

Dermatologic and ocular diseases

Use of specific IgE in assessing the relevance of fungal and dust mite allergens to atopic dermatitis: A comparison with asthmatic and nonasthmatic control subjects

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Background: Although allergens have been implicated as aggravating factors in atopic dermatitis (AD), there is little epidemiologic data on the significance of specific IgE.

Objective: We sought to compare sensitization to dust mite and fungi between patients with AD and asthmatic and nonasthmatic control subjects.

Methods: Total IgE and specific IgE to *Dermatophagoides pteronyssinus*, *Alternaria alternata*, *Aspergillus fumigatus*, *Candida albicans*, *Malassezia furfur*, and *Trichophyton rubrum* were measured in 73 patients with moderate to severe AD. Total IgE and IgE specific for *D pteronyssinus*, *A alternata*, and *M furfur* were also measured in sera from 156 asthmatic and 212 nonasthmatic control subjects.

Results: Positive correlations were found between total IgE and IgE antibodies specific for each of the antigens. IgE specific for *M furfur* was observed more frequently in adults compared with children with AD ($P < .01$). AD sera had higher levels of total IgE and a higher prevalence of positive sera to *D pteronyssinus* (95% vs 42% and 17% for subjects with AD, asthmatic subjects, and nonasthmatic subjects, respectively), *M furfur* (53% vs 1% and 0.5%), and *A alternata* (49% vs 29% and 18%). Among the sera from subjects allergic to mites, the contribution of IgE specific for *D pteronyssinus* to the total IgE levels was similar regardless of the clinical status. **Conclusions:** Our results demonstrate that moderate-to-severe AD is strongly associated with sensitization to dust mite and *M furfur* (odds ratios, 45.6 and 132 vs pooled control sera). These results suggest that both environmental allergens and colonizing fungi contribute to the severity of disease, which is consistent with the view that mite avoidance and antifungal treatment can be beneficial in the treatment of these patients. (J Allergy Clin Immunol 1999;104:1273-9.)

Key words: Atopic dermatitis, dust mite, fungi, specific IgE antibodies, *Malassezia furfur*, *Alternaria alternata*

Although there is a large body of evidence documenting an association between IgE antibody to allergens and atopic dermatitis (AD), there is only partial acceptance of the etiologic significance of allergens in the disease. It has been demonstrated that the high serum total IgE levels seen in patients with AD represent specific responses to antigens to which the individual has been exposed.¹ However, there is surprisingly little data comparing sensitization in subjects with AD to that found in other populations of subjects with allergic diseases or nonallergic control subjects. Several studies indicate that contact with aeroallergens may play a role in the exacerbations of AD, either by the demonstration of IgE and IgG antibodies to house dust mite allergen in patients with AD, circulating T cells which are specific for group I mite allergens, or local provocation of the skin with aeroallergens.¹⁻⁵ Another source of antigenic stimulation that has been investigated are organisms that colonize or infect the skin. *Staphylococcus aureus*, which can be recovered from the skin of greater than 80% of patients with AD, has been proposed as a source of exotoxins that can act as antigens eliciting specific IgE synthesis, as superantigens leading to T cell-mediated responses, or both.⁶ These T-cell responses are postulated to involve production of IL-4 and IL-10 and lead to the upregulation of IgE synthesis.^{7,8}

Other microorganisms have also been implicated in specific IgE responses in patients with AD.⁹⁻¹¹ *Malassezia furfur* (formerly *Pityrosporum ovale*) is a lipophilic yeast with a colonization rate as high as 92% in healthy individuals, being more frequent after puberty in temperate climates, and most frequently isolated from the upper back, chest, neck, and scalp.¹²⁻¹⁴ This fungus has been associated with the head, scalp, and neck variant of AD, but it is not clear whether infection with *M furfur* is a primary pathogenic factor or a secondary consequence of changes in the skin of patients with AD.^{9,15} The incidence of sensitization in the general population is also unclear.

