

Ragweed Skin Test Responsiveness Correlates with Specific Immunoglobulin E Levels

Jeffrey Stokes, M.D.,* Richard Kessler, B.S.,# George Philip, M.D.,§ and Thomas B. Casale, M.D.*

ABSTRACT

Evaluation for allergic rhinitis requires an objective measure of atopy. Serum eosinophils, total and specific immunoglobulin E (IgE), and skin testing have been used as this measure. The objective of our study was to examine the relationship between in vitro allergy tests and in vivo responsiveness. We compared eosinophil counts, total IgE, and the specific IgE radioallergosorbent test (RAST) measurements to end point skin test titrations with ragweed. Forty subjects ≥ 18 years of age with at least a 2-year history of moderate to severe ragweed-allergic rhinitis and a positive skin-prick test to ragweed participated in this study with 33 subjects having data for all measurements. End point skin tests were performed by intradermal injection of 0.03 mL of threefold dilutions of standardized short ragweed extract into the forearm. Ragweed-specific IgE was significantly correlated to end point wheal and erythema concentrations. The results were similar whether the end point wheal ($r = -0.714$; $p < 0.001$) or erythema ($r = -0.862$; $p < 0.001$) concentration was used, and the correlation between these two values was significant ($r = 0.97$; $p < 0.001$). However, 8 of 33 subjects had a negative specific IgE RAST value for ragweed. There was a significant relationship between ragweed-specific IgE and total IgE ($r = 0.72$; $p < 0.01$). No significant correlations were found between blood eosinophils

and either total IgE, ragweed-specific IgE, and end point wheal or erythema concentrations. Skin test responsiveness to ragweed correlated with in vitro ragweed-specific IgE levels, but these tests are not equivalent indicators of the degree of IgE-mediated sensitivity. (Allergy and Asthma Proc 26:103–107, 2005)

The incidence of atopy has been increasing over the last 40 years.¹ Atopic individuals have an increase in allergic antibodies (immunoglobulin E [IgE]) and eosinophils compared with the general population. For assessment of allergic rhinitis, a number of measures have been studied. Total serum IgE concentrations are highly variable in this population and can be affected by other factors besides allergy; therefore, total IgE generally is not considered a diagnostic measure of allergic rhinitis.² Allergen-specific IgE can be determined by several different procedures. *In vitro* tests, such as the radioallergosorbent test (RAST), determine the amount of allergen-specific IgE in serum.³ *In vivo* testing such as skin-prick and end point skin titrations measure the responsiveness of the patient to an allergen by wheal and flare response.⁴ Both the RAST titer and the skin test are thought to be indicators of the degree of IgE-mediated sensitivity to a specific allergen.⁵ Some investigators have indicated that RAST values can be highly specific but somewhat less sensitive than standard skin-prick tests.⁶ In a recent review of IgE-mediated hypersensitivity the authors conclude that “for food and respiratory allergy both serum IgE antibody and prick/puncture skin test results are considered equivalently acceptable, and intradermal (ID) skin tests are not needed.”⁷ End point skin titration testing is able to quantify the *in vivo* response based on wheal and flare size. This method of testing has been used as the gold standard for testing the biological relevance of allergen potency. In the United States, some allergens are standardized based on bioequivalent allergy units (BAUs)

*From the *Division of Allergy/Immunology, Department of Medicine, Creighton University, Omaha, Nebraska, #Clinical Research Operations, Merck, Incorporated, Omaha, Nebraska, and §ImmuLogic Pharmaceutical Corporation, Waltham, Massachusetts*

Address correspondence and reprint requests to Thomas B. Casale, M.D., Department of Medicine, Creighton University, 601 North 30th Street, Omaha, Nebraska 68131

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calculated from equivalent skin test responses (e.g., 10,000 BAU of grass allergen and 10,000 BAU of cat dander produce equivalent skin test responses). These units are based on the titration dose of extract to produce a 50-mm sum of erythema.⁸ However, this procedure is labor intensive and poses potential safety risks. Therefore, we attempted to determine if RAST compared favorably with the gold standard, end point skin testing. In theory, the quantitative values for the RAST titer and end point skin titration should be correlated inversely with each other. However, this still has to be proven for various antigens such as ragweed. This study also evaluated the usefulness of other *in vitro* measures of allergy, including total serum IgE and peripheral blood eosinophil levels.

