

Original article

The relevance of microbial allergens to the IgE antibody repertoire in atopic and nonatopic eczema

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Background: A propensity to microbial skin infections has been reported in atopic ("high IgE") and nonatopic ("low IgE") forms of eczema. However, the relationship between antimicrobial IgE antibodies and nonatopic disease is unclear.

Objective: We examined the relevance of microbial allergens to the allergen-specific IgE antibody repertoire in patients with atopic dermatitis.

Methods: Patients with IgE levels of less than 150 IU/mL were stratified according to sensitivity (n = 22) or no sensitivity (n = 27) to 11 common food allergens and aeroallergens. The prevalence and titers of antimicrobial IgE antibodies were compared with those of patients (n = 36) with increased total IgE levels (>150 IU/mL). Skin-derived serum chemokines were also analyzed.

Results: Patients with low IgE levels showed decreased disease severity, increased age of onset, a striking female predominance, and a distinct distribution of skin lesions. High titer IgE antibodies (sum of 8 bacterial and fungal allergens = 29.8 ± 32.6 IU/mL) and multisensitization specific for microbial allergens was characteristic of patients with high IgE levels, with an overall 84% positivity; however, antimicrobial IgE antibodies comprised 3% or less of allergen-specific IgE antibodies. By contrast, antimicrobial IgE antibodies were detected in only 20% of patients with low IgE, and titers were negligible, irrespective of sensitization to common allergens. These patients were monosensitized, and exclusive microbial sensitivity was uncommon (10%). Patients with low IgE with no sensitivity to common allergens had lower levels of serum macrophage inflammatory protein 3 α compared with their sensitized counterparts.

Conclusion: Antimicrobial IgE antibodies are uncommon in patients with atopic dermatitis with low IgE levels.

Clinical implications: Hypersensitivity to microbial allergens is an unlikely trigger for eczematous eruptions in patients with low IgE levels. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Atopic dermatitis, bacterial allergens, fungal allergens, IgE antibodies, atopy, chemokines, thymus and activation-regulated chemokine, macrophage inflammatory protein 3 α , inhalant allergens, food allergens

The causative role of allergens in atopic dermatitis (AD), more commonly known as eczema, continues to be disputed. Elicitation of eczematous lesions by means of application of allergen to the skin in atopy patch tests and improvement of skin condition after allergen avoidance might be considered as evidence for causality.¹⁻⁴ The relationship between disease severity and levels of total IgE and IgE antibodies directed against environmental allergens further supports this view. However, a significant proportion of eczema (16% to 45% in European studies) is not associated with increased IgE levels.^{5,6} Eczema has been classified into atopic and nonatopic forms based on varying criteria. For example, although some studies have defined nonatopic eczema (NAE) according to low total IgE levels (<150 kU/L), others base this classification on a lack of sensitization to inhalant or food allergens.⁵⁻⁸

In some studies the clinical features of atopic eczema (AE) and NAE have been essentially the same, although a female predominance and later age of onset have been reported for the nonatopic form.⁹⁻¹¹ At the cellular level, differences have been described in the 2 forms of eczema based on expression of cytokines and surface antigens relevant to immune cell activation. Not surprisingly, increased expression of the high-affinity IgE receptor Fc ϵ RI is a feature of antigen-presenting cells derived from patients with AD with increased total IgE levels, as well as from patients with other atopic disorders.¹¹⁻¹⁴ However, T_H2 cytokines, which are pivotal to IgE-mediated disease (IL-4, IL-5, and IL-13), are not consistently increased in the blood of patients with the extrinsic, or atopic, form of eczema compared with the nonatopic form.¹⁴

The skin of all patients with AD is susceptible to colonization and infection with a variety of bacterial and fungal organisms, and it has been postulated that IgE antibodies directed against microbial products contribute to the pathogenesis of both forms of AD. Accordingly, IgE

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Abbreviations used

AD: Atopic dermatitis
 AE: Atopic eczema
 NAE: Nonatopic eczema
 SE: Staphylococcal enterotoxin
 TARC: Thymus and activation-regulated chemokine
 TSST: Toxic shock syndrome toxin

antibodies to microbial components have been detected in 50% or more of patients with low total IgE levels and no measurable IgE antibodies to common allergen sources.^{15,16} Moreover, staphylococcal enterotoxins (SEs), which are often produced by strains of *Staphylococcus aureus* isolated from the skin of patients with AD, have been reported to alter the activity of regulatory T cells in patients with AD.^{17,18}

The present study was designed to assess the relevance of microbial allergens to AD by analyzing IgE antibodies specific for a broad panel of bacterial and fungal allergens in the context of total IgE levels and sensitization to common allergens.

METHODS

Subjects

Patients with AD were those presenting to the University of Virginia Dermatology Clinic or Allergy Clinic for routine evaluation and treatment. All patients reported physician-diagnosed AD and lacked a clinical history consistent with contact dermatitis. The presence of itchy rash was confirmed on physical examination. Age of onset, history of skin distribution, and history of other atopic disease was determined by using a questionnaire. Severity of disease was assessed by using the SCORAD index, which combines objective (extent and intensity of lesions) and subjective (daytime pruritus and sleep loss) criteria.^{19,20} Sera were obtained from 85 adult patients with AD and from 15 healthy control subjects without AD selected on the basis of total IgE levels of less than 150 IU/mL. The study was performed according to the Declaration of Helsinki protocols. Studies on human subjects were approved by the University of Virginia Human Investigation Committee. All subjects provided written informed consent.

