

Anaphylaxis after sesame seed ingestion

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Four patients with histories of systemic anaphylaxis or angioedema and urticaria after ingestion of sesame seed or sesame oil-containing products were studied for anti-sesame seed extract IgE by the radioallergosorbent test (RAST). Three of four were found to be positive. RAST inhibition after density-gradient ultracentrifugation of sesame seed extract indicates that the most active allergens appear to have molecular weights between 8,000 to 62,000 daltons.

Although systemic anaphylaxis is a well-known clinical entity, documentation of specific etiologic agents in such phenomena is often difficult. We studied four patients with suspected anaphylactic reactions to sesame seed or sesame seed oil and documented the presence of IgE anti-sesame seed globulin in three of these patients.

Additional studies of the sesame seed allergen were performed. Sucrose density-gradient ultracentrifugation and radioallergosorbent test (RAST) inhibition were employed to determine the relevant molecular weights of the IgE-binding allergens. Electrophoresis and Ouchterlony analyses indicated the relative numbers and mobility characteristics of the different antigens.

MATERIALS AND METHODS

Purification of sesame seed extract

Sesame seed concentrate (1/10) was obtained from Hollister-Stier (Spokane, Wash.). It was dialyzed to remove glycerine, then concentrated by ultrafiltration.

Rabbit immunization

Two rabbits were immunized with low-dose (0.08 mg) and two with high-dose (0.8 mg) sesame seed extract mixed with equal volumes of complete Freund's adjuvant. Primary immunization was followed by a booster injection (0.1 mg)

4 to 7 wk later. Serial bleedings of rabbit antisera were performed at 1-, 2-, and 3-wk periods after primary immunization and 1 wk after booster immunization.

Crossed electrophoresis

One percent medium endosmosis (ME) agarose (Marine Colloids, Inc., Rockville, Me.) was dissolved in Tris-barbital buffer (pH 8.6) containing 44.3 gm Tris, 22.4 gm barbital, 0.53 gm calcium lactate, and 1.0 gm sodium azide per 5 L. Fifteen microliters of sesame seed extract (3.4 mg/ml) were electrophoresed at 7 V/cm for 45 min in one dimension in a 1½ cm × 4 cm strip on a 3 cm × 4 cm glass plate. Rabbit anti-sesame seed extract (7.8 µl/cm²) in 1% agarose was layered on each side of the initial agarose strip. The plate was rotated 90°; the second electrophoresis was run overnight at 3 V/cm. Following drying of the agarose, the protein bands were stained with coomassie blue in 12.5% trichloroacetic acid.

RAST assays

Sesame seed extract and other antigens noted were coupled to cyanogen bromide-activated disks as described by Hoffman and Haddad.¹ Column-purified specific rabbit anti-IgE was labeled with ¹²⁵I.¹ Relative RAST score was graded so that each 1+ was equivalent to 4% binding of labeled anti-IgE after subtraction of normal control values. Maximal binding with allergen-coated disks and high-titered reagent sera is usually 40%, or equivalent to a 10+ score in our assay. Scores of 1+ or higher are considered significant.

Sucrose density-gradient ultracentrifugation

Sesame seed extract (3.5 mg) was layered over a linear 5% to 20% sucrose density gradient in barbital-buffered saline pH 7.4 (SW 41 rotor) and centrifuged at 40,000 rpm (Beckman Model L ultracentrifuge) for 17 and 40 hr at 5° C. Aliquots (0.5 ml) were removed from the top, tested for protein by the Lowry technique, and pooled in eight groups

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TABLE I. Patients and test results

Case No.	History of sesame seed-sensitive patients	Relative RAST score	Counts per minute anti-IgE bound*
1	72-yr-old woman developed hypotension, urticaria, and angioedema minutes after eating halvah candy; another episode of systemic anaphylaxis occurred after eating hamburger on sesame seed bun	1+	2,550
2	31-yr-old man with history of asthma and hay fever noted urticaria, throat fullness, and bronchial asthma shortly after eating salad containing sesame seed oil; in addition, he has had 6 or 7 episodes of systemic anaphylaxis requiring emergency-room treatment, usually attributable to sesame seed products, peanuts, or almonds	2+	3,053
3	33-yr-old woman with a past history of eczema, hay fever, and asthma developed angioedema of lips and mouth with pharyngeal pruritus after ingestion of sesame seeds, sesame flour, or halvah candy	4+	5,164
4	45-yr-old man claimed he had angioedema of lips and tongue if he placed one sesame seed on his tongue; he has conspicuously avoided sesame seed products for many years	Neg.	600

*Control disk without reagenic serum = 685 cpm bound; normal serum plus sesame seed disks = 614 cpm bound.