METHODS

This study was conducted just before the onset of ragweed pollen season in Omaha, NE. Forty patients aged ≥ 18 years were enrolled into the study after informed consent was obtained and 33 had data for all measurements. All patients had at least a 2-year history of moderate to severe ragweed-induced allergic rhinitis confirmed by a positive skin-prick test to standardized short ragweed extract and not controlled on over-the-counter antiallergic medication. Patients receiving oral antihistamines, nasal corticosteroids, cromolyn sodium, immunotherapy, or oral or parental corticosteroids were excluded from participating. All patients were white and the majority were women.

Prick Testing

Patients were required to respond to a prick test of short ragweed extract, defined by a wheal diameter of 5 mm or greater than that of the control diluent and the presence of erythema was measured 15–20 minutes after placement. Standardized short ragweed in 50% glycerin, 100,000 AU/mL, or 300 U/mL of Amb a 1 (Antigen Laboratories, Liberty, MO) was used and prepared fresh daily. Appropriate positive (histamine) and negative (diluent, 0.03% human serum albumin) controls were placed at the same time with a bifurcated needle (Allergy Labs of Ohio, Columbus, OH).

End Point Skin Titration

Patients were tested one time with threefold dilutions of standardized lyophilized short ragweed extract containing 100,000 AU/mL or 150 U/mL of Amb a 1 (Greer Laboratories, Inc., Lenoir, NC) reconstituted with 10.0 mL of 0.03% HSA and labeled dilution 0. Serial threefold dilutions of reconstituted ragweed extract were prepared fresh daily and stored at 2–8°C before use. Dilution 1 was prepared by adding 0.9 mL of dilution 0 to 1.8 mL of HSA to obtain dilution 1. Subsequent dilutions were prepared in a similar fashion, adding 0.9 mL of previous dilution to 1.8 mL of HSA until dilution 18 was prepared. If end point was not reached at dilution 18, titrations were made to dilution 26 to determine the end point.

Testing was performed to include four serial threefold dilutions with graded erythema responses, which bracket $\Sigma_{E(\text{erythema})} = 50$ mm and include the end point where $\Sigma_E = 0$ or $\Sigma_E \sim \Sigma_{W(\text{wheal})}$. The skin responses (Σ_E) should fall within the limits of ≥ 0 to ≤ 125 mm. Usually, this range can be obtained by using the most concentrated dilution, where $\Sigma_E = 0$ or $\Sigma_E \sim \Sigma_W$, and the next three more concentrated serial dilutions.

Aliquots of 0.03 mL of the varying concentrations of ragweed extract were injected ID into the forearm with 1.0 mL of tuberculin syringe starting with dilution 15. Wheal and erythema responses were measured as the sum of the longest and orthogonal diameters at 15 minutes postinjection. A minimum of four doses of ragweed extract was used to capture a range of erythema responses from 0 mm (or erythema response equals wheal response) to a ≥ 50 -mm response. End point concentration was determined by log linear interpolation of erythema response (0 mm) or flare response versus ragweed concentration. The end point wheal response was calculated from the same measures used for the erythema responses in an analogous fashion, using log linear interpolation.

RAST Titers and Eosinophil Count

In vitro tests were performed by SmithKline Beecham Clinical Laboratories (St. Louis, MO) according to standardized and approved methods: total IgE (IU/mL), blood eosinophils (cells/ μ L), and ragweed-specific IgE (kU/L) by RAST (% response of 0.35 calibrator) graded as <60% negative, 60–70% very low, 71–110% low, and >110% increasing positive level. All individuals had a measurable RAST titer (>0.35 kU/L) even if the response was rated as negative (<60%). Total white blood cells were measured from peripheral blood. A manual differential was performed and the total peripheral blood eosinophils were calculated (cells/ μ L).

Statistics

Statistical analysis was performed using the Spearman rank correlation coefficient. A value of $p \leq 0.05$ was considered significant.

RESULTS

Erythema and Wheal Responses

We first examined the relationship between ragweed-induced end point wheal and end point erythema responses. There was a good correlation between the log concentration of ragweed (AU/mL) calculated to elicit a wheal or erythema response of 0 mm ($p = 0.752$; $p < 0.001$; Fig. 1).

