

Respiratory allergy to ragweed: Correlation of bronchial responses to allergen with bronchial responses to histamine and circulating immunoglobulin E

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Flow-volume loops were recorded before and after quantitative inhalation of increasing concentrations of histamine (H) and ragweed (R) in 26 subjects allergic to ragweed. A threshold response (TR) was defined by a change in forced expiratory volume in 1 sec (FEV₁) greater than -10% and the presence of wheezing. In 20 subjects with reproducible TRR and TRH, 14 with asthma and hay fever and six with hay fever only, we measured: (1) total IgE with a solid-phase (paper radioimmunosorbent test [PRIST]) and a double-antibody assay; and (2) specific IgE with four tests using as solid phase paper disks (commercial or laboratory prepared radioallergosorbent test [RAST]), microcellulose, and sepharose. The double-antibody assay and PRIST produced highly correlated results ($r = 0.94$), but total IgE was not related to TRR. Although the four tests for specific IgE gave highly correlated results, they did not correlate with TRR or TRH. There was a linear correlation between TRH and TRR ($r = 0.71$). When compared with the corresponding slope reported in sensitized guinea pigs, TRR/TRH was shifted toward lower antigen doses. In summary: (1) in opposition to total or specific IgE, histamine airway responses may be important in the bronchial localization of allergic reactions to ragweed; and (2) the airways seem to be more sensitive to allergen in spontaneous asthma in humans than in anaphylactic "asthma" in guinea pigs.

The level of circulating specific IgE may explain the intensity of IgE-mediated symptoms as suggested for hay fever¹ or the degree of organ sensitivity to allergen challenge as proposed for bronchial asthma.²⁻⁹ However, as commonly observed and formally demonstrated, skin responses to allergen, which correlate very well with circulating specific IgE, cannot distinguish hay fever from asthma.¹⁰ Therefore the level of circulating IgE cannot explain the localization of IgE-mediated manifestations to a certain organ. Would the responsiveness of various target organs to

chemical mediators of anaphylaxis be important in such a process?

Our previous incidental observations¹¹ suggested that, in opposition to Tiffeneau's findings,¹² bronchial responses to inhaled allergen and acetylcholine were ostensibly interrelated. Subsequently it was found that in actively or passively sensitized guinea pigs the bronchial responses to inhaled histamine and acetylcholine correlated among themselves as well as with bronchial responses to allergen.¹³

In recent years the relationship between bronchial responses to allergen and bronchial responses to metacholine, histamine, or circulating specific IgE have been the subject of several reports from various countries.^{2-9, 14-17} Unfortunately, many times these reports, including our initial observations, raise serious questions concerning their design and methods.

Our study represents an initial step in the investigation of the mechanisms involved in the bronchial targeting of IgE-mediated reactions. It had two purposes: (1) to test the hypothesis that the hyperactivity of the bronchial smooth muscle to bron-

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TABLE I. Anthropometric, respiratory, and immunologic data in 20 subjects allergic to ragweed

Patient	Dx	Sex	Age (yr)	Height (cm)	FVC	FEV ₁	FEV ₁ /FVC	FEF _{max}	FEF 25% to 75%	FEF 50%	FEF 75%	TDH (mg)	TDA (PNU)
1	A	F	25	165	88	83	77	102	47	57	49	0.08	250
2	A	M	27	177	111	91	84	115	55	46	37	0.32	100
3	A	F	26	168	88	73	68	108	33	35	29	0.08	250
4	A	M	27	178	77	72	77	99	33	34	28	0.16	10
5	A	M	24	179	89	83	84	111	58	51	45	0.08	50
6	A	M	29	182	79	82	85	114	79	83	49	0.64	250
7	A	M	30	183	70	76	84	97	63	49	56	0.32	100
8	A	F	23	161	81	79	86	114	60	58	39	0.08	10
9	A	M	26	174	93	79	85	107	54	50	45	1.28	250
10	A	F	27	171	86	71	71	89	34	35	34	0.32	20
11	A	F	25	163	81	73	86	107	51	50	39	0.16	50
12	A	M	29	175	105	94	62	125	61	62	49	0.32	20
13	A	M	20	185	96	60	54	96	31	32	32	0.32	100
14	A	F	24	168	94	94	82	94	49	58	50	0.32	100
15	R	M	25	174	101	108	84	131	114	98	110	1.28	500
16	R	F	22	166	96	102	86	108	89	91	69	2.56	500
17	R	M	22	183	83	97	84	109	99	86	98	1.28	500
18	R	M	29	172	77	87	84	105	86	84	92	1.28	250
19	R	M	30	177	97	96	84	80	78	80	59	1.28	500
20	R	M	28	173	97	99	86	126	103	102	80	1.28	1000