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Abbreviation used

AD: Atopic dermatitis

The hypothesis that fungi colonizing the skin can play a role in the pathogenesis of AD was reinforced by reports of symptomatic improvement in some patients with AD after treatment with ketoconazole.¹⁵ Similarly, we obtained clinical improvement with prolonged use of systemic antifungal agents in the treatment of 5 patients with severe AD, in which the disease was refractory to conventional treatment.¹⁶ In the present study we assessed the quantity and relationships of specific antibodies to dust mites; to the colonizing fungi *M furfur*, *C albicans*, and *Trichophyton* species; and to the inhaled fungi *Alternaria alternata* and *Aspergillus fumigatus* in a population of 65 patients with AD. These results were evaluated both within the AD group and for 3 of the allergens in relation to results on sera obtained from 156 patients presenting with asthma and 212 random control sera. The object was to further define the relationship between IgE antibodies and AD and to define criteria for identifying patients who should be considered for allergen-specific therapy.

METHODS**Patients**

Seventy-three patients (34 females) with AD were studied. They had been referred to either the Asthma and Allergic Diseases Center or the Immunodermatology Clinic of the University of Virginia for treatment of their skin disease. The clinical diagnosis of AD was based on finding the characteristic chronic pruritic skin rash often accompanied by atopic respiratory disease (asthma, rhinitis, or both). Their ages ranged from 5 to 82 years, with 16 individuals under the age of 17 years (defined as children in this article). The severity of AD varied from moderate to severe.

The sera from patients with AD were compared with sera from a group of 156 asthmatic patients (asthma group), including 102 adults and 54 children, and with sera from a group of 212 nonasthmatic control subjects (control group), including 110 adults and 102 children. The sera in the asthma group were obtained from random patients who presented with asthma to either of 2 hospital emergency departments in Wilmington, Delaware, or from 2 middle schools in Central Virginia classified as having asthma on the basis of a questionnaire, use of treatment for asthma, or both, as well as the presence of bronchial hyperreactivity to inhaled histamine.^{17,18} The sera of the control group were from patients attending the same emergency departments with any condition other than shortness of breath and children from the 2 schools with negative answers to the questionnaire and without bronchial hyperreactivity to histamine. The asthmatic and control subjects were not evaluated for skin disease.

Measurements of total IgE and allergen-specific antibodies

Specific IgE antibodies to *D pteronyssinus*, *A alternata*, *M furfur*, *A fumigatus*, *C albicans*, and *T rubrum* were measured by using a fluoroimmunoassay (CAP system; Pharmacia, Uppsala, Sweden). Total IgE for sera from patients with AD was also measured by using the CAP system. Sera were first assayed undiluted, and when necessary, assays were repeated with the sera diluted to enable quantitative measurement of specific IgE antibodies. When specific antibodies were detected for each allergen tested, those sera from

patients with AD were tested for IgE to human insulin. If antibody was detectable to human insulin, this level was adopted as the background for the patient and subtracted from the levels of IgE to the specific allergens. If specific IgE to any of the allergens or human insulin were undetectable, the background was assumed to be indistinguishable from asthmatic or control subjects. The sera of the asthma and control groups were initially assessed by using an RIA for total IgE and a quantitative RAST for specific IgE antibody to *D pteronyssinus*.¹⁷ The total IgE has been previously standardized to international units. Once we had established that 101 negative sera (as determined by RAST) were below the level of detection by CAP, the remaining sera that were negative as determined by RAST were assumed to be negative by CAP. Where specific IgE was measurable by RAST, the assay was repeated by CAP. In 21 cases, the RAST for *D pteronyssinus* was positive, but insufficient serum was available for CAP analysis; for these, the CAP value was extrapolated from the RAST value by generating a regression analysis and reading a value based on the slope of the line (correlation coefficient, ≥ 0.9). Sera were also unavailable in 13 cases for *Alternaria* species. Again, the RAST levels were extrapolated from a regression analysis (correlation coefficient, 0.66). In prevalence analyses a CAP class II result (≥ 0.7 U/mL) was considered positive for sera from asthmatic subjects, control subjects, and subjects with AD after subtraction of the background level, as described previously.

Statistical analysis

An analysis of normality (Kolmogorov-Smirnov test) indicated nonnormal distribution for all the studied variables. After a base-10 log transformation of the values, a normal distribution was obtained for eosinophil counts and total IgE levels. We compared the log values of these variables between groups by using the Student *t* test for independent samples. Correlation coefficients were generated by using the Spearman method for nonparametric data. Prevalence data were compared by using a retrospective contingency table analysis. The confidence interval used for all statistical analyses was 95%.

