

Diagnosis of IgE-mediated reactions to food antigens by radioimmunoassay

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The radioallergosorbent test (RAST) was performed on sera from 127 pediatric age patients, who reported the occurrence of more than one episode of immediate hypersensitivity type symptoms (urticaria, angioedema, anaphylaxis, asthma, allergic rhinitis, and atopic eczema) or other acute manifestations upon the gastrointestinal tract after the ingestion of one or more foods. Fourteen different foods, as well as two purified cow's milk fractions, were studied by radioimmunoassay. Specific IgE antibodies to the clinically suspected food allergen were demonstrated in 59 per cent of all reported untoward reactions. A questionable result was obtained in an additional 12 per cent. A positive RAST to at least one food allergen was found in 75 per cent of the patients studied. This technique appears to be a promising and useful adjunct in the diagnosis of IgE-mediated food hypersensitivity.

Food intolerance has been recognized by physicians since ancient times. One of the major problems facing the physician is to determine the nature of the food intolerances. These include IgE-mediated reactions, reactions mediated by nonreaginic antibody, congenital or acquired enzyme deficiencies, nonimmunologic mediator release, and possibly other mechanisms.¹ We will use the term *food allergy* to refer to an IgE-mediated reaction to food.¹ The total spectrum of symptoms that may appear as a result of food allergy is not clearly delineated. Anaphylaxis, angioedema, urticaria, asthma, atopic eczema, allergic rhinitis, gastrointestinal upset, diarrhea, headache, and tension-fatigue syndrome are some of the symptoms reported for food allergies.² The diagnosis of food allergy rests on the clinical history, the relief of symptoms upon elimination of foods, and aggravation of the symptoms upon the reintroduction of a single food into the diet. Skin testing has given variable results,³⁻⁹ with many more positive tests than can be justified by history and provocation testing. Serologic techniques, proposed for diagnosis of food allergy, have generally been found to be unreliable. Precipitation and hemagglutination tests do not specifically detect IgE antibodies. The rat mast cell degranulation test appeared promising¹¹ but is technically difficult to perform reliably, and histamine release from either

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human basophil leukocytes or rat mast cells is too laborious a procedure for routine use.

The RAST¹² provides a reliable, reproducible method for detecting specific IgE antibodies against a wide variety of antigens. In this study, sera from 127 children who reported various symptoms of intolerance to at least one of 14 common foods were tested by RAST for specific IgE antibodies, using 14 common foods. This technique is shown to be a reliable, reproducible method for detecting IgE antibodies against foods.

MATERIALS AND METHODS

Selection of patients

The major criterion for admission to the study was a reasonably positive clinical history of food intolerance to at least one food in which the symptoms were produced consistently upon ingestion and the identity of the offending food was reasonably clear. Almost all of the patients were 9 months to 18 years of age and came from the Pediatric Allergy Clinic of the Los Angeles County-USC Medical Center. Patients reporting severe symptoms were rarely intentionally challenged, although many were fortuitously challenged. Approximately one third of the patients had unequivocal histories. These included all of the 20 patients with immediate explosive reactions (anaphylactoid or angioedema), 12 of the patients with urticaria and asthma, and the 2 patients with gastrointestinal upset whose serum gave positive RAST. The patients with atopic eczema selected for the study included one sensitive to corn and kept on an elimination diet; he was intentionally challenged once and fortuitously challenged three times; severe exacerbation of eczema occurred on each of the four challenges. The eczema group also included 3 patients controlled by milk elimination, 4 having reactions to fish, and 2 with shrimp reactions. Patients with atopic dermatitis 1 to 14 years of age were tested with all foods they or their parents felt were related to their symptoms as well as with milk, egg, fish, and wheat.

Control patients included symptom-free adults, newborn infants, and allergic symptom-free children visiting the Emergency Room or Endocrine Clinic. Each antigen system was tested with a variety of controls including adult nonatopic serum, newborn cord serum, at least 4 nonatopic children's serum (ages 1, 4, 10, 12), and sera from at least 5 atopic children of various ages with no history of food reactions. All the atopic controls had at least one positive RAST to inhalant antigens. An additional group of eleven 10 to 15-month-old children was used as controls for milk and egg.