Respiratory measurements, abbreviated as indicated in text, are expressed as percent of predicted,²⁸ with the exception of %FEV₁/FVC, which represents the ratio observed. Threshold dose of histamine (TDH) or allergen (TDA) represents the dose in milligrams and PNU, respectively, delivered to the patient. For immunologic measurements DA is double-antibody method, PRIST is paper disk radioimmunosorbent test for determination of total IgE (Pharmacia Diagnostics kit), RAST is radioallergosorbent test for specific IgE (Pharmacia kit), RAST-lab is RAST performed on activated paper disks, microcellulose is microcrystalline cellulose test, and sepharose is the quantitative IgE antiragweed antigen E test. (), Score. For this and other immunologic measurements included in the table (%B/T, U/ml), as well as the estimation of the titer from the score, see Methods.

Dx, Diagnosis; A, bronchial asthma; R, rhinitis.

choconstrictor mediators and not the serum level of specific IgE may explain why some subjects with respiratory allergy to ragweed are more sensitive than others to bronchial challenge with allergen; and (2) to determine which of the most used assays for circulating total and specific IgE are most helpful in a study concerned with the localization of IgE-mediated reactions in the respiratory tree.

MATERIAL AND METHODS

Subjects

Twenty-six subjects with bronchial asthma and hay fever and six subjects with hay fever only initially volunteered for this study. We set the following criteria for selection: age between 20 and 30 yr; ability to perform reproducible forced vital capacity (FVC) and forced expiratory volume in 1 sec (FEV₁), as defined by a variability (range/mean) of both measurements <5%; change in FEV₁ after inhalation of saline not larger than the spontaneous variability; day-to-day variability of FVC and FEV₁ <10%; asthma or hay fever or both during ragweed season; positive response (5

mm) to 0.02 ml 5 PNU ragweed extract (Hollister-Stier, Spokane, Wash.) administered intradermally; no respiratory symptoms during the study period or in the preceeding 6 wk (free interval); and no previous immunotherapy for ragweed allergy. Ten asthmatic subjects were taking oral bronchodilators (1 to 2 tablets metaproterenol or aminophylline per day), which were discontinued 12 hr prior to inhalation tests.

These 26 subjects with respiratory allergy to ragweed were submitted to inhalation tests for response to histamine and ragweed, performed 2 to 4 days apart from February to May. Both tests were repeated after a similar interval. Only 14 subjects with asthma and hay fever and six subjects with hay fever only showing identical threshold doses to inhaled histamine or allergen were included in this study.

Inhalation tests

The challenge tests consisted of the administration of progressively increasing concentrations of histamine dihydrochloride (Hollister-Stier) or ragweed extract (Calbiochem, Los Angeles, Calif.) through a Monaghan (Lit-

Total IgE		Antiragweed IgE antibodies			
DA (U/ml)	PRIST (U/ml)	RAST (%B/T)	RAST-lab (%B/T)	Micro- cellu- lose (%B/T)	Seph- arose (U/ml)
550	489	44 (3)	48 (3)	35 (3)	150
200	198	41 (3)	47 (3)	27 (2)	132
440	508	11 (1)	10 (1)	6 (1)	20
350	423	53 (4)	50 (3)	35 (3)	180
195	216	6 (1)	6 (1)	4 (1)	26
550	729	50 (3)	64 (4)	40 (4)	201
240	261	42 (3)	31 (3)	15 (2)	135
370	353	13 (1)	9 (1)	6 (1)	31
95	99	7 (1)	10 (1)	5 (1)	22
121	109	17 (2)	15 (2)	9 (2)	39
400	432	7 (1)	9 (1)	5 (1)	32
225	270	35 (3)	33 (3)	20 (3)	162
345	387	7 (1)	6 (1)	5 (5)	19
109	113	7 (1)	10 (1)	6 (1)	15
180	261	3 (0)	4 (0)	2 (0)	7
42	44	33 (3)	32 (3)	21 (3)	75
48	38	2 (0)	2 (0)	2 (0)	5
28	26	14 (2)	15 (2)	9 (2)	8
80	85	41 (3)	43 (3)	27 (3)	70
425	414	51 (4)	57 (4)	45 (4)	183

tleton, Colo.) 225 volume ventilator set in line with a Bard-Parker (Rutherford, N. J.) manifold nebulizer with the mouthpiece attachment removed. Using a computerized system with flow as primary signal¹⁸ and a Collins 13 l spirometer, the volume ventilator was set at 500 ml tidal volume, plateau flow of 2 L/min, and duration of inspiration of 0.8 sec. The nebulizer, powered through the ventilator by an air flow of 6 L/min, was activated in inspiration only and delivered 0.01 ml allergen or histamine solution per 500 ml. Eighty percent of aerosol particles ranged between 0.8 and 6.0 μ m.*

