

An immunologic evaluation of ragweed-sensitive patients by newer techniques

Richard Evans, M.D., Lieutenant Colonel, MC, USA,* Robert E. Reisman, M.D., John I. Wypych, Ph.D., and Carl E. Arbesman, M.D.
Buffalo, N. Y.

Several currently available methods for quantitation of reaginic antibody are compared. Twenty-three ragweed-sensitive patients were evaluated with the use of the following measurements: serum IgE concentration, P-K titer in man, RAST antibody level, and leukocyte histamine release. Hemagglutinating antibody titers were also determined. Significant correlations were found between P-K titers, RAST test results, and IgE concentrations. The cellular responses of histamine release to challenge by antigen and anti-IgE antibody were parallel. The RAST test, P-K titers, and IgE concentrations did not parallel the values of maximal leukocyte histamine release with challenge by antigen or anti-IgE antibody. There was a correlation between P-K titer and leukocyte sensitivity to antigen. The hemagglutinating antibody titers did not parallel any of the other measurements. It is hoped that continued application of these newer techniques will offer a better understanding of the clinical responses in ragweed-sensitive patients.

Reaginic antibodies are the immunologic hallmark of atopic disease. These antibodies belong to a specific class of immunoglobulins—IgE.¹ Classic methods of estimating their presence have been the direct skin test and the Prausnitz-Küstner (P-K) reaction.

More recently, in vitro methods designed to quantitate this antibody and measure its activity have become available. Total serum IgE concentrations can be readily determined. The radioallergosorbent test (RAST) described by Wide and co-workers² semiquantitatively measures specific reaginic antibody. In vitro assay of antigen-induced histamine release from leukocytes indicates the presence of cell fixed reaginic antibody.³⁻⁵

The purpose of this paper is to compare and evaluate the currently available techniques in ragweed-sensitive patients. A survey of a population of 23 ragweed-sensitive adults is presented. The following measurements were made: IgE concentration, P-K titer, RAST antibody level, leukocyte histamine release with challenge by ragweed antigen and anti-IgE antibody, and hemagglutinating antibody titer.

From the Allergy Research Laboratory of the Buffalo General Hospital and the Departments of Medicine and Microbiology of the State University of New York at Buffalo.

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Reprint requests to: Dr. Arbesman, 100 High St., Buffalo, N. Y. 14203.

*Present address: Walter Reed General Hospital, Washington, D. C. 20012.

MATERIALS AND METHODS

Patients

Twenty-three patients, ages 14 to 48, were selected on the basis of a clinical history compatible with marked sensitivity to ragweed pollen. All had 3+ or 4+ skin test reactions following an intradermal injection of 0.02 ml. of a 1:1,000 dilution of a 15 per cent whole ragweed extract, and none had previously received specific immunotherapy.

Antigen

A 15 per cent aqueous extract was prepared from defatted short ragweed pollen purchased from Greer Laboratories (Lenoir, North Carolina). Various dilutions of this antigen were used in skin testing and in the following *in vitro* methods: histamine release, passive transfer, and hemagglutination. A 30 per cent extract was used for the RAST test.

Antiserum

Goat antiserum specific for the Fc portion of IgE myeloma protein and absorbed with normal human plasma was used. The method of preparation of this antiserum has been described previously.⁶

RAST^{7, 8}

A 30 per cent whole ragweed extract, 2.2 ml., was conjugated to 450 mg. of cyanogen bromide-activated cellulose particles.⁹ Aliquots, 1.0 mg., of this antigen-cellulose conjugate were added to 50 μ l samples of patients' sera, and the samples were incubated overnight at room temperature in a horizontal rotator. After washing, 0.1 ml. of ¹²⁵I-labeled anti-IgE was added to each tube. This anti-IgE was the goat antiserum which had been absorbed with copolymerized E myeloma protein,¹⁰ eluted with glycine HCl, and labeled with ¹²⁵I by use of the chloramine T method.¹¹ The ¹²⁵I-labeled anti-IgE was diluted in 0.1M phosphate buffer, pH 7.4, with one per cent Tween 20 and 0.2 per cent human serum albumin and contained approximately 60,000 c.p.m. in a 0.1 ml. aliquot. After addition of anti-IgE, the samples were rotated overnight at room temperature in a horizontal rotator, washed, and counted in a gamma counter. The same serum from a ragweed-sensitive patient was included in every experiment as a reference standard. The results were interpreted as per cent of this reference standard.