RESULTS

The serum assays demonstrate that many of the patients with AD had serum IgE antibodies directed against fungi that are characteristically inhaled (*A alternata* and *A fumigatus*) and also to colonizing fungi (*C albicans*, *M furfur*, and *T rubrum*; Table I). However, the mean concentration of IgE specific for any of the fungi was approximately 50-fold lower than specific IgE to *D pteronyssinus* (Table I and Fig 1). The patients with AD also had very high total serum IgE levels and high eosinophil counts. Analyzing the data for adults and children separately, eosinophil counts were higher in children, whereas IgE antibody to *M furfur* was much more common in adults. Specific IgE to dust mites was also marginally more common in adults. Correlations were found between serum total IgE levels and specific IgE levels to mite and fungi in the patients with AD (Table II). There was no correlation between eosinophil counts and serum total or specific IgE levels in these patients. There were extensive correlations between specific IgE for each of the fungi, with correlations between *A alternata* and *A fumigatus*, between *A fumigatus* and *C albicans*, and between *C albicans* and *M furfur*. Although a correlation was found between *C albicans* and *M furfur*, it was less strong than the 0.9 previously reported.¹⁹

TABLE I. Values of eosinophil counts, serum total IgE levels, and specific antibodies to fungi and *D pteronyssinus* in the 65 patients with AD

	Total (n = 73)		Adults (n = 57)		Children (n = 16)	
	Mean (CI)*	Prevalence†	Mean (CI)	Prevalence	Mean (CI)	Prevalence
Eosinophil count (cells/mL)‡	538 (429-675)	—	479 (368-624)	—	872 (644-1180)§	—
Serum total IgE (IU/mL)§	3173 (2110-4772)	—	3675 (2380-5676)	—	1881 (624-5671)	—
IgE to <i>D pteronyssinus</i> (U/mL)	208 (120-360)	69/73	196 (107-359)	56/57#	269 (61-1187)	13/16
IgE to <i>A alternata</i> (U/mL)	4.05 (1.59-6.42)	43/73	4.47 (2.66-7.54)	33/57	2.94 (0.97-8.84)	10/16
IgE to <i>A fumigatus</i> (U/mL)	3.67 (2.34-5.76)	45/73	3.48 (2.08-5.84)	34/57	4.45 (1.52-13.0)	10/16
IgE to <i>C albicans</i> (U/mL)	3.87 (2.59-5.8)	55/73	4.29 (2.74-6.71)	46/57	2.5 (0.87-7.19)	9/16
IgE to <i>M furfur</i> (U/mL)	4.96 (2.99-8.22)	39/73	5.36 (3.16-9.08)	37/57**	1.77 (0.06-5.3)	2/16
IgE to <i>T rubrum</i> (U/mL)	2.11 (1.36-3.27)	42/71	2.20 (1.26-3.84)	31/55	1.82 (0.98-3.36)	11/16

*Geometric mean and 95% confidence interval (CI) of subjects with measurable levels.

†Percentage of patients with specific IgE of 0.7 or greater after adjustment for background.

‡Eosinophil results were available from 50 adults and 12 children.

§International units per milliliter.

||Units of allergen-specific IgE antibody per milliliter. Units are international units derived from CAP system modification of RAST control curves.

#Significantly greater than adults ($P < .05$), as determined by the Student t test.

**Significantly greater than children ($P < .05$), as determined by a retrospective contingency table analysis.

***Significantly greater than children ($P < .01$), as determined by a retrospective contingency table analysis.

TABLE II. Correlation coefficient* between serum total IgE levels and IgE antibodies to mite and fungi in the patients with AD

	IgE to <i>D pteronyssinus</i>	IgE to <i>A alternata</i>	IgE to <i>A fumigatus</i>	IgE to <i>C albicans</i>	IgE to <i>M furfur</i>	IgE to <i>T rubrum</i>
Serum total IgE	0.70†	0.35‡	0.46†	0.66†	0.54†	0.37†
IgE to <i>D pteronyssinus</i>	—	0.27§	0.41†	0.42†	0.38†	0.36‡
IgE to <i>A alternata</i>	—	—	0.70†	0.54†	0.45†	0.29§
IgE to <i>A fumigatus</i>	—	—	—	0.68†	0.53†	0.42†
IgE to <i>C albicans</i>	—	—	—	—	0.67†	0.39†
IgE to <i>M furfur</i>	—	—	—	—	—	0.22

*Values correspond to the Spearman correlation coefficient.