TABLE II. Relative RAST inhibition of density-gradient*-separated sesame seed extract

Fraction No.	Molecular weight	Percent inhibition			Relative protein OD (280 nM)
		Dilution 1:100	Dilution 1:1,000	Dilution 1:10,000	
2	<8,000	54	29	12	0.123
3	8,000 to 20,000	59	60	14	0.235
4	20,000 to 40,000	69	57	18	0.209
5	40,000 to 62,000	73	28	6	0.127
6	62,000 to 84,000	69	25	1	0.077
7	84,000 to 105,000	60	12	7	0.050
8	105,000 to 126,000	63	0	0	0.044

Data is from a single-inhibition experiment with reagenic sera from case 2. Two additional experiments with the same sera gave similar results. Serum from patient 3 was also tested with density-gradient-separated sesame seed extract and had a similar inhibition profile.

*Ultracentrifugation run for 42 hr at 5° C.

according to relative molecular weights (see Table II). Three identical sesame seed extract tubes were prepared as well as myoglobin, bovine serum albumin, and human gamma globulin (5 mg each) and used as molecular weight markers.

RESULTS

Demonstration of IgE anti-sesame seed reactivity

Sera from each of the patients mentioned in Table I and five controls were tested for IgE anti-sesame seed antibody using cellulose disks to which sesame seed protein had been coupled. As seen, two of the patients with documented systemic anaphylaxis were noted to have reagenic antibody (cases 1 and 2). In

addition, case 3 developed local angioedema after sesame seed ingestion.

In sera from case 4 no sesame seed reagin could be demonstrated.

Antigenic activity of sesame seed extract

New Zealand white rabbits were immunized with sesame seed protein as mentioned in Materials and Methods. Sucrose density-gradient ultracentrifugation (5% to 20%) of sesame seed extract was performed as described for 17 hr.

Aliquots were removed from the top of the gradient and antigenic activity of the fractions was assessed by Ouchterlony double diffusion and immunoelectropho-

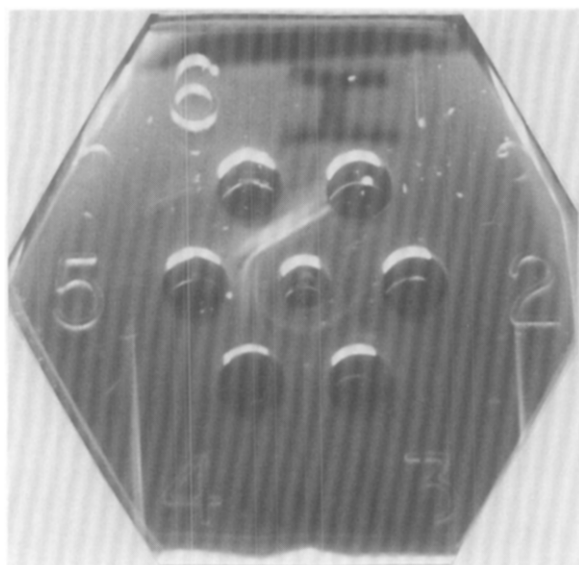


FIG. 1. Ouchterlony analysis of sesame seed extract after 17-hr density-gradient ultracentrifugation. Pooled fractions were added to agarose plates and developed with rabbit anti-sesame seed serum. Fraction 1: <12,000; 2: 12,000 to 25,000; 3: 25,000 to 40,000; 4: 40,000 to 90,000; 5: 90,000 to 150,000; 6: >150,000 daltons.

resis using hyperimmune rabbit antisera. As seen in Fig. 1, antigenic activity is demonstrated in the fractions between 25,000 to >150,000 daltons. At least five precipitating bands are noted after sucrose density-gradient electrophoresis in the higher-molecular weight fractions. In addition, the lower molecular weights (12,000 to 40,000) gave only a single precipitin band.

Conversely, patient sera possessed no precipitating antibody to sesame seed extract.

Crossed electrophoresis

In order to further delineate the number of antigenic components in sesame seed extract, crossed electrophoresis was carried out as described in Materials and Methods. As seen in Fig. 2, at least nine antigens are noted. The most potent antigens migrate towards the anode. Immunoelectrophoresis (not shown) confirmed the anode mobility of all except two of the antigens. Also, no precipitin lines occurred with normal rabbit sera and sesame seed extract.

RAST inhibition

In order to identify the most active fractions of the sesame seed extract, we studied inhibition of two different reaginic sera by density-gradient centrifugation-separated sesame seed extract. Dilutions of each of the pooled fractions (1:100, 1:1,000, 1:10,000) were incubated with equal volumes of