Serum antibody and chemokine assays

Serum total IgE levels and IgE antibodies to allergen extracts were measured by using the Pharmacia CAP assay (Uppsala, Sweden). The panel of allergens tested comprised dust mite (*Dermatophagoides farinae*); cat dander; dog epithelium; German cockroach (*Blattella germanica*); ryegrass (*Lolium perenne*); timothy grass (*Phleum pratense*); ragweed (*Ambrosia psilostachya*); common silver birch (*Betula verrucosa*); wheat (*Triticum aestivum*); egg white; milk; SEA, SEB, SEC, and SED; staphylococcal toxic shock syndrome toxin (TSST); and the fungi *Candida albicans*, *Malassezia* species, and *Trichophyton rubrum*. Serum levels of thymus and activation-regulated chemokine (TARC) and macrophage inflammatory protein (MIP) 3 α were measured by means of ELISA (R&D Systems, Minneapolis, Minn).

Statistical analysis

The Spearman test was used to assess the quantitative correlations between variables. The between-group prevalence for different

variables was compared by using the χ^2 test and the Fisher exact test as appropriate. The Mann-Whitney test was used to compare serum antibody and chemokine levels. SCORAD score and age of onset of disease were analyzed by using the Student *t* test. A *P* value of less than .05 was considered significant.

RESULTS

Classification of patients and clinical features of AE and NAE

Thirty-six of the 85 patients with AD studied had increased total IgE levels (>150 IU/mL) and were classified as the AE >150 group. Of the 49 patients with low total IgE levels (<150 IU/mL), 22 (45%) had detectable IgE antibodies directed against at least one of 11 common inhalant and food allergens (dust mite, cat, dog, cockroach, ryegrass, timothy grass, ragweed, birch, wheat, egg white, and milk). These subjects comprised the AE <150 group. The remaining 27 patients had no sensitivities to common inhalant or food allergens and were classified as the NAE <150 group. Within the NAE group, there was a strong female predominance and the lowest family history of atopy or personal history of wheeze. Eczema in both groups of patients with low total IgE levels was associated with decreased SCORAD scores and increased age of onset (Table I). Total IgE levels were strongly correlated with SCORAD scores in the AE >150 group ($r = 0.6$, $P < .001$) but not in the other 2 groups ($r < 0.3$, $P > .2$; Fig 1). A higher prevalence of lesions affecting only the hands was observed among the 2 groups with low total IgE levels, whereas lesions affecting the head and neck were least common within the AE <150 group.

Allergen-specific IgE antibody repertoire is restricted in atopic patients with low total IgE levels

Analysis of specific IgE antibodies directed against a broad panel of common inhalant and food allergens showed a high frequency of positive results and high titers among the positive results for patients with increased total IgE levels ($n = 36$, Fig 2). Both the prevalence and titers were significantly decreased for all allergens tested among patients with low total IgE levels ($n = 49$, $P < .001$). Comparison of the pattern of sensitization among patients of the AE >150 and AE <150 groups showed that dust mite was the dominant allergen for both groups, with a prevalence of 88% and 64%, respectively (Fig 3, A). Although IgE antibodies to egg or milk were present in greater than 30% of patients in the AE >150 group, these antibodies were not detected in the AE <150 group. IgE antibodies specific for 3 microbial allergens included as part of an initial screen (*C albicans*, SEA, and SEB) were measurable at high titers (up to 65 IU/mL) in 50% or more of patients with increased total IgE levels (Fig 2, A); however, these antibodies constituted a minor proportion of allergen-specific IgE antibodies ($\leq 3\%$) in these patients (Fig 3, B). By contrast, only a single patient with low total IgE levels (89 IU/mL) had IgE antibodies to *C albicans* (Fig 2, B), and no anti-SEA or anti-SEB IgE

TABLE I. Demographic and clinical characteristics of patients with AD

Group	Mean age (y) and sex	Total IgE level (IU/mL)*	SCORAD*	Onset (y)*	Distribution				Family history	Wheeze
					Head/ neck	Trunk	Limbs	Hands only		
Atopic eczema, total IgE >150 IU/mL (AE >150 group), n = 36	34 ± 12 25 F/11 M	4060 ± 5485 (159-21,266)	35 ± 16 (6-60)	6 ± 9 (1-34)	19/36 (53%)	11/36 (31%)	29/36 (81%)	0/36¶	19/25#	19/34 (56%)
Atopic eczema, total IgE <150 IU/mL (AE <150 group), n = 22	29 ± 8.7 15 F/7 M	65 ± 38 (14-143)	24 ± 13‡	11 ± 11‡	4/22	5/22 (23%)	17/22 (77%)	3/22 (14%)	13/21 (62%)	10/22 (45%)
Nonatopic eczema, total IgE <150 IU/mL (NAE <150 group), n = 27	33 ± 10 24 F/3 M†	37 ± 36 (2-41)	22 ± 9§	18 ± 14§	13/27 (48%)	4/27 (15%)	20/27 (74%)	3/27 (11%)	11/24 (46%)	10/26 (38%)

F, Female; M, male.

*Values represent the means ± SD, with ranges in parentheses.

†P < .001 compared with the AE <150 and AE >150 groups.

‡P = .01 (SCORAD) and P < .05 (onset) compared with the AE >150 group.

§P < .001 compared with the AE >150 group.

||P ≤ .05 compared with the other 2 groups.

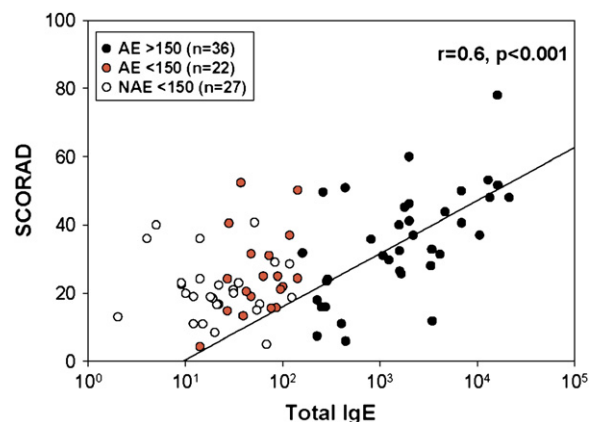
¶P = .05 compared with the AE <150 group.