† $P \leq .001$.

‡ $P \leq .01$.

§ $P \leq .05$.

The results for sera of patients with AD were compared with asthmatic and nonasthmatic control sera for concentration of specific IgE antibody to *D pteronyssinus*, *A alternata*, and *M furfur* (Table III). Mean specific IgE was higher in sera from patients with AD for all antigens. Most striking was the difference in prevalence of IgE to *M furfur* among sera from patients with AD, asthmatic subjects, and nonasthmatic control subjects (53% vs 1% and 0.5%, respectively). The highest concentrations of specific IgE measured were directed against the dust mite antigen, and the quantity of IgE antibody to *D pteronyssinus* in the patients with AD was much higher than in either of the other groups (Fig 2, A). To assess the relevance of specific IgE antibodies to dust mites in a setting of marked inequality of total IgE, the values were adjusted by dividing IgE to the mite by the total IgE for each sera in which specific IgE to *D pteronyssinus* was measurable. A comparison of this ratio demonstrated that contribution of specific IgE to *D pteronyssinus* to the total IgE (approximately 15%) was no different between any of the groups (Table III). Distribution charts for IgE to *A alternata* (Fig 2, B) and for IgE to *M furfur* (Fig 2, C) show that although the prevalence of positive results

was higher in patients with AD, this difference was less impressive for *A alternata* than for *M furfur*.

DISCUSSION

It has long been known that patients with AD have high concentrations of serum IgE and allergen-specific IgE. Some of these studies have reported a direct relationship between total IgE levels and the severity of skin involvement.^{20,21} To date, most of the published studies have used as control subjects patients with other skin diseases seen in the same clinics or nonrandom control subjects recruited locally. An ideal study would start with a random population and prospectively follow the factors hypothesized to be associated with the acquisition and severity of disease. The cumulative incidence of AD is reasonably high if all forms are considered, with perhaps 10% of individuals having evidence for a typical pruritic skin rash at some point in their lives.²² However, most AD is infantile, mild, and transient. Moderate-to-severe AD is much less common, and a large population study (ie, ≥ 4000) would be required to learn anything about those patients most affected by the disease. A recent

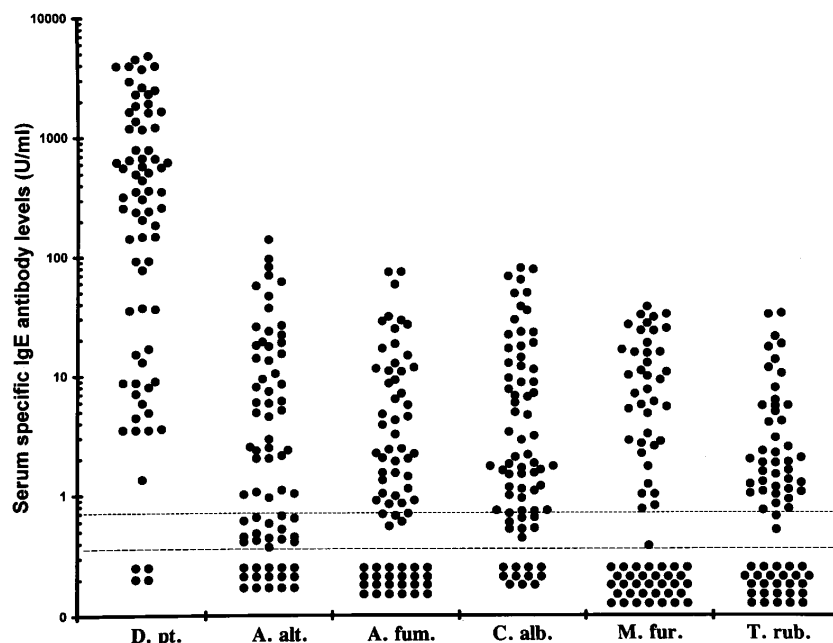


FIG 1. Quantitative measurements of IgE antibodies to *D pteronyssinus* and to fungi in the patients with AD as measured by CAP system after subtraction of background binding. The lower dashed line represents the limit of detection for this assay, and the upper dashed line indicates the level required for the sera to be considered positive for statistical analysis. *D. pt.*, *D pteronyssinus*; *A. alt.*, *A alternata*; *A. fum.*, *A fumigatus*; *C. alb.*, *C albicans*; *M. fur.*, *M furfur*; *T. rub.*, *T rubrum*.