With the ventilator in assist mode the subjects took 10 breaths through the nebulizer, holding their lips tight around its exit port. Starting with 0.005 mg histamine base or 1 PNU ragweed extract, the dose of drug or allergen, expressed as dose delivered by 10 breaths at the mouth, was increased every 15 min for histamine (doubled) and every 30 min for allergen (2, 5, 10, 20, 50, 100, 250, 500, and 1,000 (PNU) until a threshold response (TR) was obtained.

Flow-volume loops were recorded with the previously mentioned computerized system before and after nebulization of histamine or allergen until FEV₁ decreased by more than 10% and the subjects had chest tightness, dyspnea, and wheezing (threshold dose). Thus at the threshold level all subjects showed a change in FEV₁ of twice its variability and symptoms and signs of airway constriction. Mean FVC, FEV₁, peak forced expiratory flow (FEF_{max}), and forced

expiratory flows (FEF) at 50%, 75%, and between 25% and 75% of the vital capacity were obtained from the three flow-volume loops with the highest FEV₁. The reproducibility and validity of the end point used in these inhalation tests is discussed elsewhere.¹⁹

Immunologic measurements

Total IgE was measured with a commercial kit (paper radioimmunosorbent test [PRIST], Pharmacia Diagnostics, Piscataway, N. J.) as well as a double-antibody method.²⁰ For the first method the indications of the manufacturer were strictly respected. For the second method the serum to be tested or various dilutions of the international standard serum were added to 0.8 U radiolabeled myeloma IgE,* 0.1 ml sheep antihuman myeloma IgE, Fc-specific,[†] diluted 1:10,000, and phosphate-albumin buffer (0.01 M K₂HPO₄-KH₂PO₄ buffer, pH 7.5, containing 1% bovine serum albumin) to bring the resulting volume to 0.4 ml. After overnight incubation at 4° C. 0.2 ml rabbit antish sheep IgG antiserum, (Cappel Laboratories, Cochranville, Pa.) diluted 1:10, and 0.2 ml normal sheep serum, diluted 1:40, were added, and this new mixture was again incubated overnight. The tubes (Falcon tubes, Scientific Products, McGaw Park, Ill.) were then centrifuged, the supernatant decanted, and the radioactivity in the precipitate measured in a gamma counter (Beckmann 300 system, Scientific Instruments Division, Fullerton, Calif.). The optimum volumes of reactants, pH, temperature and duration of incubation, dilution of the first and second antibody, and dilution of the sheep serum were determined in preliminary trials as indicated by Morgan and Lazarow.²²

Antiragweed IgE antibodies were determined with four solid-phase radioimmunoassays; a commercial paper disk method (radioallergosorbent test [RAST] Pharmacia Diagnostics); a method based on paper disks previously activated with CNBr‡ to which ragweed extract was bound in our laboratory; a microcrystalline cellulose method²³; and a quantitative method for detecting specific IgE antibodies attached to sepharose-bound antigen E.²⁴ The selection of the solid-phase polysaccharides for antigen coupling was guided by a comparative study of various RASTs.²⁵ The methods based on the use of the same radiolabeled anti-IgE serum were run during the same week.

The commercial kit (RAST) was used as indicated by the manufacturer. The results were expressed as corrected percent counts bound divided by total counts added (%B/T). The RAST score was translated into dilution (titer) as proposed by the manufacturer. The nonspecific binding of ¹²⁵I anti-IgE with negative control sera was approximately 0.8% to 1%.

For the microcrystalline cellulose assay we followed the indications of Gleich and Jones.²³ Briefly, microcrystalline

*Myeloma IgE kindly provided by Dr. K. Ishizaka. It was labeled with ¹²⁵I using the chloramine T method.²¹

†Anti-IgE antiserum Fc-specific raised against ND myeloma (Pharmacia Diagnostics).

‡Kindly provided by Dr. E. Lindgren, Pharmacia Diagnostics.