Radioallergosorbent test

The RAST was performed by modifications of the method of Ceska, Erikson, and Varga.¹³ Crude or purified antigen was coupled to CNBr-activated paper discs. One hundred discs were coupled to 1 ml. of 1:20 extract diluted in 9 ml. of 0.1 M NaHCO₃, pH 9.6, and remaining reactive sites were blocked with ethanolamine. After thorough washing, the discs were incubated with 10 μ l of patient serum overnight. After washing, the discs were incubated with ¹²⁵I-labeled specifically purified monospecific horse antihuman IgE in buffer containing normal horse serum and polyethylene glycol.¹⁴ The discs were then thoroughly washed and counted in a crystal scintillation counter. Positive reactions were defined as greater than 1.7 times nonspecific binding and equivocal ($\pm$) reactions as 1.4 to 1.7 times nonspecific binding. The equivocal reactions were defined as the range of probabilities of 0.33 to 0.05. All positive reactions were $p < 0.05$.

The antihuman IgE was produced by immunizing a horse with PS-myeloma protein. The serum was absorbed using insolubilized¹⁰ human IgG, IgM, and IgA. The IgE-specific serum was then absorbed to an immunoadsorbent column made with ND IgE myeloma protein* and

*Kindly provided by Dr. S. G. O. Johansson.

TABLE I. Major symptom on food ingestion for each patient and correlation of at least one positive RAST with history

Symptom	No. of patients	% positive RAST
Anaphylactoid symptoms	10	100
Asthma	23	96
Angioedema	13	92
Atopic eczema	30	87
Urticaria	26	62
Diarrhea	2	50
Gastrointestinal upset	6	33
Allergic rhinitis	5	0
Positive skin test on routine testing; no overt history	6*	67
Other	6	17

*4 to milk, 2 to wheat.

eluted with 3 M NaSCN, pH 6.0. The eluted antibody was extensively diafiltered and concentrated using an Amicon PM-10 ultrafilter membrane; 5 μ g portions of antibody were labeled using 2 mCi of ^{125}I .¹⁶ After separation of unbound iodine by chromatography on Sephadex G-25, 50 per cent of the counts were bound to ND myeloma discs.

The monospecificity of the antihuman IgE serum was verified by its use in an enzyme-linked immunoassay.¹⁷ The correlation with a commercial radioimmunoassay for measurement of serum IgE (Phadebas, Pharmacia, Inc.) was $\rho = 0.97$. Data using this RAST system with inhalant allergens have been presented elsewhere.¹⁸

Allergens

Crude commercial extracts (Hollister-Stier Laboratories) were used for most antigens. α -Lactalbumin and β -lactoglobulin were purchased from Sigma Chemical Company. The chocolate extract used was a mixture of equal parts of Hollister-Stier chocolate with extracts of Baker's and Hershey's chocolate prepared in our laboratory. This mixture detected substantially more positive sera than any of the individual extracts. Carob extract was prepared in our laboratory from a carob sample supplied by Arcon, Los Angeles, California.

Serum IgE levels

Total IgE level determinations were performed by competitive binding radioimmunoassay using Phadebas kits.

RESULTS

RAST's with foods were performed on sera collected from 127 patients reporting food intolerance and a series of asymptomatic nonatopic and atopic adults and children including newborn infants. Radioactive binding levels in nonallergic adults and children were usually higher than in newborn infants, so the highest nonallergic levels were used as background. One control patient from the emergency room was found to have IgE antibody to egg white, but a review of the patient's chart showed a past history of atopic dermatitis. The 127 patients reported 299 individual food intolerances; specific IgE antibodies were demonstrated in 175 or 50 per cent of these cases; if equivocal ($\pm$) RAST levels are included, 211 or 71 per cent were positive. At least one positive RAST to food was demonstrated in 95 or 75 per cent of the patients.

