

Quantitative assessments of IgG and IgE antibodies to inhalant allergens in patients with atopic dermatitis

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Using antigen-binding radioimmunoassays, we have measured class specific antibodies against two major inhalant allergens, antigen P₁ from D. pteronyssinus and Rye I from grass pollen, in sera from 69 patients with atopic dermatitis. The results show that many of the patients have IgE ab to these allergens in keeping with their skin tests. In all cases, the IgE ab was paralleled by IgG ab to the same allergen. In many sera, IgE ab to these inhalant allergens made a significant contribution to the total serum IgE. With two other allergens to which these patients had not been exposed, specific IgE ab was detected in only one serum, whereas the 42 sera tested did not contain IgE ab to diphtheria toxin. Eleven of the adult patients with atopic dermatitis had no history of asthma and had strongly positive skin tests. This group of patients had levels of total IgE and specific ab to antigen P₁ that were very similar to those found in a comparable group of patients who had both atopic dermatitis and asthma. Our recent finding that allergens applied to the skin can induce delayed eczematous lesions provides a mechanism by which allergens could contribute to skin lesions. Our present results support the view that specific sensitivity to common allergens should be taken into account in considering the causes of these patients' skin lesions. (J ALLERGY CLIN IMMUNOL 72:27-33, 1983.)

After IgE was discovered in 1967, it was rapidly established that patients with atopic dermatitis had high or very high serum IgE levels.¹⁻⁵ Values of 5000 to 30,000 IU/ml are not uncommon in children or adults with this disease. These levels of serum IgE are most unusual except in association with atopic dermatitis or helminth infection⁶ and are greater than those found in patients with rhinitis or asthma. By RAST technique it has been reported that patients with atopic

dermatitis often have IgE ab's to both food and inhalant allergens.⁷⁻⁹ Although the reported levels of RAST binding with food allergens have often been very low, the RAST binding with inhalant allergens has usually been high.⁷⁻⁹ It is possible that some of the reported low levels of binding seen with food allergens are attributable to the increased background that can occur in the RAST in the presence of high levels of total serum IgE.⁸⁻¹⁰ Surprisingly, the high levels of serum IgE found in sera from patients with atopic dermatitis have not been seen as supporting a role for IgE ab's in this disease.¹¹⁻¹⁴ Elevation of serum IgE has been considered to be "nonspecific" or has been attributed to a failure of IgE-specific suppressor T cells.^{5, 13} Some other reports have suggested that IgE antibodies to inhalant allergens are not relevant to atopic dermatitis because they are found only in sera from patients who also have respiratory allergies, i.e., asthma and rhinitis.^{7, 12}

In the present studies we have used purified al-

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Abbreviations used:

Rye I:	Group I protein from rye grass pollen
antigen P ₁ :	Antigen P ₁ from <i>Dermatophagoides pteronyssinus</i>
SAS:	Saturated ammonium sulfate
BA:	Binding activity
PLA:	Phospholipase A from bee venom
RAST:	Radioallergosorbent technique
ab:	Antibody

lergens and antigen-binding radioimmunoassays to measure class-specific ab's in sera from patients with atopic dermatitis.¹⁵⁻¹⁹ These assays for IgE ab are not affected by total serum IgE and allow measurements of IgG ab in parallel with IgE ab. The results suggest that most of these patients have made an immune response to common inhalant allergens and that this is not dependent on the presence of respiratory allergy. The levels of IgE ab were high but the pattern of this response is similar to that seen in other allergic patients and we found no evidence for nonspecific production of IgE ab.

