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Diagnosis of Multiple Inhalant Allergies in Children by Radioimmunoassay

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ABSTRACT. A series of 302 patients reporting immediate hypersensitivity symptoms to inhalant allergens was evaluated by both puncture skin testing and the radioallergosorbent test (RAST) using 13 common inhalant allergens. Agreement between the two tests varied from 71% to 93% for the various allergens and averaged 82%. The best correlation, 93%, was obtained with mixed grass pollen allergens. If a purified allergen, rye group I, was used the correlation rose to 97.5%. The correlation was 88% for the mold *Alternaria*, and the negative-sera did not become positive when tested over a wide range of dilutions. Fifty-eight percent of the patients with house dust allergy reacted to house dust mite (*Dermatophagoides farinae*). Correlations of 71% to 93% were obtained with ten weed and tree pollens. The only patients in whom positive RASTs were found with negative skin tests to the same antigen had either severe atopic dermatitis or decreased skin response to exogenous histamine. Sera from a group of 20 patients with positive allergy history but negative skin tests also had negative RAST. The radioallergosorbent test appears to add little information to that obtained by a comprehensive allergy history and puncture skin tests in the great majority of children with inhalant allergy. The only exceptions were children with severe atopic dermatitis, suppressed skin reactivity or dermatographism. *Pediatrics*, 54:151, 1974, ALLERGY, INHALANT, RADIOALLERGOSORBENT TEST, SPECIFIC IgE.

The radioallergosorbent test was introduced by Wide, Bennich and Johansson in 1967¹ for detection of specific IgE antibodies *in vitro*. The test was modified to be quantitative² and also to determine allergen content.^{3,4} Correlations were made with skin testing,⁵⁻⁷ provocation testing,^{5,8,9} histamine release from chopped lung,^{8,9} histamine release from human basophil leukocytes,^{6,7} and passive transfer tests.^{6,10} Correlation with all these methods was excellent. Recently the RAST has been shown to be a useful method for diagnosis of food allergy^{10,11} and drug allergy.⁹

In this study the results of radioallergosorbent testing are compared to clinical results including allergy history and scratch skin test, to determine when the information obtained from RAST is redundant and when it may be of value to the physician in children with allergies to multiple inhalant allergens. Thirteen allergens were investigated including grass, tree and weed pollens, a mold and house dust mite.

MATERIALS AND METHODS

Patients

A group of 302 patients from the Pediatric Allergy Clinic at the Los Angeles County-USC Medical Center was studied. Each patient was given a complete allergy work-up including allergy history, physical examination, puncture skin testing and complete blood cell count. When needed to establish diagnosis, intradermal skin tests, chest x-ray, nasal smear, quantitative immunoglobulin determinations, bacterial and parasite cultures and provocation or challenge tests were performed. A blood sample was obtained from each patient for radioallergosorbent tests and total IgE determination during an initial visit.

The patients ranged from under 1 year of age to adulthood. Ninety-three percent were under 18 years of age and 50.5% were 8 years old or younger. Eighty-one percent of the patients studied were between 3 and 15 years.

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Allergens

Both skin tests and radioallergosorbent tests were performed with commercial extracts of 13 common inhalant allergens found in Southern California. These included grass, 4 tree and 6 weed pollens, the mold *Alternaria* and house dust mite. These extracts were supplied by Hollister-Stier Laboratories, Inc., and Allerved Laboratories. In addition, radioallergosorbent tests were performed with rye group I, a purified grass allergen prepared by a modification of the method of Johnson and Marsh.¹²

Skin Tests

Routine skin testing was performed with a scarifier (Center Laboratories) using 1/20 weight/volume glycerinated extracts; mite antigen was used at a concentration of 1/50. Skin tests were read 20 minutes after application and graded + to 4+ using the following criteria: + 0.5-cm wheal, 2+ 1-cm wheal, 3+ 1.5-cm wheal with pseudopod, 4+ 2-cm wheal with pseudopods. Negative diluent control and 1 mg/ml histamine control were always performed. A one plus test is not usually considered significant unless accompanied by a strongly positive history relative to that allergen.

Serum IgE Levels

Total serum IgE was determined by the use of Phadebas kits (Pharmacia Laboratories, Inc.).

Radioallergosorbent Tests

Radioallergosorbent tests were performed on sera from all patients with 2 plus or greater skin tests or a one plus skin test and a strongly positive history to any of the 13 allergens studied. In addition sera from 20 patients with negative skin tests and sera from nonatopic and newborn subjects were tested with each of the allergen systems.