#P = .042 compared with the NAE <150 group.

antibodies were measurable in any of these patients, including the AE <150 group (Figs 2, B, and 3, B).

IgE antibodies to bacterial products and fungi are uncommon in patients with AD with low total IgE levels

The relationship between IgE antibodies to microbial products and atopic status was further examined. Positive results for anti-SEA and anti-SEB IgE antibodies (>0.75 IU/mL, CAP class II) were found only in patients with total IgE levels of 800 IU/mL or greater. IgE antibodies to both allergens were present in 82% (14/17) of patients with IgE levels of greater than 2000 IU/mL. Total IgE levels were strongly correlated with anti-SEA and anti-SEB IgE antibodies in the AE >150 group ($r > 0.64$, $P < .001$; Fig 4, A). IgE antibodies to additional staphylococcal toxins and fungal allergens were measured in 65 sera from all patient groups to determine whether patients with low total IgE levels had mounted IgE antibody responses against other microbial products. IgE antibodies against at least one bacterial product (SEC, SED, or TSST) were observed in most of the AD >150 group (18/25 [72%]). Furthermore, 92% (11/12) of patients with total IgE levels of greater than 2000 IU/mL were sensitized to all 3 bacterial allergens. Thus multisensitization to staphylococcal products was associated with high IgE levels. By contrast, IgE antibodies against SEC, SED, or TSST were detected in only 4.5% (1/22) and 11.1% (2/18) of patients in the AE <150 and NAE <150 groups, respectively (Fig 4, B). A high prevalence of IgE antibodies against *Malassezia* species or *T rubrum* was observed in the AE >150 group (18/25 [72%]), with titers of up to 65 IU/mL. By contrast, only 13.6% (3/22) of patients in the AE <150 group and 11.1% (2/18) of patients in the NAE <150 group had detectable IgE antibodies to *Malassezia* species, *T rubrum*, or both (Fig 4, C). Monosensitization to bacterial or fungal

**FIG 1.** Correlation between total IgE levels in the sera of patients with AD and disease severity. Disease severity was assessed by using the SCORAD index.

allergens was characteristic of patients with low total IgE levels who were sensitized to any microbial source (6/8 subjects). No antimicrobial IgE antibodies were detected in patients with total IgE levels of 35 IU/mL or less ($n = 17$), regardless of sensitivity to other allergens. The overall prevalence of IgE antibodies against any microbial component was 84% for the AE >150 group, 18% for the AE <150 group, and 22% for the NAE <150 group. Among those patients with measurable titers directed against any microbial source, antimicrobial IgE antibodies constituted a similar fraction of the total IgE levels for the AE >150, AE <150, and NAE <150 groups ($1.1\% \pm 1.1\%$ [$n = 20$], $2.8\% \pm 2.4\%$ [$n = 4$], and $1.3\% \pm 1.1\%$ [$n = 4$], respectively; $P > .2$). However, when levels were analyzed for all patients tested for each of the 8 microbial allergens, the sum of IgE antibodies to all microbial components was negligible for the NAE <150 and

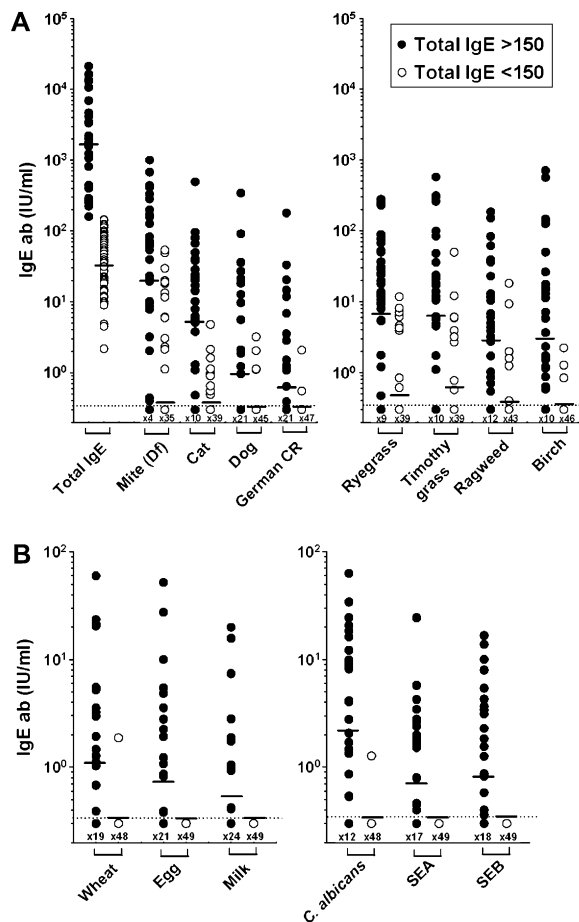


FIG 2. Prevalence and levels of allergen-specific IgE antibodies (*ab*) in the sera of patients with AD with high and low total IgE levels. Antibodies were measured by using the CAP assay in patients with AD with total IgE levels of greater than 150 IU/mL ($n = 36$) and patients with total IgE levels of less than 150 IU/mL ($n = 49$). **A**, IgE antibodies specific for inhalant allergens. **B**, IgE antibodies specific for food and microbial allergens. Two sera with total IgE levels of greater than 150 IU/mL were not available for measurement of dust mite-specific IgE antibodies. Horizontal bars represent geometric means, and dashed lines represent the limit of sensitivity of the assay. *Df*, *D farinae*.

AE <150 groups (0.4 ± 0.2 IU/mL and 0.7 ± 1.1 IU/mL, respectively) compared with the AE >150 group (29.8 ± 32.6 IU/mL, Fig 5).