MATERIALS AND METHODS

Patients

Sixty-nine patients with atopic dermatitis were studied. They had been diagnosed clinically on the basis of a characteristic rash²⁰ and had been referred for assessment in a clinical immunology outpatient department. Their ages ranged from < 1 to 60 yr, and the skin condition varied from moderate to severe. In order to compare different patient groups with similar age ranges, the 38 atopic dermatitis patients aged 11 yr or older (mean age 25) were compared with two other patient groups and one control group. The patient groups consisted of 26 subjects with mean age 29 yr (range 13 to 46) who presented with a clinical history of perennial rhinitis and 24 subjects with mean age 26 yr (range 15 to 55) having asthma of varying severity who were attending the same clinic. The control group comprised 19 subjects who had either seasonal rhinitis alone or no allergic symptoms, mean age 27 yr (range 24 to 40). From the 38 "adult" atopic dermatitis patients, two subgroups were further compared: (1) 21 subjects with a history of an asthmatic attack within the previous 2 yr and who were currently under treatment for asthma and (2) 11 subjects who had no history suggestive of reversible bronchospasm. The remaining six patients had an equivocal history of bronchospasm.

Skin tests

Prick tests were performed on all subjects, using extracts of *Dermatophagoides pteronyssinus*, mixed grass pollens, house dust, animal danders (cat, dog, horse) milk and egg (Bencard Ltd., Betchworth, Surrey, U.K.); a wheal diameter 3 mm greater than that caused by the diluent control after

10 min was regarded as a positive reaction. Where possible, intradermal skin testing was carried out with 0.03 ml of serial 10-fold dilutions of *D. pteronyssinus* antigen P₁ (from 10⁻⁷ progressively up to 10⁻¹ µg/ml).¹⁶ Intradermal skin testing was carried out on 26 "adult" patients with atopic dermatitis and on all the control subjects. The skin reactions were read at 15 min, and a wheal larger than 6 by 6 mm was regarded as the endpoint.¹⁷ Skin testing was carried out only on skin that was not eczematous, if necessary on the back; patients who had generalized eczema were tested only when they were in remission.

Measurements of allergen-specific antibodies

Antigen binding assays as previously described were used to measure IgG and IgE ab's.¹⁵⁻¹⁹ Serum dilutions, usually 0.1 ml of 1:12.5 and 1:25, were incubated with ¹²⁵I-labeled antigen for 4 hr at room temperature. Class-specific ab was then precipitated overnight at 4° C by using monospecific goat anti-IgG or goat anti-IgE. The precipitates were washed three times in borate-buffered saline, pH 8.0, and the residual radioactivity was counted in a γ-counter. The quantity of radiolabeled antigen added for each assay was in the range of 2 to 5 ng/tube. All allergens were radiolabeled by a modification of the chloramine T technique.²¹ The specific activity of the radiolabeled allergens ranged from 11.9 to 67.1 µCi/µg (10,000 to 78,000 cpm/ng). The allergens/antigens included *D. pteronyssinus* antigen P₁,¹⁶ Rye I,²² the euglobulin fraction of male rat urine (rat urine allergen) (kindly donated by Dr. J. Longbottom, The Brompton Hospital, London),²³ PLA,^{19, 24} and diphtheria toxin.¹⁸

Each assay was quantitated by using doubling dilutions of a standard serum to form an IgG control curve. A different standard serum was used for each allergen. Results for both IgG and IgE ab's were read off the control curve, multiplied by the serum dilution and expressed as units of BA per milliliter. We have previously estimated, using the RAST absorption technique, that the unit of BA for antigen P₁ represents ~1.0 ng of ab¹⁶ whereas the unit of BA for Rye I represents ~0.3 ng.¹³ Antibodies to diphtheria toxin were quantitated with a serum that had been substandardized against horse antitoxin, and the units used are equivalent to 10⁻³³ IU of antitoxin.¹⁸ Sera from different groups of patients were assayed in parallel. Where necessary, assays were repeated with lower or higher dilutions of the sera, and sera containing large quantities of BA were diluted so that only 25% of the added radioactivity was bound by all classes of ab. When assaying high dilutions of sera (>1:100), carrier immunoglobulins (0.1 ml of a 1:100 dilution of IgG myeloma serum) were added to maintain a constant amount of precipitate. All the assays for IgE ab required the addition of 0.1 ml of 1:100 IgE myeloma serum from patient P. S., kindly provided by Dr. K. Ishizaka, The Johns Hopkins University, Baltimore. In order to avoid interference by large quantities of IgG ab, the assays for IgE ab to diphtheria toxin were carried out on 33% to 50% SAS fractions of serum, and the results were

