Acta Derm Venereol 2013; 93: 340–345

CLINICAL REPORT

Sensitization to Skin-associated Microorganisms in Adult Patients with Atopic Dermatitis is of Importance for Disease Severity

Andreas SONESSON[1], Jacek BARTOSIK[1], Julie CHRISTIANSEN[1], Ingrid ROSCHER[2], Fredrik NILSSON[3], Artur SCHMIDTCHEN[1] and Ove BÄCK[1]

[1]Division of Dermatology and Venereology, Department of Clinical Sciences, Lund University, [3]Competence Centre for Clinical Research, Skåne University Hospital, Lund, Sweden, and [2]Department of Dermatology, Rikshospitalet, Oslo University Hospital, Oslo, Norway

Atopic dermatitis (AD) is a chronic inflammatory skin disease. Environmental and genetic factors, as well as microbial products from yeasts and bacteria, play a role in triggering the disease. A cohort of 619 adult patients with AD was screened for severity of AD, sensitization to *Malassezia sympodialis*, *Candida albicans*, *Staphylococcus aureus* enterotoxins and *Dermatophagoides pteronyssinus*. Serum levels of interleukin (IL)-18 were measured. Immunoglobulin E (IgE) sensitization to the combination of both yeast and mite antigens was found to be associated with more severe disease and higher levels of total IgE. AD patients with IgE sensitization to several microbial antigens had more severe disease than those with no IgE sensitization to microbial antigens. Sera from patients with IgE-associated AD showed higher levels of IL-18. Skin-associated microorganisms are exogenous factors triggering IgE-response and severity of AD. These findings are clinically important, and sensitization to these organisms should be assessed and considered in treatment strategies. *Key words: Malassezia sympodialis; Candida albicans; Staphylococcus aureus; enterotoxins; Dermatophagoides pteronyssinus.*

Accepted Jun 11, 2012; Epub ahead of print Oct 16, 2012

Acta Derm Venereol 2013; 93: 340–345.

Andreas Sonesson, Division of Dermatology and Venereology, Department of Clinical Sciences, Lund University, Biomedical Center B14, Tornavägen 10, SE-221 84 Lund, Sweden. E-mail: andreas.sonesson@med.lu.se

Atopic dermatitis (AD) is a relapsing chronic inflammatory skin disease characterized by severe pruritus, xerosis and typically distributed eczematous lesions, often seen in association with allergic rhinoconjunctivitis and asthma (1). Environmental and genetic factors are involved in the pathogenesis of AD. Individuals with AD have an impaired epidermal barrier, with abnormalities in lipid synthesis, protease activity and protease inhibitors, along with elevated transepidermal water loss and a reduction in the thickness of the stratum corneum (2, 3). Several genes associated with barrier structure and proteolytic regulatory mechanisms of the epidermis are associated with AD and have a role in the aetiology of the skin barrier dysfunction (4). The barrier dysfunction allows substances from the environment, such as products from microorganisms (*Malassezia*, *Candida* and *Staphylococcus aureus*), to penetrate into the skin. Exogenous proteases facilitate breakdown of the epidermal barrier and provide enhanced access for toxins, allergens and antigens to the basal epidermal and the dermal portions of the skin, where interactions with keratinocytes, antigen-presenting cells and immune effector cells take place (2). This all contributes to a chronic cutaneous inflammation and a complex immune response, involving both the adaptive and innate immune systems (5–7). It is reported that *Malassezia*, *Candida* and *S. aureus* play important roles in triggering the disease (8–10). Certain microbial products, such as *S. aureus* enterotoxins, are known to stimulate keratinocytes directly and mediate the release of proinflammatory cytokines (11). Interleukin (IL)-18 is a member of the IL-1 family of cytokines and is thought to participate in AD skin inflammation (12). Moreover, serum IL-18 levels are reported to be associated with allergic symptoms in children (13). IL-18 is a proinflammatory cytokine involved in T_H1 mediated responses, but also able to induce T_H2 cytokines and IgE production (14). Epidermal and systemic IL-18 are associated with AD severity, and *S. aureus* colonization appears to be correlated with epidermal IL-18 production in patients with AD (15, 16) The level of specific IgE antibodies to *S. aureus* antigens is reported to correlate with total IgE levels and disease severity in patients with AD (17). Moreover, *Malassezia* can induce specific IgE responses in AD patients with head and neck dermatitis, which are rarely seen in other atopic patients (18–20) and sensitization to *Candida* allergens contributes to the pathogenesis of AD (21).

It is reasonable to assume that microbial products are potential triggers for the maintenance of the inflammatory process in AD. It is therefore of importance to investigate the occurrence of specific IgE responses to microbial pathogens in the blood of patients with AD, as a marker of colonization and penetration of the skin. The aims of the present study were: to investigate the clinical impact of sensitization to microorganisms among adult patients with AD; and to investigate whether there were any differences in the systemic cytokine levels of IL-18,

 doi: 10.2340/00015555-1465
 ISSN 0001-5555

a cytokine produced by keratinocytes, among patients with IgE-associated vs. non-IgE-associated eczema.