*Information provided by the manufacturer.

cellulose (Brinkmann Instruments, Des Plaines, Ill.) was first activated by CNBr dissolved in *N,N*-dimethylformamide, incubated at pH 11 for 45 min, and repeatedly washed with borate buffer. To 1 gm activated microcellulose suspended in 50 ml borate buffer (pH 8), 240 mg dried ragweed extract partially purified* dissolved in 6 ml borate buffer was added. The flask was rotated overnight at 4° C. After washings with borate buffer we stopped the activation with 1 M ethanolamine and repeated washings with alternately low- and high-pH borate buffer as described by Schellenberg and Adkinson.²⁴ The absorbance of microcellulose coupled with ragweed was less than 0.03 at 280 nm, and it was suspended in RAST buffer in concentration of 1 mg/ml. The optimum amount of ragweed needed for coupling was checked by separate runs in which similar amounts of activated microcellulose were coupled with higher (300 mg) and lower (180 and 100 mg) amounts of ragweed. Using the assay described below with a positive control serum with high antiragweed IgE antibodies the %B/T increased briskly when 180 mg was used and plateaued off at 240 and 300 mg.

The assay was performed in duplicate by mixing 0.5 mg microcellulose suspension, 0.5 mg RAST diluent, 0.05 ml serum to be tested, and 0.2 ml RAST diluent. After 24-hr incubation, aspiration of the supernatant, and washings, 8 ng ¹²⁵I sheep antihuman IgE (ND) antiserum, Fc-specific,† was added to each tube and the final volume adjusted to 0.75 ml with RAST diluent. After overnight rotation at room temperature the tubes were washed and counted in the Beckmann gamma counter.

The results were expressed as (1) corrected %B/T (nonspecific binding ~1%); (2) titer [The positive control serum with high antiragweed IgE antibodies (%B/T ~65%) was diluted 1:4, 1:25, and 1:50. The %B/T observed in the undiluted sera of our patients run at the same time was compared with %B/T of the three dilutions of the positive control serum. When the observed %B/T fell between titers (e.g., 1:25 and 1:50), the lowest titer was ascribed to patient's serum (e.g., 1:25).]; and (3) RAST score (The %B/T given by the positive control serum diluted 1:5, 1:25, and 1:50 were given a score of 3, 2, and 1 respectively. Lower %B/T than those given by 1:50 dilution were considered to have a score of 0. The patients' sera were scored similarly to the procedure used for titer.).

Previously activated paper disks were coupled to ragweed by a method similar to that described for microcellulose, using 240 mg ragweed extract for 100 paper disks. The activation was stopped by repeated washings and use of 1 M ethanolamine.²⁴ According to Ceska et al.²⁶ the activated paper disks can be stored at -20° C without any decrease in their activity.

*Kindly provided by Dr. P. Shannon, Hollister-Stier.

†Purchased from Pharmacia Diagnostics, lot No. 9523. The antiserum was labeled in our laboratory.²¹ It was used in this assay, the RAST performed with previously activated paper disks coupled in our laboratory to ragweed (RAST-lab), and the Sepharose test. We had ~80,000 cpm in each tube.

Using the above-mentioned paper disks and anti-IgE labeled in our laboratory, the assay was run as indicated by the RAST manufacturer. The results were expressed as corrected %B/T, titer, and score using the same procedure described for the microcrystalline cellulose assay. The negative control sera gave %B/T of less than 1%.

The quantitative Sepharose test was performed as described by Schellenberg and Adkinson.²⁴ Briefly, ragweed antigen E* was deglycerinated by passage over a Sephadex G-25 column. Sepharose 4B (Pharmacia Diagnostics) was activated by CNBr, maintaining the pH at 11. After washing the sepharose beads with cold buffer, 10 mg antigen E were added and incubated overnight at 4° C. After washing with water, incubation for 3 hr with 1 M ethanolamine (pH 8), and repeated washings with low-pH borate buffer alternated with high-pH borate buffer, the sepharose-antigen beads were suspended in RAST buffer (50% w/v). The assay consisted of the incubation of 0.1 ml serum, 0.5 ml 1% allergosorbent, and 8 ng ¹²⁵I sheep antihuman IgE (ND) for 6 hr at 25° C. The IgE bound to allergosorbent was eluted by treatment with alkaline (pH 11.0) and sham (pH 7.5) RAST buffer. The tubes were incubated for 1 hr at 25° C using an orbital rotator. After centrifugation the supernatants were removed. The eluted IgE was measured with PRIST and the results expressed in units per milliliter using Schellenberg-Adkinson's equation.²⁴ The fraction of allergen-specific IgE bound to the allergosorbent and the fraction of antigen-specific IgE eluted at alkaline pH were found to be 89% and 90%, respectively.