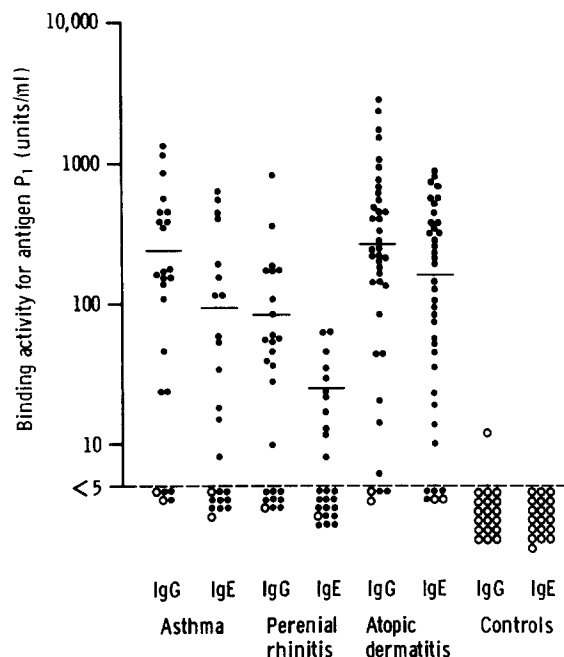


FIG. 1. IgG BA and IgE BA for antigen P₁ in sera from age-matched patients with asthma, perennial rhinitis, and atopic dermatitis and control. ●, Positive skin test to *D. pteronyssinus*; ○, negative skin test; —, geometric mean values; ----, minimum level of sensitivity of the assay.

corrected to unfractionated serum values on the basis of the total IgE in the 33% to 50% SAS fraction. Total IgE was measured by double-antibody inhibition radioimmunoassay using a standard serum substandardized from the WHO international standard 75/502 provided by the National Institute for Biological Standards and Controls, London.

Statistics

The Wilcoxon rank sum test was used to compare IgG BA and IgE BA in sera from patients with rhinitis, asthma, or atopic dermatitis. Total IgE levels were compared by Student's *t* test for unpaired samples.

RESULTS

IgE and IgG antibodies to inhalant allergens

In the sera from 69 patients with atopic dermatitis, 46 were found to contain IgE antibody (BA) against the dust mite allergen, antigen P₁. In each case these sera also contained IgG BA for the same antigen. A group of 38 patients was compared with groups of patients with asthma or perennial rhinitis of similar age range (Fig. 1). The quantities of BA for antigen P₁ were higher in patients with atopic dermatitis than in patients with perennial rhinitis (IgG BA, $p < 0.002$; IgE BA $p < 0.002$) (Table I). The levels of BA were also higher than those in sera from patients with asthma but this difference did not reach signifi-

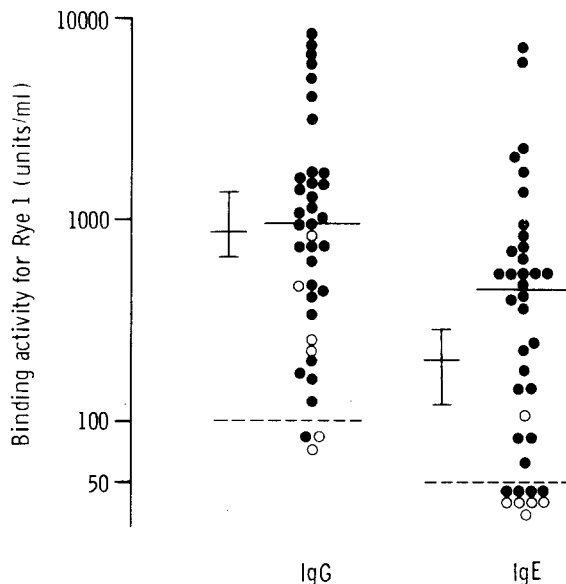


FIG. 2. Serum antibodies to Rye I. ●, Positive skin test to grass pollens; ○, negative skin test; —, geometric mean values; bars, geometric mean and 95% confidence limits for sera from patients with grass pollen hay fever assayed in parallel; dashed line, minimum sensitivity of the assay.