TABLE III. Comparison of serum total IgE level and specific IgE antibodies between groups

	AD group*		Asthma group		Control group	
	Mean (CI)†	Prevalence‡	Mean (CI)	Prevalence	Mean (CI)	Prevalence
Serum total IgE (IU/mL)	3173 (2110-4772)	—	172 (134-221)	—	44.5 (36.9-55.1)	—
IgE to <i>D pteronyssinus</i> (U/mL)	208 (120-360)	69/73	19.3 (12.4-30.3)	65/156**	12.2 (7.3-20.3)	36/212
Ratio of IgE to <i>D pteronyssinus</i> / total IgE§	0.147	—	0.158	—	0.147	—
IgE to <i>A alternata</i> (U/mL)	4.05 (1.59-6.42)	43/73¶	3.7 (2.4-5.5)	45/155††	2.0 (1.4-3.0)	38/212
IgE to <i>M furfur</i> (U/mL)	4.96 (2.99-8.22)	39/73#	0.7 (0.3-1.7)	2/144	0.6 (0.3-0.7)	1/205

*Specific IgE antibody levels in subjects with AD have been adjusted for background as described in the "Methods" section.

†Geometric mean and 95% confidence interval (CI).

‡Subjects with levels of specific IgE of 0.7 U/mL or greater related to the number of subjects tested.

§The value given is the mean of the ratios for serum in which IgE to *D pteronyssinus* was measurable.

||Odds ratio of 84 (95% CI, 27-291) versus control subjects; odds ratio of 24 (95% CI, 8-82) versus asthmatic subjects; odds ratio of 46 (95% CI, 15-151) versus pooled control subjects.

¶Odds ratio of 6.6 (95% CI, 3.5-12.3) versus control subjects; odds ratio of 3.5 (95% CI, 1.9-6.5) versus asthmatic subjects; odds ratio of 4.9 (95% CI, 2.8-8.6) versus pooled control subjects.

#Odds ratio of 234 (95% CI, 33-616) versus control subjects; odds ratio of 81 (95% CI, 18-207) versus asthmatic subjects; odds ratio of 132 (95% CI, 36-267) versus pooled control subjects.

**Odds ratio of 3.5 (95% CI, 2.1-5.8) versus control subjects.

††Odds ratio of 1.9 (95% CI, 1.1-3.2) versus control subjects.

report from Germany found that 0.7% of a cohort of 2000 children had "severe" eczema, but the mean total IgE level among this severe group was only 350 IU/mL.²³ The present study was designed to compare a severely affected clinic population with a large number

of asthmatic subjects enrolled serially from an emergency department or enrolled as part of a population-based study at 2 local schools and random control subjects from the same locations.