Five-millimeter filter paper disks were activated with cyanogen bromide by the method of Wide.¹³ After extensive washing and acetone drying, the activated disks were stored at -15°C. Disks were coupled to antigen in 0.1M NaHCO₃, pH 9.2. The remaining reactive sites were blocked with ethanolamine and the disks extensively washed. The coupled disks were stored in incubation buffer: 0.9% NaCl, 0.05M sodium phosphate, pH 7.4, 0.3% human serum albumin, 0.05% sodium azide, and 1% Tween 20. Each lot of coupled disks was tested with known positive reactors' sera. The reproducibility of binding to the disks was $\pm 4\%$ (average of $28,316 \pm 1,200$ cpm).

Anti-human IgE was raised by immunization of a horse with PS myeloma protein in Freund's complete adjuvant. The antiserum was adsorbed with

human IgG (Gamastan, Cutter Laboratories, Inc.), IgM, IgA and normal serum coupled to an agarose column.¹⁴ The immunoglobulin fraction was prepared from the adsorbed serum by ammonium sulfate fractionation.¹⁵ The specific anti-IgE antibody was purified by adsorption to a column of purified ND myeloma protein (kindly provided by Dr. S. G. O. Johansson) coupled to agarose. After extensive washing the specific anti-IgE antibody was eluted with 3M NaSCN, pH 6.0.¹⁶ The specifically purified antibody was diafiltered and concentrated using an Amicon PM-20 membrane. The monospecificity of the antibody was verified by its use in an enzyme-linked immunosorbent assay for IgE. The correlation with a commercially available radioimmunoassay (Phadebas, Pharmacia, Laboratories, Inc.) was $\rho = .97$.¹⁷

The radioallergosorbent test was performed by the method of Ceska and Varga¹⁸ in a somewhat modified version. Serum, 0.01 ml, and 0.1 ml of incubation buffer without Tween 20 were incubated with a single antigen coupled disk in a 12 × 75-mm plastic test tube for 16 hours. The disks were washed four times with 1% Tween in 0.9% saline. Four micrograms of monospecific, specifically purified class specific anti-human IgE was radioiodinated with 2 microcuries of ¹²⁵I using the chloramine T method.¹⁹ After removal of free iodide by gel filtration on Sephadex G-25, the radioactive antibody was stored in 2 ml of 8% human serum albumin and 0.5 M Tris HCl, pH 7.4. Immediately prior to use, the radioactive antibody was diluted in incubation buffer containing 4% polyethylene glycol 20,000 and 6% normal horse serum to give a final concentration of 10 to 15 ng of antibody per milliliter. Dilute antibody, 0.1 ml, was added to each disk and the tubes were incubated from four to 16 hours. The ratio of counts specifically bound to nonspecifically bound was approximately constant over this range of incubation time. After incubation the disks were washed four times with Tween-saline and counted for one minute in a crystal scintillation counter. Positive and negative control sera were run with each allergen system with each batch.

The scoring of RAST tests is given in Table I. At least one serum gave a + + + + RAST with each of the 14 allergen systems.

RESULTS

A series of 302 patients reporting immediate hypersensitivity-type symptoms (seasonal allergic rhinitis, extrinsic asthma, angioedema-urticaria related to inhalant exposure) was examined in the Pediatric Allergy Clinic. As shown in Table II, 72% reported asthma, 53% allergic rhinitis, 20%

TABLE I
SCORING OF RADIOALLERGOSORBENT TESTS

Score	Times Background (cpm)	Sample Experiment (cpm)
Background	1-1.2	4000-5000
±	1.2-1.5	5001-7000
+	1.5-3	7001-15000
++	3-5	15001-25000
+++	5-7	25001-35000
++++	7-9	35001-45000
+++++	9-11	45001-55000
++++++	11-13	55001-65000
+++++++	13-15	65001-75000
+++++++	15-17	75001-85000
cpm added		274000

TABLE II

ALLERGIC SYMPTOMS REPORTED BY PATIENTS IN THIS STUDY

Symptom	No. of Cases	%
Asthma	213	73
Allergic rhinitis	157	53
Atopic dermatitis	59	20
Urticaria-angioedema	46	16
Miscellaneous	9	3
Total cases	302	

atopic dermatitis and 16% urticaria-angioedema. Total serum IgE levels were measured on 252 of these patients and the results are presented in Table III; 38.5% of the patients' sera were found to have elevated serum IgE levels of more than 300 international units (IU). This group with elevated IgE levels included most of the patients who reported atopic dermatitis as current and/or past problems. However, over 60% of the atopic patients did not have elevated serum IgE levels.