cance ($p > 0.1$, for both classes). The patients with atopic dermatitis were divided into those who were being treated for asthma and a group of 11 patients who had never had symptoms of reversible bronchospasm. The results showed that the group of "adult" patients who had never had asthma had equally high levels of total IgE and specific IgE ab to antigen P₁ (Table I).

Thirty-two (out of 38) patients with atopic dermatitis had positive skin tests to grass pollen, and 29 of these patients had detectable IgE BA for the grass pollen allergen Rye I (Fig. 2). All these 29 sera also contained IgG BA for Rye I; in addition there were six sera that contained IgG BA for Rye I but no detectable IgE BA (Fig. 2). The levels of IgE BA for Rye I were significantly higher than those found in sera from hay fever patients ($p < 0.002$) (Fig. 2). For both allergens, there was an excellent quantitative correlation between IgG BA and IgE BA in sera from patients with atopic dermatitis: for antigen P₁ $r = 0.82$, $p < 0.001$; for Rye I $r = 0.789$, $p < 0.001$.

Relationship between serum IgE BA and skin sensitivity to antigen P₁

In patients with atopic dermatitis we found a good correlation between the presence of IgE antibodies and skin sensitivity as demonstrated by positive prick tests (see Figs. 1 and 2). In order to quantitate the patients' skin sensitivity, we carried out intradermal

TABLE I. Antibodies to antigen P₁ and total serum IgE in patients with rhinitis, asthma, and/or atopic dermatitis

Patient group	Total IgE ^A (IU/ml)	Incidence of positive skin test to Dpt	Antibodies to ^B antigen P ₁ (U/ml)	
			IgG	IgE
Rhinitis n = 26	410 (58-3,708)	25/26	83 (10-805)	25 (8-63)
Asthma n = 24	494 (32-5,500)	22/24	244 (23-1,325)	96 (8-618)
Atopic dermatitis ^C				
All patients n = 38	4,008 (125-23,000)	36/38	263 (6-2,400)	160 (14-838)
With asthma n = 21	5,288 (1,350-23,000)	20/21	274 (20-1,575)	165 (24-768)
No asthma n = 11	5,159 (2,100-17,712)	11/11	268 (6-1,775)	200 (14-700)

Dpt = diphtheria toxin.

Values in parentheses represent range of results observed.

^A Measured by radioimmunoassay; results are geometric means. Statistical analysis of log values for total IgE with Student's t test showed that the two atopic dermatitis groups were not significantly different ($p > 0.1$) but that each group was significantly different from both the asthma group and the rhinitis group ($p < 0.001$).^B Mean values for sera showing positive IgE antibodies to antigen P₁ (see Fig. 1). Statistical analysis with the Wilcoxon rank sum Test showed no significant difference for IgG and IgE antibodies between the two atopic dermatitis groups, or between either group and the asthma patients ($p > 0.1$). The results for the patients with rhinitis were significantly lower than all the other groups ($p < 0.01$).^C Patients with atopic dermatitis were divided into those who had had treatment for asthma within the last 2 yr and those who had never been treated for asthma and had no symptoms suggestive of reversible bronchospasm. Six patients were excluded from this classification because they had an equivocal history.**TABLE II.** Specific IgE ab to antigen P₁ and Rye I as a percent* of total IgE in patients with atopic dermatitis