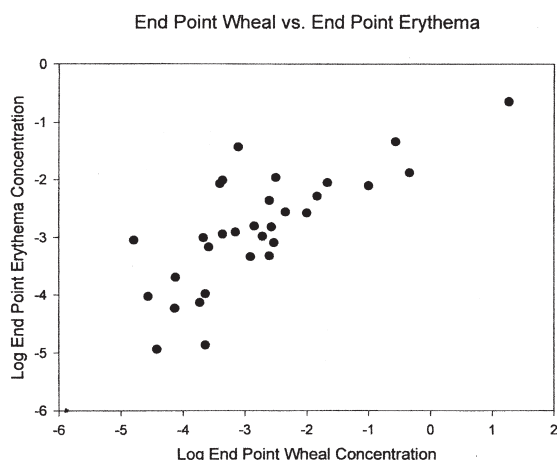


Figure 1. Correlation between end point wheal and erythema responses for ragweed. Values are log concentrations of ragweed (AU/mL) calculated to elicit a wheal or erythema response of 0 mm ($r = 0.752$; $p < 0.001$).

Relationship Between Specific IgE and End Point Responses

Next, we examined the relationship between ragweed-specific IgE levels and ragweed-induced end point erythema and wheal responses (Fig. 2). Specific IgE to ragweed correlated with both the end point wheal ($r = -0.714$; $p < 0.001$) and the erythema ($r = -0.862$; $p < 0.001$) log concentrations (Fig. 2). However, eight of 33 subjects had a negative specific IgE RAST value for ragweed (<60%) despite a positive skin-prick test. Moreover, patients with similar levels of ragweed-specific IgE had differences in end point concentrations of up to two logs.

Relationship Between Total and Specific IgE

We also assessed the relationship between total and ragweed-specific IgE levels. As shown in Fig. 3, there was a significant correlation between total and specific IgE ($r = 0.72$; $p < 0.001$). However, two values (total IgE >800 IU/mL) appeared to drive the slope somewhat. Otherwise, the total and ragweed-specific IgE values were not strikingly related.

Relationship Between Total IgE and End Point Responses

The relationships between total IgE values and ragweed-induced end point wheal and erythema responses were assessed subsequently. We found that total IgE values correlated better with ragweed-induced end point erythema than wheal responses ($r = -0.494$, $p < 0.01$; and $r = -0.320$, $p > 0.05$, respectively; Fig. 4).

Eosinophils and IgE

Because blood eosinophil levels are thought to correlate with IgE-mediated disease severity, we examined the relationship between peripheral blood eosinophil counts and

measures of IgE. No significant correlation was found between absolute peripheral blood eosinophil counts and either total or ragweed-specific IgE levels. We also examined whether eosinophil levels were correlated to *in vivo* ragweed-induced skin test responses. Neither the ragweed-induced end point erythema nor the wheal responses were correlated to peripheral blood eosinophil counts (Table I).

DISCUSSION

As alternative measures of IgE-mediated allergen sensitivity, RAST titers (measuring the allergic immune response in the bloodstream) and end point skin titration (measuring the allergic immune response in the skin) could be expected to correlate well. However, this still is not proven for various antigens such as ragweed. This study was performed to determine the correlation between RAST titers and end point skin test concentrations for ragweed in patients with confirmed ragweed allergy by history and standard skin-prick tests. This study also evaluated other blood-stream measures of allergy, including total serum IgE and peripheral blood eosinophil levels; these end points are known to be elevated with atopic disease although they do not measure the allergen-specific immune response.

In 1982, Turkeltaub and colleagues established the end point skin test technique that we used.⁹ These authors concluded that this method of end point titration was highly reproducible and was suitable as a primary bioassay of allergenic activity. The Food and Drug Administration uses a similar technique to determine bioequivalent units.³

In the United States, the wheal value generally is given greater weight when interpreting skin-prick tests.¹⁰ On the other hand, erythema has been suggested as the key measure for ID skin test titration and end point titration.⁹ Erythema is larger and can be measured with equal or better precision than the wheal, and near the end point dilution, it yields a steeper dose-response line than wheal, indicating more accurate and precise test results requiring fewer replicates and patients than flatter dose responses seen with the wheal.³ In our study, both the wheal and the erythema responses to end point titration correlated with RAST values. However, the erythema response was better correlated with the RAST value and with the total IgE level (for which only the erythema response correlated significantly). Therefore, although end point wheal and erythema responses were significantly correlated with each other, the erythema response appeared to be the more useful ID skin test end point.