For all four solid-phase radioimmunoassays used to determine the circulating antiragweed IgE antibodies, we determined the specific IgE antibodies remaining in the supernatant at the end of the first step of the assay (i.e., after incubation with patients' sera). The supernatants were run with microcellulose and RAST assays and produced %B/T ~1%. This was taken to represent complete absorption of these antibodies to the allergosorbent.

RAST inhibition test²⁷ was performed with RAST-lab test. The same positive control serum mentioned above was used as the source of high-titer antiragweed IgE antibodies. In the first step of the assay 0.1 ml ragweed extracts used for inhalation tests were tested over a 1,000-fold range (10 PNU to 10,000 PNU) by incubation with 0.05 ml test serum. After performing the remaining steps of RAST, the 50% inhibition point was determined by eye fitting. With this method the concentration of ragweed antigen E in the extracts used for inhalation was determined by comparing these extracts with a Hollister-Stier extract with known concentrations of antigen E (control extract). The control extract was carefully diluted until its 50% inhibition point was identical to the 50% inhibition point produced by the ragweed extracts used for inhalation tests. At this stage we considered that all extracts had a similar content in antigen E (i.e., 22 µg/10,000 PNU).

*Obtained from National Institute of Allergic and Infectious Diseases, Research Resources Branch, Bethesda, Md.

For statistical analysis we used the linear regression analysis²⁸ as performed by a Hewlett-Packard calculator (HP97).

RESULTS

The anthropometric data, average basal respiratory measurements, threshold doses to inhaled histamine and allergen, and the results of various immunologic measurements are presented in Table I. In asthmatic subjects the degree of bronchial obstruction was mild, whereas in subjects with hay fever, with the exception of one flow measurement in subject 19, the pulmonary function tests were within normal limits. The threshold dose of inhaled histamine ranged between 0.08 and 2.56 mg histamine base (dose delivered), whereas the threshold dose of inhaled ragweed ranged between 10 and 1,000 PNU (dose delivered). At the threshold level the $-\Delta FEV_1$ ranged between 10% and 17%, with an average of 14.1% for allergen and 13.8% for histamine.

As indicated in Table I, for total IgE measurements there was a good correlation between results obtained by double-antibody method and PRIST ($r = 0.94$, $p < 0.01$).

Whatever the mode of expression of the results, the four solid-phase radioimmunoassays for IgE antiragweed antibodies produced highly interrelated results ($p < 0.01$). The coefficient of correlation RAST–RAST-lab, RAST-microcellulose assay, and RAST-lab–microcellulose assay was 0.97 for score and 0.91, 0.94, and 0.95 for titers. The units per milliliter of IgE antiragweed antigen E (Sephacose method) correlated very well with %B/T counts, RAST score, or titer as determined by RAST (0.94, 0.88, and 0.89, respectively), RAST-lab (0.93, 0.91, and 0.92), or microcellulose assay (0.91, 0.85, and 0.86).

In asthmatic subjects the degree of bronchial patency as expressed by %FEV₁/FVC correlated very poorly with threshold doses of histamine or allergen ($p > 0.05$); the correlation coefficient was 0.17 for histamine and 0.14 for allergen.

In asthmatic subjects threshold doses of histamine or allergen were not related ($p > 0.05$) to the total IgE levels measured with the double-antibody method (0.36 and 0.39 for histamine and allergen, respectively) or PRIST (0.38 and 0.24). In the entire group threshold doses of allergen were not related to total IgE determined with either method (0.06 for double-antibody method and 0.1 for PRIST). However, in the antibody method and 0.1 for PRIST). However, in the entire group the threshold doses of histamine did relate to total IgE measured with the double-antibody method (0.55) or PRIST (0.50) ($p < 0.05$).