In the majority of studies looking at specific IgE lev-

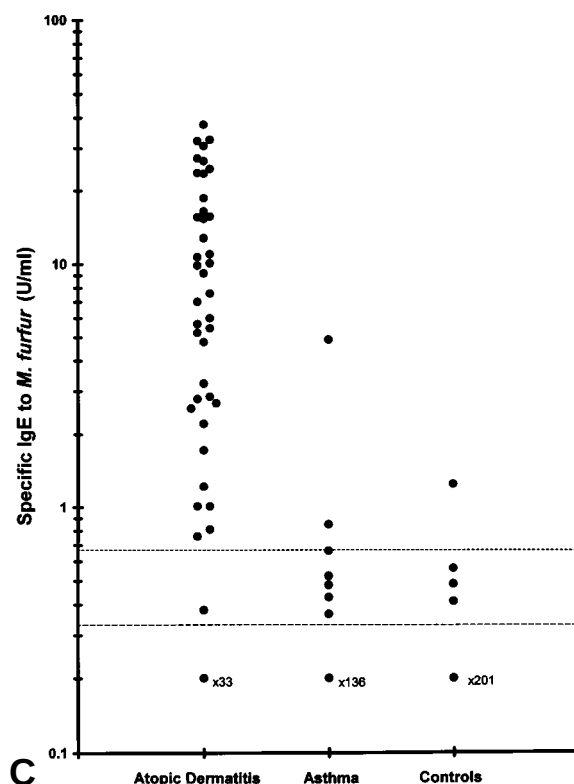
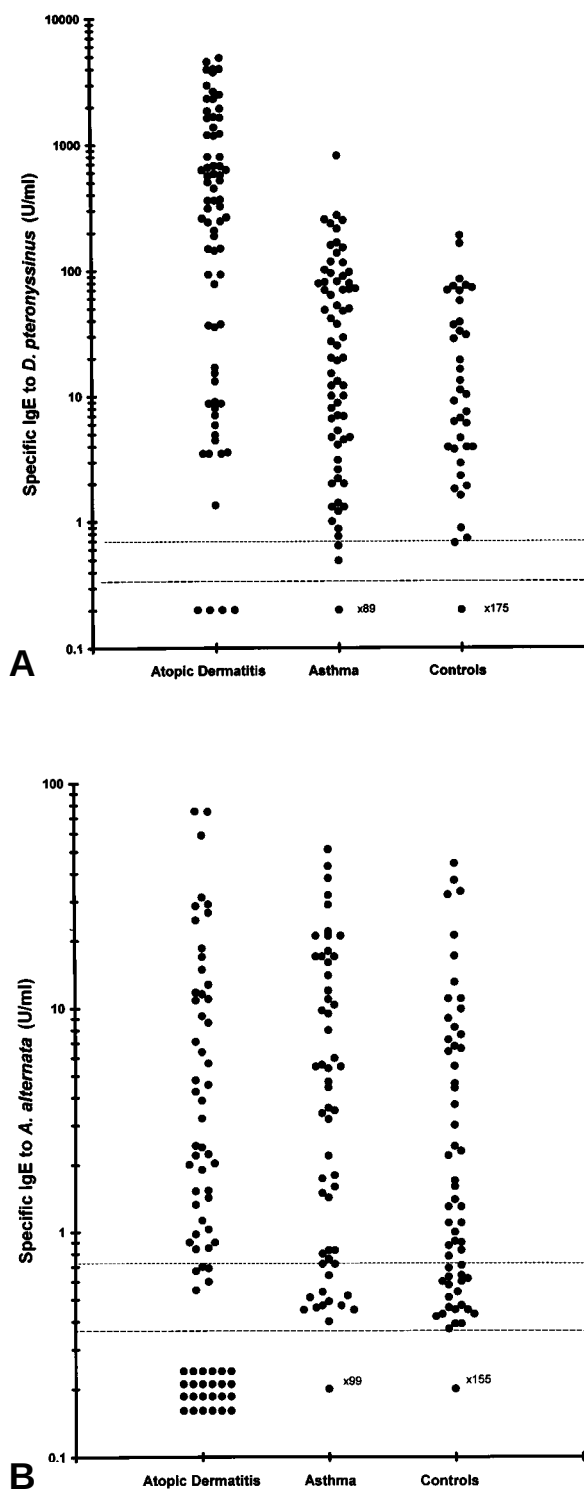


FIG 2. CONT.

els, dust mite has been shown to be the dominant indoor allergen associated with symptoms of AD. Detailed studies have documented serum antibodies, in vitro T-cell responses, and in vivo delayed patch test responses to purified mite allergens. Furthermore, the clinical improvement after dust mite avoidance measures can be dramatic.²⁴⁻²⁶ There is similar evidence relating to other indoor aeroallergens, particularly those derived from the domestic cat.

Organisms growing on the skin are recognized as contributing to exacerbations of AD. There is good evidence that patients become sensitized to proteins secreted by *Staphylococcus aureus*.⁷ Fungi growing on the skin could also contribute to disease expression.^{10,11} Indeed, we have recently reported 5 severe cases in which addition of oral itraconazole to the treatment regimen produced marked clinical improvement.¹⁶ Subsequent experience in our clinic has supported the view that fungi, yeast, or both on the skin can contribute to AD. Previous studies from Europe had incriminated the lipophilic yeast *M. furfur* and suggested that it might be specifically relevant to head and neck dermatitis.^{9,15,27}

The present study evaluated IgE antibodies to potential skin-colonizing organisms, including *Malassezia*, *Candida*, and *Trichophyton* species, and compared them with antibodies to fungi that are generally referred to as sources of inhalant allergens (eg, *Alternaria* and *Aspergillus* species) and dust mite. The first conclusion of the study was that IgE antibodies to all fungi tested are