End point skin test responses, both wheal and flare, correlated with ragweed-specific IgE values. However, 25% of patients, all of whom had positive skin test responses, had negative or equivocal RAST values. Thus, a substantial percentage of patients had a negative RAST for ragweed-specific IgE despite having positive skin tests to ragweed and a history of ragweed-induced allergic rhinitis symptoms. Indeed, typical *in vitro* tests have established specificity and sensitivity in allergy diagnosis inferior to that of

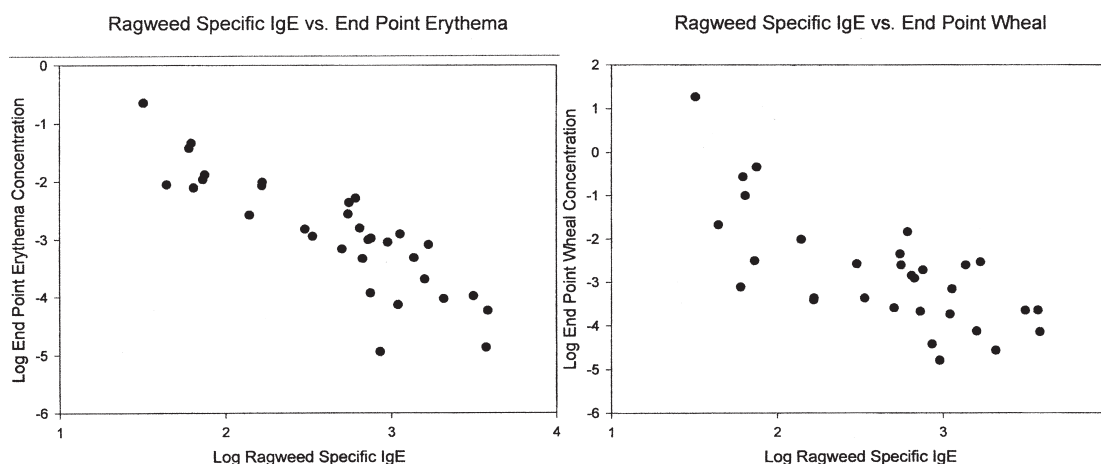


Figure 2. Correlation between log ragweed-specific IgE (kU/L) and (A) log end point wheal and (B) erythema concentrations.

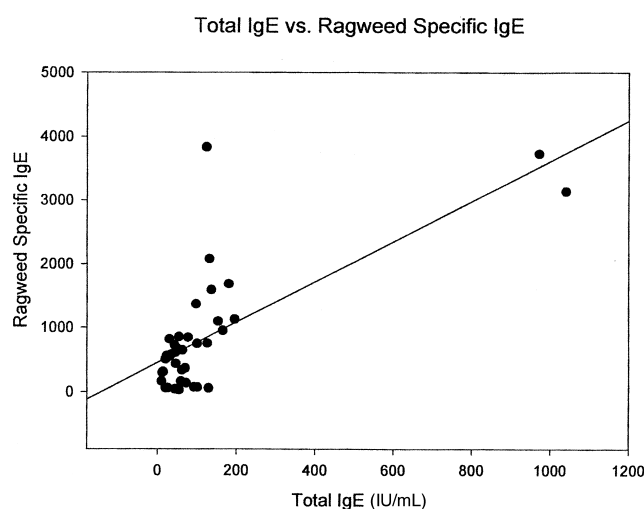


Figure 3. Correlation between total IgE and RAST ragweed-specific IgE (kU/L).

in vivo skin testing assessment.¹¹ A newer version of RAST testing is currently available, called the Pharmacia CAP RAST. This “second-generation” RAST-type assay has shown greater reproducibility than the early RAST tests including the one used in this study.¹² In addition, second-generation RAST assays have shown superior sensitivity and specificity, making them theoretically more diagnostically equivalent to *in vivo* testing techniques. However, no studies have systematically compared these new assays to end point titration.