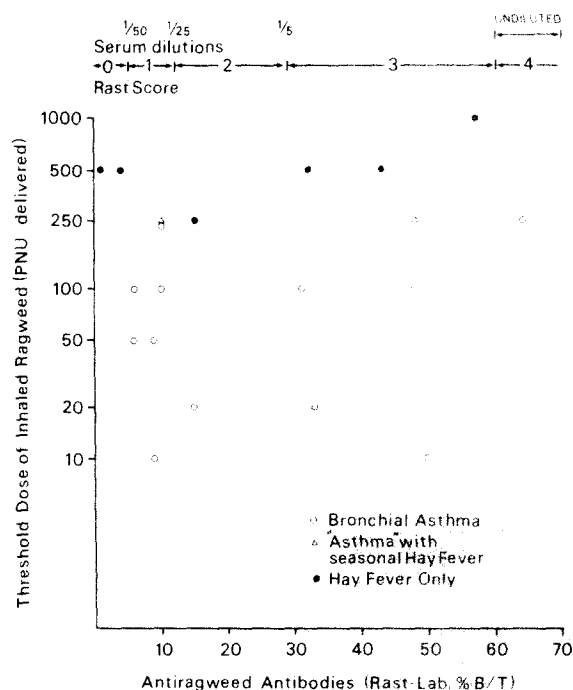


FIG. 1. Relationship between IgE antiragweed antibodies and threshold dose of inhaled allergen.

Regardless of the method used or the mode of expressing the results, neither in the asthmatic subjects nor in the whole group were the threshold doses to histamine or allergen correlated with the level of IgE antiragweed antibodies ($p > 0.05$). In the entire group, for RAST r was 0.01 and 0.2 for %B/T (for histamine and allergen responses, respectively), 0.1 and 0.1 for score, and 0.16 and 0.17 for titers; for RAST-lab r was 0.2 and 0.4 for %B/T, 0.12 and 0.12 for score, and 0.14 and 0.17 for titer; for microcellulose assay r was 0.1 and 0.4 for %B/T, 0.4 and 0.4 for score, and 0.17 and 0.22 for titer; for the quantitative sephacose assay r was 0.14 and 0.10. In asthmatic subjects, for RAST r was 0.1 and 0.1 for %B/T, 0.03 and 0.05 for score, and 0.05 and 0.4 for titer; for RAST-lab r was 0.13 and 0.22 for %B/T, 0.03 and 0.05 for score, and 0.05 and 0.04 for titer; for the microcrystalline cellulose assay r was 0.02 and 0.24 for %B/T, 0.04 and 0.14 for score, and 0.04 and 0.20 for titer; for the sephacose assay (units per milliliter of specific IgE) r was 0.1 and 0.12. For all these plots p was larger than 0.05. These findings are illustrated in Fig. 1 for threshold doses of allergen and circulating specific IgE measured with RAST-lab and in Fig. 2 for threshold doses of histamine and IgE antiragweed antigen E measured with the Sepharose method.

As shown in Fig. 3 the threshold doses of inhaled histamine and inhaled ragweed were well correlated

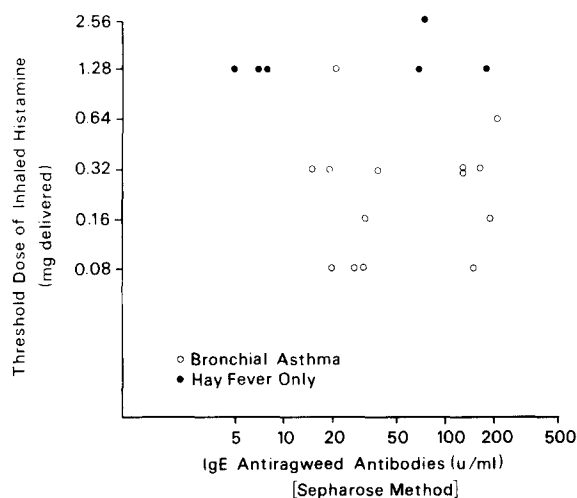


FIG. 2. Relationship between IgE antiragweed antibodies and threshold dose of inhaled histamine.

in the asthmatic subjects ($r = 0.44$, $p < 0.05$) as well as in the whole group ($r = 0.71$, $p < 0.01$).

DISCUSSION

The main findings of our study were that in a group of subjects with asthma and hay fever with average levels of circulating specific IgE the bronchial responses to inhaled ragweed correlated with the bronchial responses to inhaled histamine but not with the level of circulating antiragweed IgE or total IgE.

In agreement with other studies^{30, 31} we found an excellent relationship between total IgE determined by a double-antibody method and total IgE determined with a solid-phase, sandwich method (PRIST). The high interrelation of the results of four solid-phase radioimmunoassays, including the quantitative sepharose method, suggests that, at least for the levels of specific IgE measured in this study, these methods are equally informative.