Serum MIP-3 α levels distinguish atopic and nonatopic patients with low total IgE levels

Because analysis of IgE antibody responses to microbial components did not distinguish atopic from nonatopic patients with low total IgE levels, we analyzed alternate serum markers that might delineate these 2 groups. Altered expression of the chemokines TARC and MIP-3 α has been reported in the skin lesions and sera of patients with AD.^{21,22} In patients with AD, the severity of disease as judged by SCORAD score was significantly correlated with serum levels of TARC ($r = 0.42$, $P < .001$, $n = 72$) but not MIP-3 α ($r = 0.19$, $P = .14$, $n = 62$).

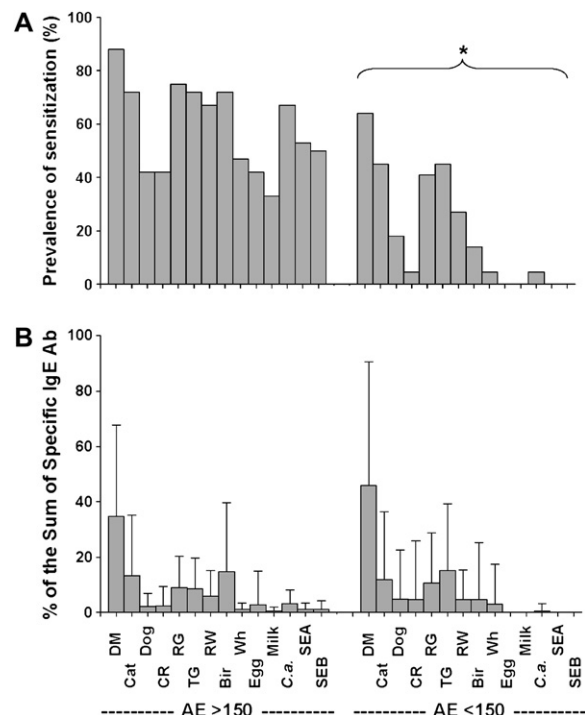


FIG 3. Pattern of sensitization to allergens in atopic patients with AD. Sera were analyzed from the AE >150 ($n = 36$) and AE <150 ($n = 22$) groups. **A**, Comparison of the prevalence of sensitization to all allergens ($*P < .03$ for all allergens). **B**, Contribution of each allergen to the allergen-specific IgE antibody repertoire expressed as the percentage of the sum of specific IgE antibodies (*Ab*) for all 14 allergens tested. *DM*, Dust mite; *CR*, cockroach; *RG*, ryegrass; *TG*, timothy grass; *RW*, ragweed; *Bir*, birch; *Wh*, wheat; *C.a.*, *C. albicans*; *SEB*, *Staphylococcus epidermidis*.

However, levels of both chemokines were highest in the AE >150 group. Surprisingly, levels of MIP-3 α , but not TARC, were significantly reduced in the NAE <150 group compared with the AE <150 group and a control group comprising subjects without AD with low total IgE levels (<150 IU/mL, Fig 6). Thus low serum MIP-3 α levels delineated patients with NAE from patients with AE with low total IgE levels.

DISCUSSION

Comprehensive analysis of the allergen-specific IgE antibody repertoire reveals that patients with AD who have low total IgE levels (<150 IU/mL) in their sera comprise a heterogeneous group. Although 45% of these patients had measurable IgE antibodies to common inhalant or food allergens, 55% did not. Classification of subjects with low IgE levels based on sensitization status identified clinical differences. For example, although a higher prevalence of lesions affecting only the hands was observed in both groups with low total IgE levels, head and neck lesions were infrequent only in those patients with low IgE levels sensitized to common allergens. Head and neck dermatitis has been associated with colonization by, and sensitization to, *Malassezia* species.^{23,24} Although