Antigen P ₁			
Geometric mean	1.96%	n = 46	
Range	0.1%-27%		
Rye I			
Geometric mean	2.37%	n = 44	
Range	0.12%-35%		

* Values were calculated for all the sera showing positive BA. The absolute values for the quantity of IgE ab represented by 1 U of IgE BA have been published previously and were obtained by the RAST absorption technique.^{15,16,26}

skin testing with antigen P₁. The concentration of antigen P₁ required to give a >6 by 6 mm wheal ranged from >1 to 10⁻⁷ μg/ml antigen P₁ (Fig. 3). Intradermal skin testing confirmed the relationship between skin sensitivity and demonstrable IgE ab. However, the quantitative relationship was very poor. It appeared that there was a plateau for skin sensitivity at around 10⁻⁵ to 10⁻⁶ μg/ml antigen P₁ (Fig. 3). Even patients with very high levels of serum IgE BA did not show greater skin sensitivity, and this was true

for both patients with asthma and patients with atopic dermatitis.

Relationship between skin tests, antibodies to antigen P₁, and age

Among the patients less than 7 yr old the prevalence of positive skin tests to *D. pteronyssinus* extract was lower, 10/22 (Fig. 4). However, seven of 10 skin test-positive patients had detectable IgE BA for antigen P₁. Interestingly, the quantities of IgE BA for antigen P₁ were often very high (i.e. >100 U/ml) in these young children (Fig. 4), and in all cases parallel elevations of IgG BA for antigen P₁ were present (data not shown). Thus children with atopic dermatitis can develop vigorous IgE and IgG ab responses to a house dust antigen within their first few years. The results suggest that there was a progressive increase in the proportion of these patients who were sensitive to dust mite antigens.

Absence of IgE antibodies to diphtheria toxin, PLA, and rat urinary allergen

Almost all the patients and controls had been immunized with diphtheria toxoid in the past. In keeping

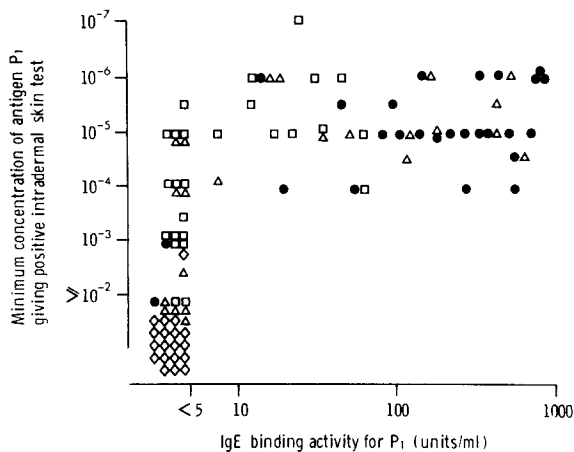


FIG. 3. Skin sensitivity to antigen P₁ related to serum IgE BA for antigen P₁. Skin sensitivity is the lowest concentration of antigen P₁ (μ g/ml) giving a wheal >6 by 6 mm. ●, Patients with atopic dermatitis; Δ , asthma patients; $\square$, perennial rhinitis patients; $\diamond$, controls. The controls included seven patients with grass pollen hay fever.

with this, many of the sera contained IgG diphtheria antitoxin (Fig. 5). However, IgE diphtheria antitoxin was not detected in sera from either the 21 controls or the 43 patients with atopic dermatitis. We also assayed these sera for IgE ab to two other allergens, PLA and a purified allergen from rat urine. None of the patients with atopic dermatitis had a history of bee keeping or allergic reactions to bee stings and none had worked with rats. IgE BA for PLA was not detected in any of the 62 sera tested and only one patient had detectable IgE BA for rat urinary allergen. None of the 25 control sera tested had IgE BA for either allergen.

