl, 0.6 mM CaCl₂, 1 mM MgCl₂, 0.03% human serum albumin, pH 7.6). After centrifugation, the supernatant was used in the histamine release experiments. Histamine in samples was determined by an automated fluorometric method.⁵ The amount of histamine release was expressed as the percentage of the total amount of histamine present in the cell suspension.

Skin Prick Test

One to two mg of dry acetone-treated food material and two drops of phosphate buffered saline (PBS) were placed on the internal surface of the forearm. After mixing the food material with PBS, the skin was punctured through the fluid with a blood lancet. After 20 minutes the reactions were read. The reactions were graded in standardized plus signs.⁷

Results

Taugh-Specific IgE Antibodies

Table I shows that IgE antibodies against taugh could be demonstrated in serum PvE and in some other sera as well. Table I further shows that sera with total IgE levels up to 400 IU/ml will give negligible non-specific, non-immune IgE binding to taugh-Sepharose. At higher total IgE levels some non-specific IgE-binding will occur. At a serum total IgE level of 1500–1700 IU/ml, this non-specific IgE binding will result in five to six percent binding of the added radioactivity (Table I). To study the within-assay precision of the taugh-IgE-RAST, five samples of serum PvE were tested in the same assay. The average binding of ¹²⁵I anti-IgE was 13.0 $\pm$ 0.8 (sd) %.

Table I. Taugh- and peanut-specific IgE was measured by RAST in serum from 10 patients. The non-specific IgE-binding to taugh-Sepharose was investigated with 5 control sera (C₁–C₅) from persons, non-allergic to food. n.d.: not done.

Patient	Taugh-Sepharose 1 mg	RAST 3 mg	Peanut disc	Total IgE IU/ ml
—	1	1		
PvE	13	18	18	260
Vs	11	14	14	150
I	12	25	7	1400
V	4	9	22	280
Vu	2	3	12	250
Kn	3	6	17	1700
Ln	n.d.	15	33	2600
K	n.d.	5	15	340
dB	1	n.d.	16	<100
La	1	n.d.	17	190
C ₁	n.d.	5		1500
C ₂	1	n.d.		200
C ₃	2	n.d.		200
C ₄	2	n.d.		400
C ₅	2	n.d.		400

Percentage of binding of radioactivity

* BSA = bovine serum albumin (Sigma).

The Cross-Reactivity Between Tauegh and Peanut

In serum PvE, peanut-specific IgE was present as well as tauegh-specific IgE (Table I). Therefore, sera that were found positive in the peanut IgE RAST were also tested for tauegh-specific IgE antibody. Indeed, in many of these sera, IgE antibodies against tauegh were detected. Some of the sera that scored positive in the peanut-IgE RAST were negative in the tauegh-IgE RAST (e.g., sera La, dB and Vu, Table I). This suggests that there is only a partial cross-reactivity between peanut and tauegh allergens. The extent of cross-reactivity between peanut and tauegh allergens was further investigated by RAST inhibition. (Figure 1). From the horizontal distance between the two curves in Figure 1 it can be calculated that approximately 50 times more peanut extract than tauegh extract was needed for 50% inhibition of the tauegh-IgE RAST.

Allergens in Tauegh and Small Green Beans

Tauegh is made by inducing small green beans to sprout. The question arose whether the IgE-binding components in tauegh are newly formed or are already present in green beans. Table II shows that IgE from serum PvE also became bound to green bean extract.

Release of Histamine by Tauegh Extract

Figure 2 shows the results of an experiment in which leukocytes from patient PvE were incubated with peanut and tauegh extracts. From the horizontal distance between the two curves in Figure 2 it can be calculated

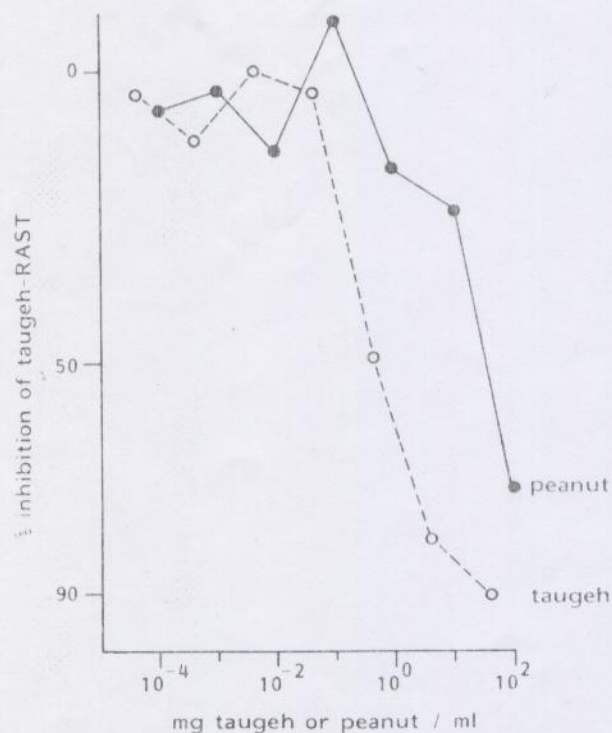


Figure 1. Inhibition of the tauegh-IgE-RAST by various amounts of acetone-treated tauegh or peanut, extracted in RAST-buffer (see Materials and Methods).