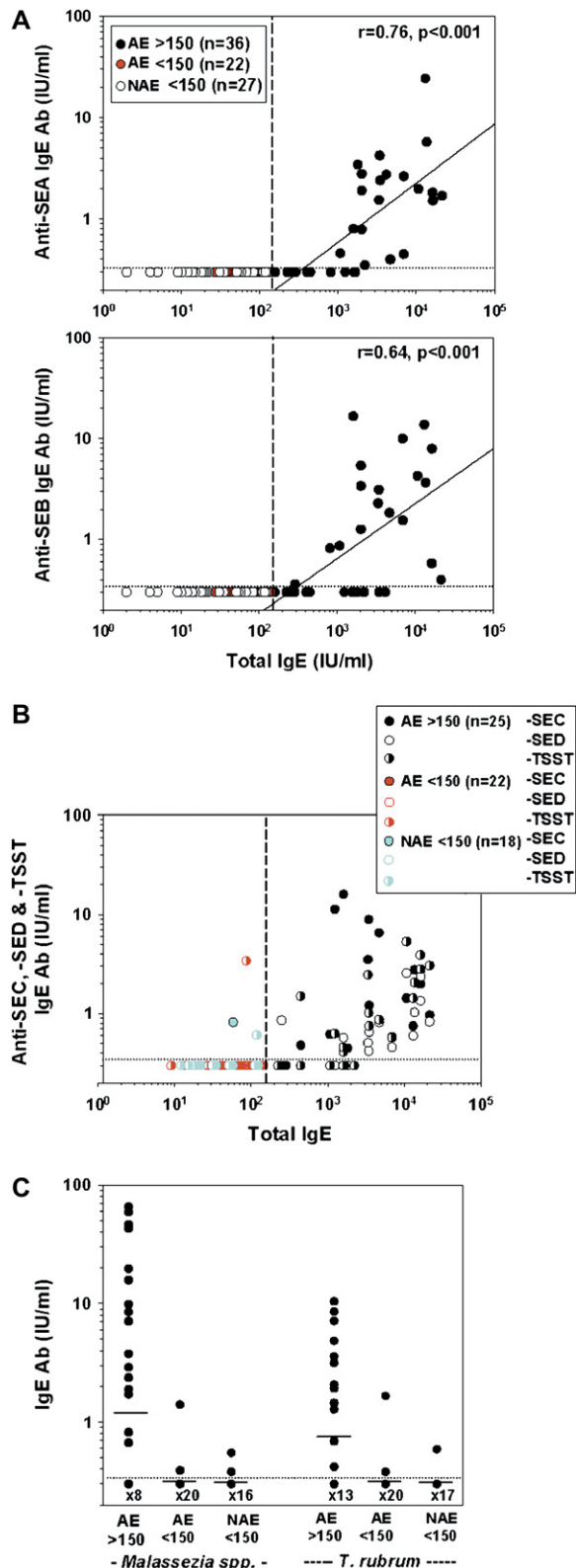


FIG 4. IgE antibodies (Ab) to bacterial and fungal allergens. **A**, Correlation between levels of total IgE and IgE antibodies to SEA and SEB for each of the 3 patient groups. **B**, Relationship between levels of total IgE and IgE antibodies to SEC (solid circles), SED

the prevalence of sensitization to *Malassezia* species was equally low among both groups with low IgE levels, we did not test for skin colonization, which might account for these differences. Although a decreased family history of atopy and a personal history of wheeze was a feature of both groups with low total IgE levels, a striking female predominance (8:1 ratio) was unique to patients who lacked sensitization to common allergens. Collectively, these differences raise important questions regarding the clinical nature of the disease in patients with low total IgE levels. Subjects were recruited to our study based on physician-diagnosed eczema and the lack of a clinical history consistent with contact dermatitis to avoid excluding patients with low IgE levels who had AD. By using these criteria, the majority of subjects with low total IgE levels (88%) also met the criteria of Hanifin and Rajka,²⁵ which are used to distinguish AD from other eczematous skin eruptions. All 6 subjects who did not meet these criteria had low total IgE levels and no sensitivities to common allergens; however, only 2 of these 6 individuals displayed skin lesions restricted to their hands. Thus inadvertent inclusion of patients with contact dermatitis was not a caveat of our study.

A large body of literature supports a relationship between total IgE levels and disease severity in patients with AD. Moreover, a link between severe refractory AD and atopy has recently been reported.²⁶ In our study the severity of disease was increased in patients with AD with high IgE levels, and in contrast to patients with low IgE levels, disease severity correlated with total IgE titers. Such observations support the view that allergenic triggers contribute to the development or persistence of severe disease.

In keeping with previous studies by our group,²⁷ the allergen-specific IgE antibody repertoire was dominated by dust mite, irrespective of IgE levels in the serum. Moreover, IgE antibodies to animal dander and pollens were relatively common in all patients with AD. By contrast, the prevalence and titers of IgE antibodies to food and microbial allergens were high in patients with increased total IgE levels compared with those in patients who had low IgE levels. Differential sensitization to food allergen was not explained solely by early age of disease onset because none of the 7 patients with early-onset disease (<1 year of age) in the AE <150 group had IgE antibodies to egg. One interpretation of the shift in allergen hierarchy in relation to total IgE levels is that in an adult population food and microbial allergens are relatively weak inducers of IgE antibody production. Consistent with this, IgE antibodies specific for these allergens comprised a minor proportion of allergen-specific IgE antibodies (<5%) among patients with AD with high IgE levels despite a high prevalence of sensitization (>38%).

(open circles), and TSST (half-solid circles) for the AE >150 (black), AE <150 (red), and NAE <150 (blue) groups. **C**, Levels of IgE antibodies to *Malassezia* species and *T. rubrum* for serum samples shown in Fig 4, B. Horizontal bars represent geometric means, and dashed lines represent the limit of sensitivity of the assay.

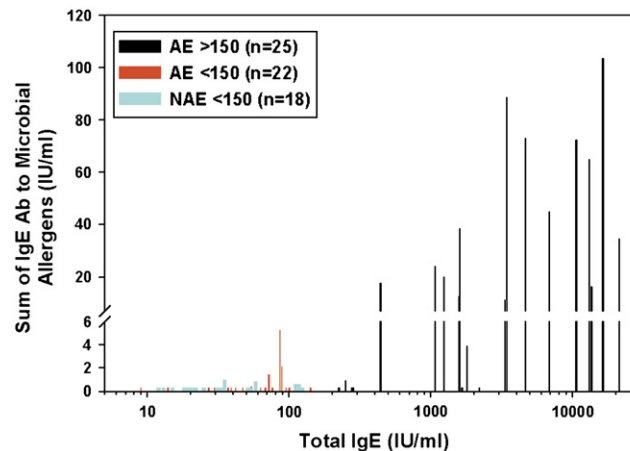


FIG 5. Relationship between the sum of IgE antibodies (Ab) to all microbial allergens and total IgE levels. Each bar represents the sum of IgE antibodies directed against *C albicans*, *Malassezia* species, *T rubrum*, SEA, SEB, SEC, SED, and TSST for a single patient.