Quantitative measurement of IgE

The concentration of IgE in the patients' sera was measured by single radioimmunoassay in antibody-containing agar. This is a modified Mancini one-step method with the use of ¹²⁵I-labeled goat anti-IgE serum in the agar.¹²

Histamine release techniques

Leukocytes were isolated from the peripheral blood of the ragweed-sensitive donors by dextran sedimentation, washed, and suspended in a Tris buffer with added Ca⁺⁺, Mg⁺⁺, and human serum albumin.³ Histamine release was induced by addition of whole ragweed extract (WRE) or anti-IgE. After incubation at 37° C. for 60 minutes, the histamine released was measured fluorometrically.^{3, 13} The concentration of antigen or antiserum required to elicit release of 50 per cent of the total cellular histamine was used as an indication of the cell sensitivity, and the maximal per cent of histamine released was regarded as an indication of the cell reactivity.¹⁴

Passive transfer

A standard P-K technique was employed. Serial two- or threefold dilutions of donors' sera were prepared, and 0.1 ml. was injected intradermally into each of 2 nonatopic recipients. The sites were challenged 24 hours later with 0.02 ml. of a 1:50 dilution of 15 per cent WRE.

TABLE I. Age, years of symptoms, hemagglutinating antibody titer, P-K titer, RAST test, IgE concentration, and leukocyte histamine release in 23 ragweed-sensitive patients

Patient	Age (yr.)	Symptoms (No. yr.)	RAST test (% standard)	P-K titer	IgE concentration (ng./ml.)	Hemagglutination titer	Leukocyte histamine release			
							Antigen		Anti-IgE	
							Reactivity (%)	Sensitivity (μ g protein/ml.)	Reactivity (%)	Sensitivity (μ g protein/ml.)
S. C.	14	3	205	4,320	1,400	640	74	9.0×10^{-4}	45	—
J. B.	17	15	191	4,320	1,450	40	62	1.3×10^{-4}	27	—
M. G.	20	12	142	1,080	125	10	43	—	17	—
R. L.	21	12	99	2,160	120	20	90	7.5×10^{-4}	52	8.0×10^{-2}
T. C.	23	11	75	800	150	160	75	6.5×10^{-4}	45	—
G. S.	22	15	69	540	115	10	83	4.5×10^{-4}	34	—
V. L.	18	6	57	540	140	10	8	—	16	—
S. K.	23	15	56	270	80	320	50	5.0×10^{-2}	11	—
B. T.	45	32	42	90	95	20	52	5.0×10^{-3}	12	—
P. K.	23	6	39	90	156	160	36	—	12	—
J. B.	32	10	36	30	216	40	94	1.8×10^{-3}	55	2.0×10^{-2}
P. M.	20	8	30	30	200	20	0	—	56	2.6×10^{-1}
J. J.	38	26	30	90	146	<10	43	—	24	—
B. G.	19	15	29	540	56	80	65	1.3×10^{-3}	13	—
C. G.	31	25	29	90	312	10	53	8.0×10^{-2}	40	—
J. H.	23	6	27	90	56	40	82	1.0×10^{-4}	55	7.5×10^{-2}
J. G.	48	36	27	30	80	<10	46	—	14	—
R. G.	24	12	17	30	15	<10	75	1.9×10^{-3}	20	—
S. N.	23	10	16	90	210	160	60	5.9×10^{-2}	30	—
M.M.	19	3	8	<10	80	40	0	—	0	—
J. G.	26	24	7	10	40	40	78	1.3×10^{-2}	48	—
S. N.	23	7	7	<10	56	20	0	—	0	—
L. M.	22	3	5	<10	25	10	0	—	0	—

One standard ragweed serum was placed in serial dilution in all recipients to assure comparability.

Hemagglutination

Hemagglutinating antibody titers of all sera were determined by a modified Feinberg-Flick technique.¹⁵

Statistical analysis

The Spearman rank correlation coefficient¹⁶ was employed in a statistical analysis of the data.

RESULTS

The data are shown in Table I. Correlations are shown in Table II. The P-K titers varied from less than 10 to 4,320. The RAST antibody concentration varied from 5 to 205 per cent of the standard serum. The IgE serum concentrations ranged from 15 to 1,450 ng per milliliter. There is a highly significant correlation ($p = <0.001$) between the P-K and RAST results and a significant correlation ($p = <0.01$) between the IgE concentration and the RAST values. P-K titers and IgE concentration did not correlate so well ($p = <0.05$).

Four of the 23 patients' leukocytes did not release histamine when challenged in vitro by antigen (WRE), and those of 3 of these 4 patients did not release

TABLE II. Spearman rank correlation coefficient (r_s) and significance level (p) of the correlations among RAST test, P-K titers, IgE concentration, hemagglutination. Titer and leukocyte histamine release in 23 ragweed-sensitive patients