DISCUSSION

Most patients with atopic dermatitis described here were sensitive to dust mite and grass pollen allergens as judged by skin tests and the presence of serum IgE antibodies. In many cases the levels of these IgE antibodies were very high and were, in all cases, paralleled by IgG antibodies. As has been reported previously for asthma and allergic rhinitis, there was a quantitative correlation between IgE and IgG antibodies.^{15, 16, 25} Thus the results for two important inhalant allergens show that patients with atopic dermatitis have a strong tendency to make both IgE and IgG antibodies against common environmental allergens. Applying previously reported absolute values for the units of IgE ab to our present results suggested that many of the sera contained >200 ng/ml specific IgE ab to antigen P₁.^{15, 16} From these absolute values it is possible to express the IgE ab as a percentage of

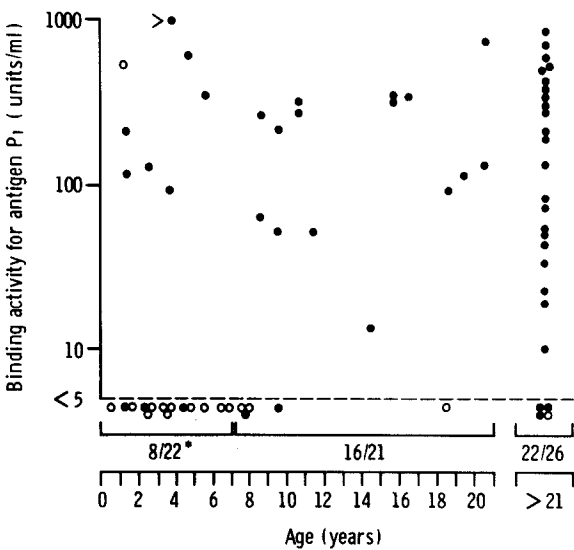


FIG. 4. Serum IgE BA for antigen P₁ in patients of various ages with atopic dermatitis. *Solid symbols*, Patients with positive skin tests to *D. pteronyssinus*; *asterisk*, patients less than 7 yr old. The proportion of patients showing detectable IgE BA is indicated: for patients <7 yr old, between 7 and 21 yr; over 21 yr.

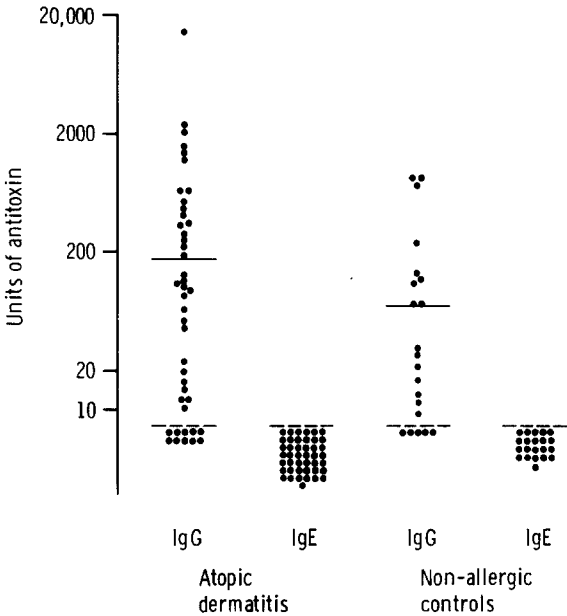


FIG. 5. IgG and IgE diphtheria antitoxin levels in sera from 43 patients with atopic dermatitis and 21 nonallergenic controls. *Dashed line*, Minimum level of sensitivity of the assay.

the total IgE (Table II). These percentages are lower than those reported previously for ragweed hay fever^{26, 27} or grass pollen hay fever¹⁵ or for a large group of patients with dust mite allergy.¹⁶ Nonetheless, the values shown in Table II indicate that in

many cases IgE ab to these two allergens represented >5% of the total IgE and in some sera these two specificities of IgE ab alone represented a major proportion of the total IgE. Since most allergen extracts contain several allergenic proteins and many of our patients showed positive skin tests to five or more different inhalant allergens, it seems likely that IgE ab to inhalants can make a major contribution to the high serum IgE levels seen in patients with atopic dermatitis.