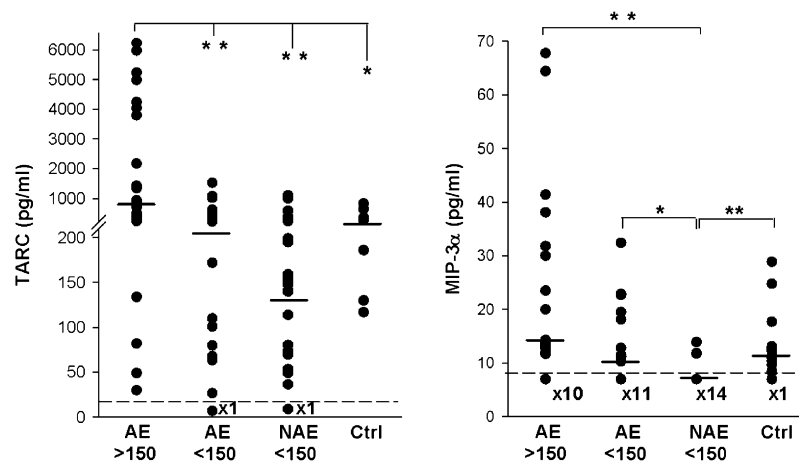


FIG 6. Serum levels of TARC and MIP-3α in patients with AD. Serum TARC levels were measured in 31 patients in the AE >150 group, 21 patients in the AE <150 group, 22 patients in the NAE <150 group, and 7 control subjects (Ctrl). Serum MIP-3α levels were measured in 25 patients in the AE >150 group, 21 patients in the AE <150 group, 16 patients in the NAE <150 group, and 15 control subjects. Horizontal bars represent geometric means, and dashed lines represent the limit of sensitivity of the assay. * $P < .05$, ** $P < .005$.

Obviously this is based on the assumption that units for antimicrobial IgE antibodies are equivalent to those for other allergens.

We analyzed IgE antibodies specific for a variety of skin-colonizing microbes, including fungi (*C albicans*, *Malassezia* species, and *T rubrum*), as well as staphylococcal products (SEA, SEB, SEC, SED, and TSST) produced by bacteria isolated from the skin of patients with AD.²⁸⁻³¹ Antimicrobial IgE antibodies were readily detected at high titers in the majority of patients with increased total IgE levels but were uncommon in patients with low total IgE levels. This contrasts with a European study that reported detection of IgE antibodies against the same microbial components in greater than 50% of patients with AD with total IgE levels of less than 150 kU/L.¹⁵ In another study IgE antibodies to *Malassezia sympodialis*, which is the most frequent species of *Malassezia* isolated

from the skin of patients with AD, were detected in 54% of total patients with AD and in 50% of patients with “intrinsic” AD with low total IgE levels.¹⁶ Neither study directly compared antimicrobial IgE antibody titers between patients with high and low total IgE levels. Here we report IgE antibodies specific for *Malassezia* species in 68% of patients with AD with increased total IgE levels, a prevalence comparable with that observed for *C albicans* (67%). However, antifungal IgE antibodies were measured in 11% or less of patients with AD with low IgE levels, regardless of sensitization to other allergens. In the present study we stratified patients with low IgE levels into 2 groups based on the presence or absence of sensitization to common allergens. Interestingly, we observed no difference in the prevalence of sensitization to microbial allergens between these 2 groups, despite a modest increase in IgE titers among those with sensitivity to common

allergens. Moreover, exclusive sensitization to microbial allergens was uncommon among nonatopic patients with low IgE levels (22%), representing only 10% of all patients with AD with low IgE levels. Collectively, our findings suggest that among patients with AD with low total IgE levels, antimicrobial IgE antibodies are uncommon and independent of sensitization to “extrinsic” allergens.

As shown previously by our group for other allergens, the antimicrobial IgE antibody levels were significantly correlated with total IgE levels in patients with AD.²⁷ For the AD group with high total IgE levels, strong correlations ($r \geq 0.4$, $P < .03$) were observed for most inhalant and food allergens (mite, cat, dog, timothy grass, ragweed, birch, and egg) but not all (exceptions were cockroach, rye grass, wheat, and milk; data not shown). One interpretation of the correlation between total IgE levels and allergen-specific IgE antibodies is that the CAP assay is not specific. Aside from the lack of consistency in this relationship for individual allergens, we observed an inverse correlation between the sum of allergen-specific IgE antibodies and total IgE titers ($r = -0.36$, $P = .04$) among patients with AD with increased total IgE levels (data not shown). Indeed, allergen-specific IgE antibodies accounted for less than 20% of total IgE in patients with AD with total IgE levels of greater than 4000 IU/mL, whereas antimicrobial IgE antibodies accounted for approximately 1%. These observations argue against nonspecific binding of allergen-specific IgE antibodies to the solid phase in the CAP assay.

Among patients with AD with high IgE levels, a key question is whether increased total IgE levels is a consequence of sensitivity to allergens or whether increased IgE production predisposes to atopy. It has been proposed that independent mechanisms determine the likelihood of becoming sensitized to allergens and that enhanced production of IgE is not associated with the propensity for sensitization.³² However, it is difficult to reconcile this stance with our observations in patients with AD, which show a close relationship between total IgE levels and allergen-specific IgE antibodies. There are numerous reports in the literature purporting to identify “atopy genes” that could explain a predisposition to production of IgE.³³ Such studies have often focused on candidate genes encoding T cell–derived cytokines that might enhance or suppress IgE synthesis. However, analysis of atopic and nonatopic patients with AD has yielded differences in T_H2 cytokine gene polymorphisms and cytokine expression that cannot simply be explained by a global increase in T_H2 cytokines in the atopic form of the disease.¹⁴

We and others have reported an increased frequency of circulating $CD25^+CD4^+$ T cells in patients with AD with increased IgE levels.^{17,34} These cells exhibit phenotypic properties consistent with regulatory T cells on the basis of high expression of the chemokine receptors CCR4 and CCR6.^{34,35} Rather than measuring T cell–derived cytokines, we analyzed serum chemokines produced in the skin that might recruit regulatory T cells to sites of allergic inflammation. Surprisingly, we observed that serum levels of the chemokine ligand for CCR6 (MIP-3 α /CCL20), but

not the ligand for CCR4 (TARC/CCL17), were significantly decreased in patients with nonatopic eczema with low IgE levels compared with their atopic counterparts. Thus although low serum levels of both TARC and MIP-3 α were characteristic of nonatopic patients with low total IgE levels, only MIP-3 α was a distinguishing marker for this group. In keeping with previous reports, serum levels of TARC were significantly correlated with disease severity.^{21,36} This is consistent with increased TARC expression in lesional skin of patients with AD.^{21,37} By contrast, serum levels of MIP-3 α were not correlated with disease severity. Consistent with this, MIP-3 α levels have been reported to be only minimally increased²² or else unaltered^{38,39} in the lesional skin of patients with AD compared with in healthy skin.