In this study, during the first step of the assays the specific IgE antibodies were completely absorbed on the solid phase used. The absence of detectable IgE antibodies against ragweed in the supernatant of these assays means that the airway responses to allergen or histamine are compared with "total" specific IgE rather than with an unspecified fraction of specific IgE antibodies absorbed on the solid phase.^{2, 4-9, 15-17}

In our inhalation tests the duration of aerosol administration, the particle size, the fluid output of the nebulizer, and the volume and flow of inspired air were defined and kept constant throughout the study. Moreover the airway responses were checked for reproducibility, and the main physiologic measurement (FEV₁) had a preset limit of variability. The arbitrary

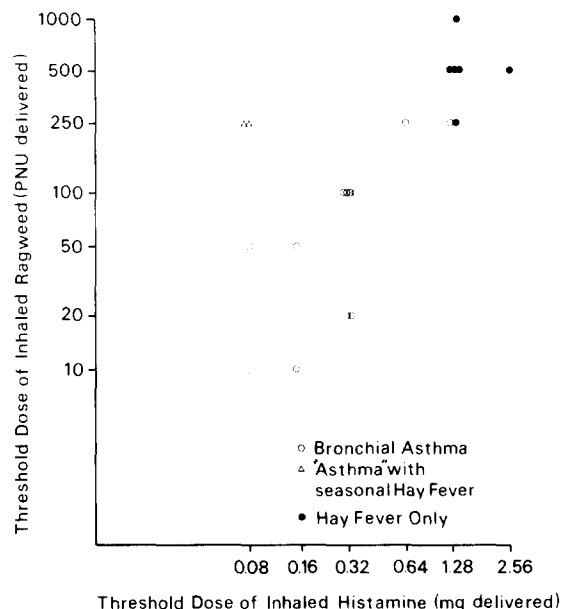


FIG. 3. Relationship between threshold dose of inhaled histamine and threshold dose of inhaled ragweed. NOTE: r was 0.71 in the entire group, 0.44 in all asthmatics, and 0.84 in 12 asthmatics with clear-cut asthma (and hay fever) during ragweed season (i.e., excluding the two subjects with questionable asthma but definite hay fever in that season).

magnitude of response requested for "significant" or "positive" airway responses ($-\Delta FEV_1 > 10\%$) was related to both the degree of variability of FEV₁ (which is consistent with the basic equation of analysis of variance) and the presence of clinical symptoms and signs of bronchoconstriction.

In the present study there was a poor correlation between bronchial responses to inhaled ragweed and circulating antiragweed IgE as determined by any of the four solid-phase assays used (Fig. 1). These findings are not completely in contradiction with reports claiming a fairly good correlation between bronchial sensitivity to allergen and circulating specific IgE.^{2-5, 7-9, 15-17} First, in those studies as well as in our study, for any degree of bronchial sensitivity to allergen there was a wide range of specific IgE. This is more apparent when specific IgE is plotted as dilution rather than score or %B/T (Fig. 1).² Second, mediocre RAST scores,^{4, 5, 7-9, 15-17} RAST titers,² or leucocyte histamine release³ were usually poorly correlated with the degree of bronchial sensitivity to allergen. Even if high levels of circulating specific IgE can modulate the bronchial responses to allergen in asthmatic subjects,^{2-5, 7-9, 15-17} it seems unproductive to use this immunologic measurement for investigating the factors responsible for the localization of ragweed allergy along the respiratory tract. This is not

surprising, because we do not know the relationship between specific IgE bound to lung mast cells and circulating specific IgE.

The threshold doses of inhaled histamine were not related to the level of circulating antiragweed IgE antibodies (Fig. 2), suggesting that in humans specific IgE antibodies may not be an important determinant of bronchial hyperreactivity to histamine. Woolcock et al.³² have reached a similar conclusion. These human observations have an animal counterpart; in vivo in guinea pigs, neither homocytotropic nor heterocytotropic antibodies could alter the bronchial responses to histamine.³³

In subjects with allergic asthma, total IgE determined by the double-antibody assay or PRIST did not correlate with the threshold dose of inhaled histamine or ragweed. Bryant and Burns have also failed to uncover a relationship between atopic status, presumably with high total IgE, and histamine responses of the airways.³⁴ In this study, consistent with all other published reports³⁵⁻³⁸ except one,³⁹ subjects with hay fever had higher threshold doses of inhaled histamine, metacholine, or allergen than the asthmatic subjects. Thus if in asthma total IgE is comparable³⁴ with or higher⁴⁰ than in hay fever, in a mixed population with asthma and rhinitis one would expect to find, as we did, a correlation between threshold doses of histamine and total IgE. The biologic significance of this relationship, if any, is obscure.