The absence of IgE ab against two other allergens or against diphtheria toxin suggests two conclusions. First, the mechanisms that restrict or suppress IgE ab production after "high dose" immunization with diphtheria toxoid are not impaired in patients with atopic dermatitis. Second, in these patients as in other allergic patients, the production of IgE antibodies is dependent on adequate allergen exposure. There is a report of IgE diphtheria antitoxin and IgE tetanus antitoxin in sera from allergic children, using the RAST.²⁸ It is possible that diphtheria or tetanus IgE antitoxin is produced transiently after injections; however, those results were seen in sera with >500 IU/ml IgE and some of them may represent nonspecific binding of IgE to the solid phase.¹⁰ Certainly there is no clinical evidence that allergic patients have an increased risk of immediate reactions after immunization with diphtheria toxoid.

The overall correlation between positive skin tests and IgE antibodies in the present series was very good. It must be recognized that skin testing patients with atopic dermatitis requires additional care because inflamed or excoriated skin often gives poor wheal and flare responses. As reported by others, the appearance of the skin responses may be unusual. These patients often show very little flare relative to the size of the wheal, which may reflect abnormal autonomic control of the blood vessels.^{14, 20} In this study we did not find a quantitative correlation between skin tests and serum IgE BA (Fig. 3). Our results suggest that there is an upper limit for skin sensitivity, at around 10^{-6} $\mu\text{g/ml}$ pure allergen. This upper limit was seen in patients who have asthma as well as those with atopic dermatitis. The quantitative correlations reported previously^{17, 29} may not apply with purified allergens in patients who have very high specific IgE ab.

Several clinical studies have strongly supported a role for inhalant allergens, particularly house dust, in the pathogenesis of atopic dermatitis.³⁰⁻³³ Some recent developments have lent support to this concept. The demonstration that dust mites are a major source of house dust allergens³⁴ has led to the use of potent dust mite extracts and has made it possible to purify the principle allergen of *D. pteronyssinus*.¹⁶ Thus de-

tailed assessment of in vivo and in vitro sensitivity to house dust allergens is now possible^{7, 16, 35}; furthermore, the quantities of allergen in house dust can be measured.^{36, 37} Many of the patients in the present series were exposed to large quantities of antigen P₁ in their houses: up to 5 $\mu\text{g/gm}$ of fine dust.^{36, 38} It has often been noted that most patients with atopic dermatitis are not aware that inhalant allergens exacerbate their skin rash. However, they would be aware of a relationship to a ubiquitous allergen like house dust only if the response was rapid. It is becoming increasingly obvious that the symptoms of allergic diseases cannot simply be ascribed to anaphylactic release of mediators from mast cells and may well involve some form of prolonged or delayed reaction to allergens. Recently it has been demonstrated that allergen applied to the skin of patients with atopic dermatitis can give rise to a delayed eczematous reaction.³⁸ These delayed skin lesions are infiltrated with basophils, eosinophils, and mononuclear cells and appear to be similar to cutaneous basophil hypersensitivity in guinea pigs.^{39, 40} By comparison with the guinea pig model, it seems likely that the production of these lesions is dependent on both anaphylactic ab's and sensitized T cells.

In conclusion, our present results confirm that many patients with atopic dermatitis have IgE ab to an important house dust allergen. In many of these cases the presence of IgE antibodies does not appear to be related to the patients' inhalant allergies. These IgE antibodies are part of an immune response that includes IgG ab and there seems no reason to regard such responses as nonspecific. Although it is not at all clear that IgE (or IgG) antibodies can mediate eczematous skin lesions, it seems that these patients also have some form of cellular immunity to the same allergen. Thus positive skin tests and serum IgE ab should be regarded as markers for a complex form of hypersensitivity. In patients who have IgE ab and are exposed to the relevant allergens, we believe that the allergens should be considered as a potential cause of their skin lesions.

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