The radioallergosorbent test was performed on each patient's serum for any of the 13 inhalant allergens for which a positive or questionable scratch skin test and/or history was obtained. The results are presented in Table IV; results with negative control sera are not included. A total of 947 tests were performed. Agreement between RAST and skin test-history was found in 747 or 79%. Agreement with individual allergens varied from 71% to 93%. Positive radioallergosorbent tests were found in several sera from patients with negative skin tests to the same allergens. These patients all had severe atopic dermatitis or subnormal skin-test response to exogenous histamine.

The best correlation was obtained with the mixed grass system. Ninety-three percent of 213

TABLE III
SERUM IgE LEVELS OF PATIENTS IN THIS STUDY

Level (IU)	No. of Patients	% of Patients
Less than 50	48	19.0
50-100	24	9.5
100-150	30	11.9
150-200	29	11.5
200-300	24	9.5
		61.5%*
300-500	28	11.1
500-1000	32	12.7
1000-2500	28	11.1
More than 2500	9	3.6
		38.5%†

*Elevated.

†Within normal range.

TABLE IV

AGREEMENT OF RADIOALLERGOSORBENT TEST WITH SKIN TEST AND ALLERGY HISTORY FOR 13 COMMON INHALANT ALLERGENS

Allergen	No. Positive RAST	No. Positive Skin Test/History	% Agree- ment
Grass Mix	199	213	93
Olive Tree	69	89	78
Chinese Elm Tree	49	67	73
Walnut Tree	36	51	71
Coast Live Oak Tree	28	39	72
Russian Thistle	38	50	76
Sagebrush Mix	41	56	73
English Plantain	33	41	80
Scale Mix	39	46	85
Lambsquarters	31	39	79
Western Ragweed	41	44	93
Alternaria	60	69	87
House dust mite	83	143*	58
Total including mite	747	947	79
total excluding mite	664	804	83

* Positive skin tests to house dust extract and/or mite.

sera of patients with a 2+ or greater scratch skin test to any of mixed grass pollen extract, bermuda, annual and blue mix, rye mix, wild oat, brome, Johnson and/or orchard grass gave a positive RAST with mixed grass extract. This positive

TABLE V
CORRELATION OF RADIOALLERGOSORBENT TEST AND SKIN
TEST WITH RYE GROUP I ALLERGEN

Skin Test Score	RAST Score					
	0	±	+	++	+++	++++ and Greater
0	23		2			
+		1	3			
++	1	2	21	2		
+++	1	2	29	3	2	4
++++			23	11	13	9
+++++			2	2		6
Total	25	5	80	18	15	19
%	15	3	49	11	9	12
Overall correlation	$\frac{158}{162} = 97.5\%$					

RAST group included four patients with positive skin tests to only bermuda grass. One hundred and sixty-two of the patients' sera radioallergosorbent tested with mixed grass were tested with a purified grass allergen, rye group I.¹² The group tested included the 14 noncorrelating sera from those tested with mixed grass. The results are presented in Table V which compares the RAST score with the skin test score for rye group I. The overall agreement between positive skin test-positive RAST and negative skin test-negative RAST was 97.5%. The two patients with a positive RAST and negative skin test both had severe atopic dermatitis. Several patients' sera had negative RAST to mixed grass and positive RAST to rye group I. A similar high correlation between reactivity to mixed grass pollens and rye group I was reported by Norman and Lichtenstein²⁰ using histamine release from human basophil leukocytes. There is some correlation between skin test score and total amounts of specific IgE antibody as measured by RAST, but there is substantial scatter.

Alternaria has been reported to be an allergen system in which patients produced a substantial amount of nonreaginic antibody as well as IgE.²¹ The results in Table VI compare RAST scores for *Alternaria* with skin-test scores. The overall agreement found with sera from 82 patients was 88%. The three patients with negative skin tests whose sera gave 2+ RAST all had severe atopic dermatitis. Seven patients with 2+ to 4+ skin tests were found whose sera give negative RAST. In an attempt to see if this was due to the presence of blocking antibodies, RAST was repeated on these sera using serum amounts of 0.1 to 50 µl. The results using various dilutions were uniformly nega-

tive. Some correlation was observed between RAST and skin test scores, but less than with rye group I.

A group of 143 sera from patients with positive skin tests to house dust and/or mite were tested with RAST to an extract of *Dermatophagoides farinae* grown on wheat. Fifty-eight percent of the sera gave positive RAST (Table IV). In order to estimate the role of wheat antigens in this system, sera from two patients with ++++ skin tests to wheat and strongly positive RAST to wheat were tested using this mite preparation; both gave negative RAST. Similar results were obtained with five strongly positive grass sera. We were not able to detect any wheat antigen in the mite preparation.