In summary, our findings show that IgE antibodies directed against microbial allergens are common in patients with AD with increased total IgE levels, and the titer of these antibodies can be high. By contrast, we provide evidence of a limited contribution of antimicrobial IgE antibodies in patients with AD with low IgE levels. Although it is possible that other allergen sources remain to be identified in nonatopic patients, the nature of the relationship between total and specific IgE antibodies suggests that this is unlikely. Deficiencies in the innate immune response to skin-colonizing organisms have been described in patients with AD with atopic and nonatopic disease.^{40,41} Our findings do not exclude an important role for such organisms in the pathogenesis of AD. However, they strongly suggest that microbial antigens do not trigger hypersensitivity reactions in patients with AD with low total IgE levels.

REFERENCES

1. Ring J, Darsow U, Behrendt H. Role of aeroallergens in atopic eczema: proof of concept with the atopy patch test. *J Am Acad Dermatol* 2001; 45(suppl):S49-52.
2. Tan BB, Weald D, Strickland I, Friedmann PS. Double-blind controlled trial of effect of housedust-mite allergen avoidance on atopic dermatitis. *Lancet* 1996;347:15-8.
3. Platts-Mills TA, Mitchell EB, Rowntree S, Chapman MD, Wilkins SR. The role of dust mite allergens in atopic dermatitis. *Clin Exp Dermatol* 1983;8:233-47.
4. Ricci G, Patrizi A, Specchia F, Menna L, Bottau P, D'Angelo V, et al. Effect of house dust mite avoidance measures in children with atopic dermatitis. *Br J Dermatol* 2000;143:379-84.
5. Schmid-Grendelmeier P, Simon D, Simon H-U, Akdis CA, Wuthrich B. Epidemiology, clinical features, and immunology of the “intrinsic” (non-IgE-mediated) type of atopic dermatitis (constitutional dermatitis). *Allergy* 2001;56:841-9.
6. Novak N, Bieber T. Allergic and nonallergic forms of atopic diseases. *J Allergy Clin Immunol* 2003;112:252-62.
7. Schafer T, Heinrich J, Wjst M, Adam H, Ring J, Wichmann HE. Association between severity of atopic eczema and degree of sensitization to aeroallergens in schoolchildren. *J Allergy Clin Immunol* 1999;104:1280-4.
8. Schafer T, Kramer U, Vieluf D, Abeck D, Behrendt H, Ring J. The excess of atopic eczema in East Germany is related to the intrinsic type. *Br J Dermatol* 2000;143:992-8.
9. Kagi MK, Wuthrich B, Montano E, Barandun J, Blaser K, Walker C. Differential cytokine profiles in peripheral blood lymphocyte supernatants and skin biopsies from patients with different forms of atopic dermatitis, psoriasis, and normal individuals. *Int Arch Allergy Immunol* 1994;103:332-40.