The major symptoms and per cent with positive RAST for 127 food-intolerant patients with good clinical histories are shown in Table I. Patients whose major

TABLE II. Number of positive RAST's with 14 common foods

Allergen	Number + (%)		Number ± (%)		Number negative	Controls
Cow's milk	25	(63)	8	(20)	7	0/15*
α-Lactalbumin	16	(59)	3	(11)	8	0/20*
β-Lactoglobulin	13	(45)	1	(3)	15	0/20*
Egg white	19	(58)	5	(15)	9	3/20*†
Codfish (fish)	16	(64)	5	(20)	4	0/15‡
Peanut	14	(93)	0		1	0/15‡
Chocolate	13	(38)	4	(12)	17	0/20‡
Walnut	11	(61)	3	(17)	4	0/15‡
Wheat	11	(61)	2	(11)	5	0/20*
Orange	10	(45)	3	(14)	9	0/15‡
Tomato	8	(80)	1	(10)	1	0/11‡
Corn	5	(50)	1	(10)	4	0/20*
Almond	5	(71)	0		2	0/11‡
Shrimp	3	(60)	0		2	0/11‡
Bivalves§	3	(100)	0		0	0/11‡
Oat	3	(100)	0		0	0/20*

*Includes nonatopics only, some atopics without overt symptoms have IgE antibodies reacting with these antigens.

†Three nonatopic children 11, 12, and 14 months had equivocal RAST reactions to egg white.

‡Includes atopic and nonatopic controls.

§Clam, oyster, scallop, abalone.

symptoms were anaphylactoid, angioedema, asthma, and eczema yielded an excellent correlation, i.e., at least one positive RAST corresponding to their reported clinical food intolerances. Positive RAST's were found in all of the patients who reported immediate explosive symptoms and in 80 per cent of the patients with unequivocal histories and/or challenge tests. Most of the noncorrelating cases were either chocolate or orange intolerances. Sera from the 10 eczema cases described under patient selection all showed positive RAST to the offending allergen. The number of food intolerances reported by each patient varied from most commonly one or two to as many as 13 of the 14 panel foods in one eczema patient. IgE antibodies were found by RAST in 83 per cent of the intolerances reported by eczema patients. About two thirds of the patients with either episodes of urticaria or a positive skin test to the food gave positive RAST's. Nineteen patients had other symptoms such as allergic rhinitis, tension-fatigue, gastrointestinal upset, diarrhea, and headache; only 4 of these (21 per cent) were positive by RAST.

The overall results of RAST with 14 foods are given in Table II. Several allergens, especially peanut, bivalve,* and oat, showed extremely high correlation between history and RAST. Fish, milk, wheat, walnut, tomato, almond, and shrimp all gave better than 60 per cent correlation. The substantial number of patients with positive RAST to orange, tomato, and chocolate indicated that IgE antibodies reacting with these foods are not uncommon. Newborn sera and sera from nonatopic children and adults gave negative RAST with all systems but egg white. Three of nine sera from 10- to 15-month-old children gave equivocal RAST to egg white. Others were negative. Atopic control sera gave negative

*Clam, oyster, scallop, abalone.

TABLE III. Number of patients with either a + or a $\pm$ RAST and number of patients with a + clinical history for each set of symptoms

	Anaphylactoid symptoms, angio- edema, asthma	Eczema	Urticaria, skin test	Other
Codfish	11/11	4/5	6/7	0/2
Cow's milk	11/12	15/15	5/5	2/8
α -Lactalbumin	7/9	11/13	0/1	1/4
β -Lactoglobulin	4/9	9/15	1/1	0/4
Peanut	3/3	8/8	3/3	0/1
Orange	5/7	7/9	1/5	0/1
Egg white	8/9	7/8	9/14	0/2
Wheat	8/10	2/2	3/5	0/1
Chocolate	7/12	6/10	4/8	0/4
Walnut	5/5	3/3	6/9	0/1
Tomato	2/2	4/5	2/2	0/1
Corn	2/4	3/4	1/2	0
Almond	1/1	2/2	2/4	0
Shrimp	1/1	2/2	0/2	0
Bivalves	1/1	0	0	2/2
Oat	0	3/3	0	0
Total	76/96 79%	86/104 83%	43/68 63%	6/25 24%

results with fish, peanut, orange, chocolate, walnut, tomato, almond, shrimp, and bivalves. Positive reactions were found with some atopic sera to cereal grains, milk, proteins, and egg white; these are currently under further investigation.