The ten other allergen systems in Table IV are all seasonal tree and weed pollens of major allergenic importance in Southern California. Correlations of 71% to 93% were obtained between scratch skin test and history. Strong reactors (4+ skin test and at least 4+ RAST) were found for every allergen system. The highest amounts of specific IgE were found in the mite and grass systems, probably as a result of the almost year-round exposure. Very high levels of specific IgE to other allergens were associated with either massive exposure, for example trees in the patient's yard, or atopic dermatitis.

Most of the patients in this study had positive RAST to two or more of the 13 allergens studied. The number of positive RAST for different allergens per patient is shown in Table VII. Ten patients (3.3%) had ten or more positive RAST for these 13 common allergens; six of these ten patients had atopic dermatitis at the time specimens were obtained.

TABLE VI
CORRELATION OF RADIOALLERGOSORBENT TEST AND SKIN
TEST WITH *Alternaria*

Skin Test Score	RAST Score					
	0	±	+	++	+++	++++
0	20			3		
+	1					
++	1	2	6	1	1	
+++	4	2	13	5		
++++	2	1	10	6	2	
+++++						2
Total	28	5	29	15	3	2
%	34	6	35	18	4	2
Overall correlation	$\frac{72}{82} = 88\%$					

DISCUSSION

The development of the radioallergosorbent test¹ and the technique of histamine release from human basophil leukocytes²² provided reliable, reproducible *in vitro* methods for the demonstration and determination of specific IgE antibodies. In order to assess the clinical usefulness of these methods, they must be evaluated with a wide variety of patients and allergens. In this study a group of 302 children, mainly with multiple inhalant sensitivities and primary symptoms of asthma (72%) and allergic rhinitis (53%), was evaluated both clinically and by the radioallergosorbent test using 13 common inhalant allergens. Overall agreement between allergy history/puncture skin test and the radioallergosorbent test for the 12 allergens for which the same extract was used in both skin testing and radioimmunoassay was 83%. This result compared favorably with those reported by others^{1,5-8,23} who report agreements of 73% to 96%.

These studies demonstrate the reliability of the skin test when accompanied by a positive history. In our study all of the patients who had positive RAST and negative skin tests fit into two categories: (1) patients with severe atopic dermatitis, and (2) patients with suppressed skin reactivity sometimes due to antihistamine medication, manifested by a decreased skin response to exogenous histamine. A group of 20 patients with positive allergy history but negative skin tests was evaluated by radioallergosorbent test and no positive RAST were found. Three of these patients were tested by inhalation provocation and negative results were obtained. These data indicate that scratch skin testing is a reliable method for detecting specific

IgE antibodies and that *in vitro* testing is rarely necessary.

One of the major factors limiting the reliability of allergy testing is the variability of crude allergen extracts. The use of purified allergens may overcome these difficulties. However, many substances contain multiple antigens, for example antigens E, K, Ra.3 and Ra.5 from ragweed.²⁴⁻²⁷ Although there are multiple allergens in grass pollen,^{28,29} there appears to be one major allergen shared by most grasses. This antigen has been isolated and identified as rye group I.¹² In this study a large group of patients with skin test reactivity to any Southern California grass pollen was tested by radioallergosorbent test with a preparation of rye group I. Of these patients, 97.5% gave a positive RAST including four who had skin tests to only bermuda grass pollen extract. It appears that the great majority of persons who develop grass sensitivity produce IgE antibodies which can react with rye group I. This situation is not dissimilar to that found with ragweed antigen E.⁸

The radioallergosorbent test appears to add little information to the results of allergy history and skin testing in the great majority of cases of inhalant allergy in children. The cases in which the RAST adds substantial information include children with severe atopic dermatitis, suppressed skin reactivity and dermatographism. The RAST can also be used for detecting immediate hypersensitivity reactions to foods^{6,10} and drugs.⁹ As a research tool for the study of immunological changes during immunotherapy the radioallergosorbent test is quite valuable since it allows the quantitation of specific IgE² as well as its detection. We have observed that patients with very high levels of specific IgE in their serum seem to have a substantially higher risk of having systemic reactions during immunotherapy.³⁰ This may reflect the empirical observation that children with atopic dermatitis and high levels of IgE have more systemic reactions than those with only respiratory problems.

TABLE VII

NUMBER OF POSITIVE RADIOALLERGO-SORBENT TESTS PER PEDIATRIC ALLERGY PATIENT FOR 13 COMMON INHALANT ALLERGENS

No. of Allergens Positive	No. of Patients	% of Patients
0	60	19.8
1	74	24.5
2	53	17.5
3	29	9.6
4	22	7.3
5	17	5.6
6	13	4.3
7	11	3.6
8	6	2.0
9	7	2.3
10	3	1.0
11-13	7	2.3
Total	302	

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