Table II. Serum from patient PvE and a control-serum (C) from a person, non-allergic to food were tested by RAST for IgE against small green bean-extract.

Patient	RAST Small green bean-Sepha- rose	Total IgE IU/ml
—	1	
PvE	11	295
C	3	260

Percentage of binding of radioactivity

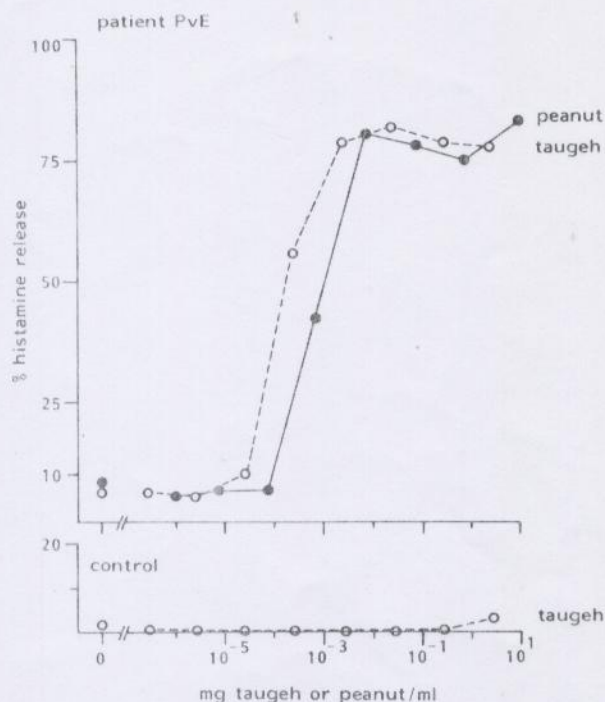


Figure 2. Histamine release from leukocytes of patient PvE after challenge in TACM, 50% D₂O with various amounts of acetone-treated tauegh or peanut, extracted in TACM-buffer (see Materials and Methods). Leukocytes from a non-allergic donor (control) were incubated with this tauegh extract in TACM, 50% D₂O.

that approximately five times more peanut extract than tauegh extract was needed to induce 50% release of histamine from leukocytes of patient PvE. At the concentrations of tauegh allergen tested, leukocytes from a normal donor did not release histamine. Leukocytes from another normal donor were unresponsive toward peanut extract (data not shown).

Skin Prick Test

When patient PvE was skin tested with tauegh, a 2½+ reaction was observed. When three control subjects were skin tested with tauegh, no reaction was observed. Skin prick tests with PBS were negative. Skin reactions to histamine were in the normal range.

Discussion

Tauegh is produced by inducing small green beans to sprout. During this process the vitamin C content of

this food rises sharply. Taugeh is an important component of egg roll, a typical dish in Chinese restaurants. Taugeh is also present in some salads. The positive reaction obtained after skin testing patient PvE with taugeh was strongly suggestive of an IgE-mediated allergy toward taugeh. The results of the RAST (Table I) and the histamine release test (Figure 2) further confirmed the presence of an IgE-mediated allergy toward taugeh in patient PvE. Sera with total IgE levels comparable to this level in serum PvE gave much less IgE-binding to taugeh-Sepharose than serum PvE (Table I). This demonstrates that the IgE-binding of serum PvE to taugeh-Sepharose was not caused by non-specific, non-immune binding of IgE to taugeh-Sepharose. Leukocytes from a normal donor did not release histamine on incubation with taugeh extract. This demonstrates that the release of histamine from leukocytes of patient PvE was not caused by a cytotoxic effect of taugeh extract on leukocytes.

In the histamine release test with leukocytes of patient PvE, taugeh extract was ~ five times more potent than peanut extract (Figure 2). At the inhibition of the taugeh IgE RAST, taugeh extract was ~50 times more potent than peanut extract (Figure 1). Table I shows that there is no constant ratio between the IgE-binding to taugeh and the IgE-binding to peanut when various sera were tested by RAST. Taken together, these data show that although a positive RAST for taugeh and a positive RAST for peanut often go together, the cross-reactivity between peanut and taugeh allergens is only partial.

In conclusion, this paper describes a new food allergy toward taugeh, and documents the IgE-mediated mechanism by skin test, RAST and histamine release test. The RAST data obtained thus far show that a substantial percentage of the patients with IgE antibodies against peanut also have IgE-antibodies against taugeh. Therefore, it should be realized that peanut allergic

patients might also experience adverse reactions to taugeh.

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"A man who doesn't worry at all doesn't care a whole lot. I should not want a man who did not care a whole lot operating on me or mine. Perhaps worry is a device of nature to make us try to do our very best. If we knew we should not worry, we might be tempted at times to be careless."

J. Chalmers Da Costa

The Trials and Triumphs of the Surgeon
 Ch. I