In the subjects studied the bronchial responses to histamine or allergen were not influenced by the degree of bronchial patency, expressed as %FEV₁/FVC ($p > 0.05$). This finding suggests that in our subjects bronchial caliber, along with the level of specific and total IgE, did not correlate with histamine or allergen airway responses.

An important observation of this study was the demonstration of a significant correlation between reproducible threshold doses to inhaled histamine and reproducible threshold doses to inhaled ragweed (Fig. 3). This observation, consistent with previous experiments based on challenge tests that were not checked for reproducibility,^{6, 7, 11} suggests that the degree of hyperreactivity to cholinergic agents or histamine may play a role in the localization in the respiratory tract of the sensitivity to ragweed. It might be hypothesized that the higher the nonspecific hyperreactivity to histamine of the bronchial muscle, the smaller the amount of histamine released during IgE-mediated reaction that would be required to constrict this muscle. Two observations, both shown in Fig. 3, suggest that in addition to hyperreactivity to histamine other factors may be important in determining the degree of bronchial sensitivity to allergen.

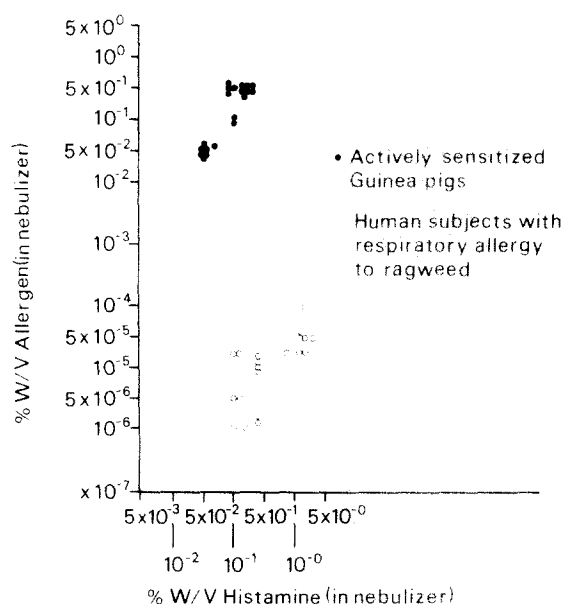


FIG. 4. Relationship between reproducible threshold airway responses to inhaled histamine and allergen in subjects with respiratory allergy to ragweed and in guinea pigs actively sensitized to egg albumin. NOTE: Using the regression equation $y = a + bx$, in guinea pigs a was -0.05 and b was 285 . In humans a was 68.4 and 62.2 in the entire group and in asthmatics, respectively, whereas b was 134.8 and 265.23 . The results on actively sensitized guinea pigs were taken from Popa et al.¹³

First, although subjects with hay fever tended to have higher threshold responses to allergen than asthmatic subjects, there was some overlap between subjects with hay fever and those with asthma³⁵ (Fig. 3). Second, there were two subjects (*open triangles*, Figs. 1 and 3) with low, "asthmatic" threshold doses to histamine but high "hay fever" threshold doses to allergen. These subjects, known to have severe asthma attacks to other allergens and occasionally requiring hospitalization for status asthmaticus, had many hay fever symptoms but minimal wheezing during the ragweed season. One may wonder if in these two subjects the affinity of the Fc fragment of the circulating antiragweed IgE antibodies for lung mast cells was lower than the affinity of other specific IgE antibodies for the same cells.

It seems that the reproducible threshold doses to inhaled histamine and inhaled allergen are linearly related not only in Hartley pigs actively sensitized to egg albumin^{13, 33} but also in patients with respiratory ragweed allergy. Egg albumin and antigen E have comparable molecular weight ($43,000$ and $37,500$, respectively) and isoelectric point.^{41, 42} The histamine content of the lung and that released by compound 48/80 per unit weight are fairly similar in the two species.⁴³ Yet for unknown reasons the guinea pig is

less sensitive to inhaled allergen than are humans; i.e., percent antigen E (in nebulizer) to percent histamine is several hundred times less than percent egg albumin to percent histamine (Fig. 4). The relationship between allergen and histamine responses may emerge as an important parameter for an experimental model of asthma.

Obviously a "definitive" study concerning the determinants of bronchial sensitivity to allergen requires more subjects than we have investigated (eventually separated as atopic and nonatopic), the inclusion of other physiologic and immunologic parameters, dose-related responses to both the chemical mediator and the allergen, and possibly also challenges with more than one allergen.

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