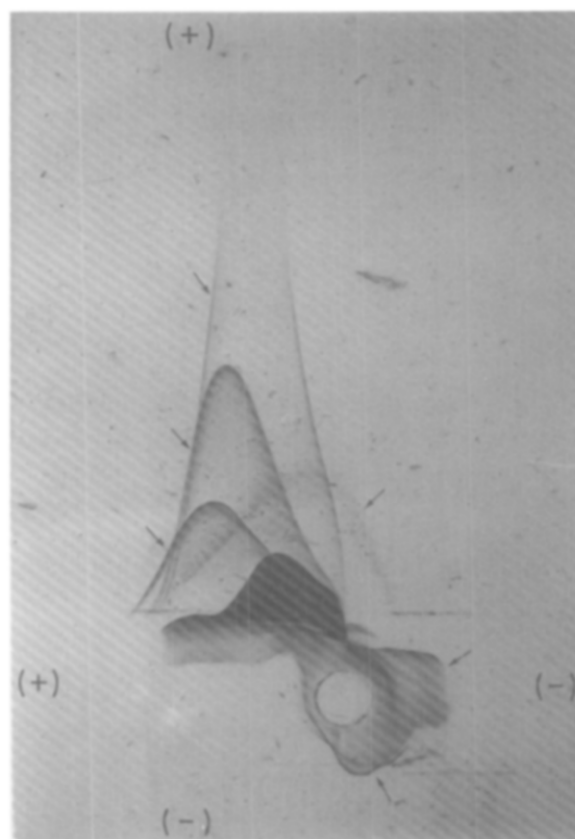


FIG. 2. Crossed electrophoresis of sesame seed extract. Note the anodal migration (a) of at least seven of the antigens. Two single cathodally migrating antigens (-) are also seen. Arrows indicate the most potent anodally migrating antigens as well as the areas of two of the cathodally migrating antigens.

sesame seed reaginic sera (50 μ l) and relative RAST inhibition activity was compared with the noninhibited serum ^{125} I-anti-IgE binding. As seen in Table II, the relative OD (280 nm) values indicated that most of the protein had molecular weights between 8,000 to 62,000 daltons and also showed the greatest RAST inhibition. Yet, the largest number of precipitin bands was seen in higher-molecular weight fractions (90,000 to >150,000).

Of interest as well is the finding that fraction 3 (Fig. 1, 20,000 to 40,000) has only a single precipitin band against rabbit anti-sesame seed extract, while fraction 4 (40,000 to 90,000) has two precipitin bands. Thus, it should be possible to separate the most active fractions by molecular weight-segregating methods.

RAST activity with nonrelated seeds and nuts

In order to assess possible cross-allergenicity with other seeds such as flax, poppy, sunflower, and cot-

ton, RAST testing was performed with these antigens, as well as peanut and almond, against sera of sesame-allergic patients. One of the four patients had positive RAST activity to almond and was clinically sensitive to nuts. The remaining sera were found to be uniformly negative to the above-named botanically unrelated seed allergens.

DISCUSSION

A literature search for sesame seed hypersensitivity revealed five reported cases: one discussed by Rubinstein,² another by Uvitsky,³ and three by Torsney.⁴ All patients were similar to several of our patients in that they developed shock, lip stinging, palatal edema, and/or asthma upon ingestion of various sesame seed products. Several had systemic anaphylaxis after Halvah ingestion. One was shown to have a half-dollar-sized wheal-and-flare reaction to an intradermal skin test that necessitated epinephrine because of progression of the reaction. Yet, sesame oil in the previous cases²⁻⁴ was not thought to be a provoking factor.

The fact that RAST testing was able to corroborate the diagnosis in three out of four of our patients is noteworthy, since it avoids any potential hazard of skin testing. In addition, leukocyte histamine release experiments were contemplated, but impractical because two of the patients resided over 1,000 mi from our laboratory and a third was lost to follow-up. RAST testing has been shown to be beneficial in confirming IgE-mediated reactions to other foods.¹ The explanation for the negative RAST scores in one patient is uncertain.

Several hypotheses have been proposed to explain negative RAST tests in patients with definite food sensitivities. One such pathway may be the formation of different antigens during digestion. The investigations by Spies⁵ on hydrolysis of milk proteins lends some credence to this hypothesis. Parish⁶ entertained the possibility that anaphylactic IgG antibodies might occur in milk sensitivity. However, these explanations may be more germane to delayed food reactions than to immediate explosive reactions.

A more feasible explanation may be the diminution of specific IgE antibodies over time to the point that they are undetectable. Our negative RAST patient (case 4) admitted not being exposed to any sesame seed products for several years. This process has been implicated in other IgE-mediated responses such as

Hymenoptera as discussed by Reisman et al.⁷ and penicillin sensitivities as described by Kraft et al.⁸

There appears to be no cross-allergenicity between botanically unrelated seeds, nuts, and sesame seed. This appears logical since sesame seed appears alone in the food Pedaliaceae family according to food-classification tables. We did not examine any cross-antigenicity between these seeds and rabbit sesame seed antiserum.

The data obtained from our sucrose density-gradient studies reveals that several antigenic components of sesame seed extract as demonstrated by rabbit antisera range from <8,000 to >125,000 daltons. Density-gradient-separated sesame seed extract has indicated that the lower-molecular weight fractions (8,000 to 62,000) appeared to be most active when judged by RAST inhibition of crude sesame seed extract. In contrast, however, fractions 90,000 to >150,000 stimulated qualitatively greater amounts of precipitating antibody. Among other foods known to produce immediate hypersensitivity reactions such as cod M protein, egg, and milk, the molecular weight range of most of the allergens is approximately 12,000 to 80,000 daltons.⁹ Additional studies of the cellular and serologic reactions with relevant sesame seed antigens should shed further light on the mechanisms of anaphylaxis to sesame seed.

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