10. Fabrizi G, Romano A, Vultaggio P, Bellegrandi S, Paganelli R, Venuti A. Heterogeneity of atopic dermatitis defined by the immune response to inhalant and food allergens. *Eur J Dermatol* 1999;9:380-4.
11. Oppel T, Schuller E, Gunther S, Moderer M, Haberstok J, Bieber T. Phenotyping of epidermal dendritic cells allows the differentiation between extrinsic and intrinsic forms of atopic dermatitis. *Br J Dermatol* 2000;143:1193-8.
12. Novak N, Teipel C, Koch S, Brix K, Bieber T, Kraft S. Evidence for a differential expression of the FcepsilonRI gamma chain in dendritic cells of atopic and nonatopic donors. *J Clin Invest* 2003;111:1047-56.
13. Novak N, Allam JP, Hagemann T, Jenneck C, Laffer S, Valenta R, et al. Characterization of FcepsilonRI-bearing CD123 blood dendritic cell antigen-2 plasmacytoid dendritic cells in atopic dermatitis. *J Allergy Clin Immunol* 2004;114:364-70.
14. Novak N, Kruse S, Kraft S, Gieger E, Klucken H, Fimmers R, et al. Dichotomic nature of atopic dermatitis reflected by combined analysis of monocyte immunophenotyping and single nucleotide polymorphisms of the interleukin-4/interleukin-13 receptor gene: the dichotomy of extrinsic and intrinsic atopic dermatitis. *J Invest Dermatol* 2002;119:870-5.
15. Novak N, Allam J-P, Bieber T. Allergic hyperreactivity to microbial components: a trigger factor of "intrinsic" atopic dermatitis? *J Allergy Clin Immunol* 2003;112:215-6.
16. Casagrande BF, Fluckiger S, Linder MT, Johansson C, Scheynius A, Cramer R, et al. Sensitization to the yeast *Malassezia sympodialis* is specific for extrinsic and intrinsic atopic eczema. *J Invest Dermatol* 2006;126:2414-21.
17. Ou LS, Goleva E, Hall C, Leung DY. T regulatory cells in atopic dermatitis and subversion of their activity by superantigens. *J Allergy Clin Immunol* 2004;113:756-63.
18. Cardona ID, Goleva E, Ou LS, Leung DY. Staphylococcal enterotoxin B inhibits regulatory T cells by inducing glucocorticoid-induced TNF receptor-related protein ligand on monocytes. *J Allergy Clin Immunol* 2006;117:688-95.
19. Stalder JF, Taieb A. Severity scoring of atopic dermatitis: the SCORAD index: consensus report of the European Task Force on atopic dermatitis. *Dermatology* 1993;186:23-31.
20. Kunz B, Oranje AP, Labreze L, Stalder J-F, Ring J, Taieb A. Clinical validation and guidelines for the SCORAD index: consensus report of the European Task Force on Atopic Dermatitis. *Dermatology* 1997;195:10-9.
21. Kakinuma T, Nakamura K, Wakugawa M, Mitsui H, Tada Y, Saeki H, et al. Thymus and activation-regulated chemokine in atopic dermatitis: serum thymus and activation-regulated chemokine level is closely related with disease activity. *J Allergy Clin Immunol* 2001;107:535-41.
22. Nakayama T, Fujisawa R, Yamada H, Horikawa T, Kawasaki H, Hieshima K, et al. Inducible expression of a CC chemokine liver- and activation-regulated chemokine (LARC)/macrophage inflammatory protein (MIP)-3 alpha/CCL20 by epidermal keratinocytes and its role in atopic dermatitis. *Int Immunol* 2001;13:95-103.
23. Kieffer M, Bergbrant I-M, Faergemann J, Jemec GB, Ottevanger V, Stahl Skov P, et al. Immune reactions to *Pityrosporum ovale* in adult patients with atopic and seborrheic dermatitis. *J Am Acad Dermatol* 1990;22:739-42.
24. Kim TY, Jang IG, Park YM, Kim HO, Kim CW. Head and neck dermatitis: the role of *Malassezia furfur*, topical steroid use and environmental factors in its causation. *Clin Exp Dermatol* 1999;24:226-31.
25. Hanifin JN, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockholm)* 1980;92(suppl):S44-7.
26. Salt BH, Boguniewicz M, Leung DY. Severe refractory atopic dermatitis in adults is highly atopic. *J Allergy Clin Immunol* 2007;119:508-9.
27. Scalabrin DM, Bavbek S, Perzanowski MS, Wilson BB, Platts-Mills TA, Wheatley LM. 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. *J Allergy Clin Immunol* 1999;104:1273-9.
28. Sandstrom Falk MH, Tengvall Linder M, Johansson C, Bartosik J, Back O, Sarnhult T, et al. The prevalence of *Malassezia* yeasts in patients with atopic dermatitis, seborrheic dermatitis, and healthy controls. *Acta Derm Venereol* 2005;85:17-23.
29. Breuer K, Haussler S, Kapp A, Werfel T. *Staphylococcus aureus*: colonizing features and influence of an antibacterial treatment in adults with atopic dermatitis. *Br J Dermatol* 2002;147:55-61.
30. Leung DYM, Harbeck R, Bina P, Reiser RF, Yang E, Norris DA, et al. Presence of IgE antibodies to staphylococcal exotoxins on the skin of patients with atopic dermatitis. Evidence for a new group of allergens. *J Clin Invest* 1993;92:1374-80.
31. McFadden JP, Noble WC, Camp RD. Superantigenic exotoxin-secreting potential of staphylococci isolated from atopic eczematous skin. *Br J Dermatol* 2000;128:631-2.
32. Jackola DR, Blumenthal MN, Rosenberg A. Evidence for two independent distributions of serum immunoglobulin E in atopic families: cognate and non-cognate IgE. *Hum Immunol* 2004;65:20-30.
33. Ober C, Hoffjan S. Asthma genetics 2006: the long and winding road to gene discovery. *Genes Immun* 2006;7:95-100.
34. Reefer AJ, Satinover SM, Nguyen J, Solga MD, Lannigan J, Wilson B, et al. Analysis of discrete regulatory T cell subsets in patients with atopic dermatitis reveals Th2-promoting properties [abstract]. *J Allergy Clin Immunol* 2007;119(suppl):S238.
35. Hirahara K, Liu L, Clark RA, Yamanaka K, Fuhlbrigge RC, Kupper TS. The majority of human peripheral blood CD4⁺CD25^{high}Foxp3⁺ regulatory T cells bear functional skin-homing receptors. *J Immunol* 2006;177:4488-94.
36. Jahn-Rozyk K, Targowski T, Paluchowska E, Owczarek W, Kucharczyk A. Serum thymus and activation-regulated chemokine, macrophage-derived chemokine and eotaxin as markers of severity of atopic dermatitis. *Allergy* 2005;60:685-8.
37. Zheng X, Nakamura K, Furukawa H, Nishibu A, Takahashi M, Tojo M, et al. Demonstration of TARC and CCR4 mRNA expression and distribution using in situ RT-PCR in the lesional skin of atopic dermatitis. *J Dermatol* 2003;30:26-32.
38. Keller M, Spanou Z, Schaeferli P, Britschgi M, Yawalkar N, Seitz M, et al. T cell-regulated neutrophilic inflammation in autoinflammatory diseases. *J Immunol* 2005;175:7678-86.
39. Kim BE, Leung DYM, Streib JE, Boguniewicz M, Hamid QA, Howell MD. Macrophage inflammatory protein 3α deficiency in atopic dermatitis skin and role in innate immune response to vaccinia virus. *J Allergy Clin Immunol* 2007;119:457-63.
40. Howell MD, Boguniewicz M, Pastore S, Novak N, Bieber T, Girolomoni G, et al. Mechanism of HBD-3 deficiency in atopic dermatitis. *Clin Immunol* 2006;121:332-8.
41. Howell MD, Novak N, Bieber T, Pastore S, Girolomoni G, et al. Interleukin-10 downregulates anti-microbial peptide expression in atopic dermatitis. *J Invest Dermatol* 2005;125:738-45.