One could argue that because the subjects chosen had already failed conventional therapy, the specific ragweed IgE levels may be greater than the typical patient with seasonal allergic rhinitis controlled with over-the-counter medications. Thus, they may also have higher ragweed-specific IgE levels as a group, but the individual subject RAST values varied significantly even in this more homogeneous and severe population.

Total blood IgE concentrations did show a significant relationship with ragweed-specific IgE, although it ap-

peared that this relationship was driven somewhat by data from the two subjects with IgE levels >900 IU/mL. Because ragweed-specific IgE is a subset of the total IgE, one might expect a positive relationship between these two measures. As stated previously, these subjects may be more severe as manifested by previous failure with over-the-counter medications. Thus, a greater percentage of their total IgE could have been specific for the ragweed wheal. On the other hand, subjects were not excluded from the study if they were sensitive to multiple aeroallergens. Thus, one might also expect that the total IgE would not be correlated with ragweed-specific IgE. These caveats probably result in the “loose association” between total IgE and ragweed-specific IgE as shown in Fig. 3. When end point skin tests were evaluated, only end point erythema was significantly associated with total IgE levels.

Eosinophils are inflammatory cells associated with atopic diseases, including allergic rhinitis and asthma. Nonetheless, peripheral blood eosinophils did not correlate with *in vitro* or *in vivo* measures of IgE-mediated sensitivity in this study. These data suggest that peripheral blood eosinophil counts are related to aspects of allergic inflammation that are distinct from the hallmark IgE sensitization of atopic diseases, as assessed specifically in this study of ragweed-induced seasonal allergic rhinitis.

CONCLUSION

End point skin titrations showed excellent correlation between erythema and wheal responses, although erythema appears to be a better measure, as noted in other studies. RAST testing established a good relationship with end point measures, but the sensitivity was low and 25% of patients had a negative or equivocal response. The newer RAST tests such as the CAP RAST may show a greater sensitivity than the traditional RAST assays, making this correlation stronger, but studies still have to be done to examine this. Total IgE concentrations correlated with skin test responses and specific IgE levels, but the correlations

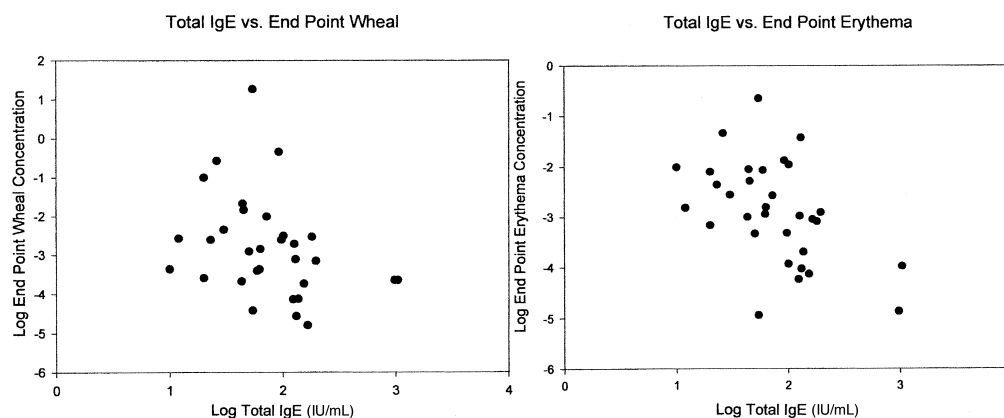


Figure 4. Correlation between log total IgE and (A) log end point wheal and (B) erythema concentrations.

TABLE I

Relationship of Blood Eosinophils to Total and Specific IgE and Skin Test Responses

	Eosinophils	
	<i>r</i>	<i>p</i>
Log total IgE	0.233	NS
Log ragweed-specific IgE by RAST	0.276	NS
Log end point wheal concentration	−0.117	NS
Log end point erythema concentration	−0.248	NS

were weak. Total blood eosinophil counts showed no relationship with any other measure of allergic sensitivity performed. This study confirms that *in vivo* skin test responses are better than *in vitro* measures of the atopic state associated with ragweed-induced allergic rhinitis.

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