The results in Tables I and II are combined in Table III, which shows positive and $\pm$ RAST's as a function of antigen and symptom. A variety of symptoms were presented by the different patients reporting intolerance to each antigen. Patients with positive RAST to orange include 3 with asthma, 5 with eczema, and one each with angioedema and urticaria; a patient with asthma and 2 with eczema were $\pm$ by RAST. The patients allergic to wheat included 4 patients who lived around a flour mill where primary exposure was by inhalation; all of these were positive by RAST. These 4 patients were also positive to grass pollen extract by RAST, but 2 of them had substantially more antibody to wheat than to either grass pollen extract or purified Rye Group I¹⁹ allergen by RAST. None of these four patients had symptoms by ingestion of wheat. Tomato allergy was manifested primarily by eczema and urticaria-angioedema. Of the 6 patients with positive RAST to corn, 3 reported symptoms to a variety of corn-containing products and products of which corn syrup was an ingredient. One of these patients developed angioedema 3 times after eating hard candy containing corn syrup; another patient had acute exacerbation of his eczema multiple times after ingestion of various foods containing corn products.

Four patients reported allergic symptoms when present in the immediate vicinity of the cooking of food products—3 from fish and the other from egg. All 4 of these patients had positive RAST's to the offending allergen with extremely high levels of specific IgE antibody.

Sera from 24 patients with histories of milk intolerance and positive RAST to milk were tested with two purified proteins from milk reported to be major

TABLE IV. RAST results on 24 patients' sera with IgE antibodies against milk

Antigen	Number +	Number $\pm$	%
α -Lactalbumin only	6	4	42
β -Lactoglobulin only	3	1	17
α -Lactalbumin and β -lactoglobulin	9	0	38
Whole milk only	1	0	4

TABLE V. RAST with cow's milk proteins

Patient	Symptom on milk ingestion	α -Lactalbumin c.p.m. bound*	β -Lactoglobulin c.p.m. bound*
Newborn		684	972
H	Control adult nonatopic	701	985
ER1	Control nonatopic	735	1,030
73-16	Control atopic	823	1,006
SZ	Anaphylaxis	4,386	9,378
72-24	Urticaria	784	1,652
73-32	Eczema	1,793	2,326
73-147	Eczema-cleared on elimination	1,879	1,100
73-234	Eczema	1,098	2,493
73-245	Asthma	1,747	1,827

*Average of duplicate determinations.

allergens, α -lactalbumin and β -lactoglobulin.¹ The results are presented in Table IV—38 per cent reacted to both antigens, 42 per cent to α -lactalbumin only, 17 per cent to β -lactoglobulin only, and 4 per cent to neither. Data from several patients with milk intolerance are shown in Table V.

Chocolate is commonly regarded to be an important allergen.¹ In this study, chocolate gave the lowest correlation (38 per cent of any of the 14 foods). Individual chocolate extract gave substantially lower correlations. Three extracts, one commercial and two prepared in our laboratory, were combined to arrive at these results. In addition, 3 of these patients' sera gave positive RAST with carob extract, and 4 more gave $\pm$ tests with carob.

RAST experiments with foods exhibit typical dose-response curves with dilution of serum, and binding can be inhibited by adding soluble food antigen.

DISCUSSION

Food intolerance consists of a large variety of different types of diseases.¹ In some of these diseases specific enzyme or biochemical defects can be identified. The most important type of food intolerance is food allergy, IgE-mediated immediate hypersensitivity reactions. Food allergy can include cases of anaphylaxis, urticaria, angioedema, asthma, eczema, acute gastrointestinal upset, and diarrhea. Specific IgE antibodies against the offending allergen have been demonstrated in sera from patients with each of these symptoms in the work reported here. The highest correlations with history were found in the patients with classic immediate hypersensitivity symptoms, anaphylactoid, angioedema, asthma, urticaria, and atopic eczema. The two patients with positive RAST whose major symptom was gastrointestinal upset (vomiting and paroxysmal spasm) reported immediate explosive symptoms after ingestion of the offending food. The case of diarrhea confirmed by RAST was due to milk.