FIG 2. Titers of IgE to specific allergens in sera from patients with AD, asthmatic subjects, and nonasthmatic control subjects. The dots or numbers below the lower dashed line indicate the number of sera that were less than the limit of detection of the assay, and those above the upper dashed line are those that were considered positive for statistical analysis. **A**, IgE antibody to *D. pteronyssinus*; **B**, IgE antibody to *A. alternata*; **C**, IgE antibody to *M. furfur*.

common among patients with AD, and in many cases the absolute levels are very high (ie, ≥ 20 ng of IgE antibody/mL; CAP class 4). Furthermore, IgE antibodies to *A alternata* and *M furfur* were significantly more common among patients with AD than among patients with asthma or asymptomatic control subjects. However, most impressive was the presence of significant concentrations of IgE antibodies to dust mite allergens in almost all of our patients with moderate or severe disease. The concentrations of IgE antibodies to mite allergens in sera from patients with AD were far higher than those found in sera from patients with asthma and higher than IgE antibodies to any of the fungi in the AD group as well. Although antibodies to mite constitute a similar proportion of the total serum IgE in patients with AD, asthmatic subjects, or control subjects, this finding is striking given how high the total IgE values were in the AD group. Taken together, the serology shows that patients with AD react consistently to dust mites and supports the view that mite exposure is a major cause of AD.

The data for fungi are different from those for dust mites and cannot be interpreted so simply. The evidence for a role for *M furfur* is based on the prevalence of positive antibodies because sensitization is rare among patients with asthma or control subjects. The odds ratios were very high for the association between sensitization to this yeast and AD. The higher IgE antibody titers to *M furfur* among adults compared with children with AD may reflect a cumulative exposure. However, colonization with *M furfur* is common among normal individuals and is not reported to be more common for patients with AD.^{14,27,28}

Elevations of IgE antibodies to *Alternaria* species have been associated with asthma, rhinitis, or both.²⁹ The hypothesis of a pathogenic role for *Alternaria* species in patients with AD was proposed in the early 1950s, with the demonstration of flare-ups of the disease after inhalation challenge with *Alternaria* species.^{24,30} It is not unusual for patients to report exacerbations on exposure to indoor mold, but the lack of an accepted and consistent means of measuring indoor exposure make it difficult to investigate those cases.³¹ In this report, although specific IgE to *A alternata* was higher than in the asthma or control groups, the quantity was not as impressive as with IgE specific for *D pteronyssinus* nor were the difference in prevalence as striking as with *M furfur*. However, the presence of specific IgE to *A alternata* is not merely a reflection of coexistent respiratory allergy because several of the sera with high levels of specific IgE to the fungus were from patients with AD without a history of asthma or allergic rhinitis. In our data there were correlations between IgE antibodies to several of the fungi that were higher than the correlation to total IgE. This could suggest either cross-reactivity between different fungi or environmental conditions that predispose to exposure to several of the fungi.

The evidence reported here adds further strength to the argument that common environmental allergens, particularly those derived from dust mites play an important role

in the disease. Three major lines of evidence support a causal relationship between exposure to dust mite antigens and the eczematous skin rash. First, there is a strong and consistent association between sensitization to mite allergen and AD. Second, improvement of the disease has been demonstrated with specific mite allergen avoidance. Finally, the application of purified mite allergen to the skin can induce a local eczematous response in sensitized individuals. Our results demonstrating the virtual absence of IgE to *M furfur* among control sera but over 50% prevalence among adults with moderate-to-severe AD, when considered in the context of previous reports of substantial improvement after treatment with oral antifungal agents, supports the hypothesis that *M furfur*, and potentially other colonizing fungi, such as *Candida* and *Trichophyton* species, play a role as exacerbating agents in patients with AD. The ability to measure specific IgE in the sera of patients with AD can be of considerable value when the epidemiology of sensitization is known. It is frequently impossible to measure exposure to allergens, yet where avoidance measures or treatment regimens exist, knowledge of sensitization can be helpful. The present results are based on in vitro measurements that avoid established problems of skin testing patients with AD. The serologic quantitation of IgE antibody to fungi could prove a valuable diagnostic tool assisting in tailoring an appropriate therapeutic approach to each patient. Patients with AD who are poorly responsive to routine treatment should be evaluated for IgE antibody to both dust mites and *M furfur*. Although it seems logical to extend this recommendation to other inhalant allergens and commensal organisms, this remains to be established.

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