	RAST test	P-K titer	IgE concentration	Hemag- glutina- tion titer	Cell reactivity to antigen	Cell sensitivity to antigen
P-K titer						
r_s	0.8925					
p	<0.001					
IgE Concentration						
r_s	0.6038	0.5040				
p	<0.01	<0.05				
Hemagglutination titer						
r_s	0.2115	0.3077	0.2846			
p	N.S.	N.S.	N.S.			
Cell reactivity to antigen						
r_s	0.2908	0.3915	0.0761	0.2846		
p	N.S.	N.S.	N.S.	N.S.		
Cell sensitivity to antigen						
r_s	0.3276	0.6238	0.1749	0.3098	0.8923	
p	N.S.	<0.01	N.S.	N.S.	<0.001	
Cell reactivity to Anti-IgE						
r_s	0.2851	0.2831	0.4227	0.1186	0.6861	0.5343
p	N.S.	N.S.	<0.05	N.S.	<0.001	<0.01

histamine when challenged in vitro by anti-IgE. Leukocytes of five other patients did not release more than 50 per cent of the total histamine when challenged with antigen. Of the 20 patients whose cells responded to anti-IgE, only those of 4 released more than 50 per cent of the total histamine. Because of this low level of maximal histamine release to anti-IgE (cell reactivity to anti-IgE), an estimation of cell sensitivity (antibody required for 50 per cent histamine release) was not possible.

When the cell reactivity to anti-IgE is compared with the cell reactivity to antigen (WRE), there is a highly significant correlation ($p = <0.001$). In addition, there is a highly significant correlation ($p = <0.001$) between the cell reactivity with antigen challenge and cell sensitivity with antigen challenge. In the analysis of this correlation, the donors whose cells released less than 50 per cent histamine were given an equivalent rank which was less than that of the more sensitive donors but greater than that of those whose cells did not release histamine at all. In all but 2 of the patients whose cells released histamine, the amount of histamine released to antigen challenge was greater than the amount released when challenged by anti-IgE.

RAST, P-K titer, and IgE concentration did not parallel cell reactivity to antigen or anti-IgE. The cell sensitivity to antigen did not correlate with RAST or IgE concentration. There was a correlation ($p = <0.01$) between P-K titer and cell sensitivity to antigen for histamine release. The hemagglutinating antibody titers ranged from less than 10 to 320 and did not parallel the other measurements. There was no correlation between duration of symptoms or patient age with the tests performed.

DISCUSSION

The methods used in this study reflect different components of the allergic state. The P-K test is a time-honored method of quantitating the amount of specific reaginic antibody present in a patient's serum. This test is very sensitive and will demonstrate as little as 0.001 μ g of antibody nitrogen.¹⁷

The RAST test is also a sensitive measure of specific antibody of the IgE class. A sensitivity in the range of picograms per microliters of IgE antibody has been reported.⁷ The RAST test is more quantitative than the P-K reaction and eliminates the variable of a recipient. Larger numbers of serum samples can be handled in the RAST test, and a clear differentiation among the samples is more readily achieved. The highly significant correlation between RAST test results and the P-K titers implies that the tests are measuring the same antibody.

Allergic patients have increased concentrations of IgE in sera.¹⁸ We found a rough correlation between P-K titer and IgE concentration and a significant correlation between RAST test results and IgE concentration. It is probable these patients' sera contained antibodies of the IgE class to more than ragweed antigen alone.

Antigen-induced leukocyte histamine release is also dependent upon the presence of reaginic antibody. Fixation of IgE to leukocytes, specifically basophils, has been demonstrated.¹⁹ The sensitivity of allergic patients' cells to antigen for histamine release parallels the severity of symptoms which the patients experience.²⁰ This correlation has been found to be more significant than the correlation between P-K titers and symptom scores.²¹

In this study, we found no correlation between the tests for serum reaginic antibody (P-K titer or RAST) and the cell reactivity to antigen or anti-IgE for histamine release. There was a correlation between P-K titer and cell sensitivity to antigen for histamine release. There was not a correlation between RAST test results and the cell sensitivity to antigen, however. Therefore, the response of sensitized leukocytes to antigens for histamine release appears to be dependent upon some factors in addition to serum reaginic antibody concentrations. This has been suggested by Levy and co-workers.^{14, 22} These authors used the technique of passive sensitization of selected nonallergic donor leukocytes to estimate reaginic antibody titers.

The cellular responses of histamine release to antigen and anti-IgE were regularly parallel: The cell reactivity to antigen and anti-IgE and the cell reactivity and sensitivity to antigen showed highly significant correlations. We were able to confirm the previous report that sensitized leukocytes respond to antigen with greater amounts of histamine release than that which is induced by anti-IgE.¹⁴ It has been suggested⁵ that leukocytes of allergic individuals release more histamine than normal leukocytes when reacted with anti-IgE. In this study the correlations between serum IgE concentration and cell reactivity to anti-IgE was roughly significant. Other studies have reported a more significant correlation between these two factors,^{14, 22} but important exceptions must

be noted. It is probable that the cells of allergic donors have a greater propensity to release histamine than do cells of normal donors.

In this patient population, age and years of symptoms did not parallel any of the test results. The hemagglutination reaction, a measure of IgG and IgM class antibodies, also did not parallel the other test results.

We anticipate that immunotherapy and subsequent pollen exposure will alter many of these patients' test results. Approximately one third of these patients are now receiving immunotherapy, and we plan to continue to study the entire group. By this means, a better understanding of the clinical responses of ragweed-sensitive patients can be derived.

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