Diagnosis of multiple food allergy has been quite difficult in the past. The most important criterion is clinical history, but it is not always easy to tell whether or not the patient or parent gives a reliable history and whether the reaction was due to food ingestion. Skin testing with many foods is unreliable³⁻⁹ and can be dangerous. Scratch testing with fish, shellfish, egg, and peanut may cause systemic reactions. At least 3 patients in this study had systemic reactions from skin testing. Many foods give false positive skin tests. In some patients with severe eczema it is not possible to visualize adequate wheal-and-flare responses. Provocation tests particularly with patients exhibiting anaphylactoid and angioedema symptoms can be dangerous. In vitro tests other than RAST have either not been shown to be specific for IgE or are difficult to perform reliably.¹¹ The RAST seems a promising, reliable, and reproducible technique for diagnosis of food allergy.

Specific IgE antibodies against a food were demonstrated in 75 per cent of 127 pediatric patients with histories of multiple episodes of food intolerance. About two thirds of the patients reported a single food intolerance out of the 14 foods tested. Two patients with severe eczema were found to have IgE antibodies to as many as 13 of the 14 foods. These two patients with severe eczema also had IgE antibodies to 13 common inhalant antigens found in this area when tested by RAST. These patients had negative RAST's to several uncommon allergens. Their IgE levels were 15,000 and 112,000 I.U. per milliliter.

Patients who were exquisitely sensitive to foods by clinical history generally had very high levels of specific IgE. These included 4 patients with inhalant sensitivity to foods. In general, the patients with the most severe symptoms and/or eczema had the highest levels of specific IgE, but there were some exceptions. Patients whose primary symptom was urticaria tended to have lower levels of specific IgE.

This study provides clear evidence of production of IgE that reacts with antigens in 14 common foods, including some foods such as orange and tomato that are thought to cause nonspecific irritation of the skin and contact dermatitis. The 10 cases with IgE reacting with orange and 8 with IgE reacting with tomato strongly indicate that these foods can act as immunogens inducing specific IgE antibodies as well as irritants and contact sensitizers.

The sera with IgE antibody against milk exhibited several specificities; 38 per cent reacted with both α -lactalbumin and β -lactoglobulin, 42 per cent with α -lactalbumin only, 17 per cent with β -lactoglobulin only, and 4 per cent with other antigens in whole milk.

Chocolate also seems to be a heterogenous allergen. Three different chocolate extracts each gave a different spectrum of patients reactivities. All three extracts were mixed together for this study. Forty per cent of the patients giving positive RAST to chocolate gave positive RAST to carob. This suggests that chocolate allergy may be due to contaminants in chocolate rather than to chocolate itself.

The most striking conclusion from this study is the reliability of patient (and parent) histories. If the history included multiple incidence of acute symptoms closely following ingestion of or exposure to the offending food, the reliability is

excellent. In general, the RAST is reported to have about 80 per cent correlation with provocation tests in inhalant allergy.²⁰ In our laboratory in a study involving 200 patients, a 98 per cent correlation was found between RAST with a purified rye grass group I antigen and a 2+ or greater scratch test to rye, Bermuda annual blue, Johnson, brome, and/or orchard grass skin test in patients with a positive history.¹⁸

Specific IgE antibodies were found for 59 per cent of all the food intolerances reported by history in this study. The RAST results for an additional 12 per cent were $\pm$. These figures are comparable with the correlations reported for many inhalant allergens.²⁰

This study did not include patients with vague symptoms, those with reported gastrointestinal upset or headache several hours after ingestion, infants with nonacute milk intolerance, patients with urinary tract problems, or those with psychological problems. These categories of patients can be studied in the future. Most of the patients in this study had classic immediate hypersensitivity symptoms or acute distress on food ingestion. The large number of positive RAST's to food in the eczema group indicate a strong association of food allergy with atopic dermatitis. The highest levels of specific IgE to foods were generally found in patients with either active or a past history of eczema. The observation that ingestion of certain foods causes an acute exacerbation in some of these patients suggests a role of food allergy in the etiology of atopic dermatitis.

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Select the ONE best answer for the following question from the Allergy Foundation of America Self-Assessment Program:

Question 3. Patients with which of the following diseases will have the highest serum levels of IgE?

- (A) Asthma
- (B) Ascariasis
- (C) Atopic dermatitis
- (D) Chronic urticaria
- (E) Allergic contact dermatitis

The correct answer and bibliographic reference will be found on page 179 of this Journal.