MATERIALS AND METHODS

Study population

Adult (≥18 years) patients with AD who fulfilled the diagnostic criteria of Hanifin & Rajka (22) were included at the initial clinical visits to the dermatology clinic in Lund, Skåne University Hospital during the period 1998 to 2007. Subjects were screened for sensitization to skin-associated microbe antigens and aeroallergens, and characterized (Table I, Fig. 1). The severity of the AD was scored clinically, using a scoring system developed by Rajka & Langeland (23), which is a practical system for global grading AD based on engaged surface area, chronicity and sleep disturbance. Current or previous rhinoconjunctivitis and/or asthma, based on anamnestic information, were also registered. AD patients who were not sensitized to the investigated antigens ($n=106$) were used as a counterpart to the IgE-associated group ($n=513$), which was composed of subjects sensitized to skin-associated microbe antigens and/or aeroallergens (Fig. 1). In the IgE-associated group, a total of 386 patients had their AD severity score recorded (Table I, Fig. 2A and 3A).

Skin prick test, total and specific IgE analysis and levels of cytokines

Skin prick test (SPT) to birch, timothy, mugwort, dog, cat, horse, cod, rye, wheat, shrimp, cow's milk, egg white, *Dermatophagoides pteronyssinus*, *D. farinae*, *Alternaria alternata* and *Cladosporium herbarum* (ALK-Albelló, Hørsholm, Denmark) was performed on the forearm of the patients. Total serum IgE levels and specific serum IgE levels to *M. sympodialis* (m70), *C. albicans* (m5), *D. pteronyssinus* (d1), Staphylococcal enterotoxin A (SEA; m80), Staphylococcal enterotoxin B (*SEB*; m81) and Staphylococcal toxic shock syndrome toxin-1 (TSST-1; Rm226) were measured (ImmunoCAPTM system, Phadia AB, Uppsala, Sweden). Concentrations of serum IL-18 were determined by human IL-18 enzyme-linked immunoassay (ELISA) kit (Medical & Biological Laboratories Co. Ltd, Nagoya, Japan) and performed in accordance with the manufacturer's instructions in frozen serum samples, obtained at the initial visits.

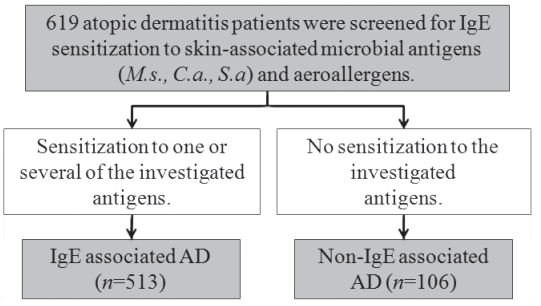

Fig. 1. Study design and group definitions. *M.s.*: *M. sympodialis*. *C.a.*: *C. albicans*, *S.a*: *S. aureus*. AD: atopic dermatitis; IgE: immunoglobulin E.

The study was approved by the local ethics committee of Lund, participants gave informed consent to participate in the study, and the study was in accordance with the protocols of the Declarations of Helsinki.

Statistical analysis

Wilcoxon-Mann-Whitney rank sum test was used to describe differences in the median values between two groups, with a p-value <0.05 being considered significant. Fisher's test was used for comparison of categorical data. Spearman's rank-order correlation (r_s) was used to examine correlations between serum IL-18 and total IgE levels or specific serum IgE levels to *SEB*. Linear-by-linear (LBL) test was used for comparison of ordered groups. Kruskal-Wallis (KW) test was used for multiple comparisons, and *post-hoc* comparisons were made by Nemenyi-Damico-Wolfe-Dunn (NDWD) test. The statistical software used was R version 2.10.1 (24), and the package coin version 1.0–14 (25) for exact non-parametric tests.

Table I. *Characteristics of the patients with atopic dermatitis (AD)*

Variables	IgE-associated[a] ($n=513$)	Non-IgE-associated[b] ($n=106$)	p-value
Male, *n* (%)	179 (35)	23 (22)	0.009[c]
Female, *n* (%)	334 (65)	83 (78)	
Age, years, mean, (range)	27 (18–82)	24.5 (18–80)	NS
Severity, median (range, *n*)	6 (3–9, 386)	5 (3–9, 89)	<0.001[d]
Severity, mean (standard deviation, *n*)	5.8 (1.8, 386)	5.0 (1.7, 89)	
Total IgE, kU/l, median (range)	265 (2–77,880)	17 (0–213)	<0.001[d]
Total IgE, kU/l, mean (standard deviation)	1,715 (5,445)	34 (44)	
Atopic rhinoconjunctivitis-positive, *n* (%)	354 (76)	36 (35)	<0.001[c]
Atopic rhinoconjunctivitis-negative, *n* (%)	112 (24)	67 (65)	
Asthma-positive, *n* (%)	195 (43)	24 (24)	<0.001[c]
Asthma-negative, *n* (%)	260 (57)	77 (76)	
Sensitized[e] to			
M. sympodialis, *n* (%), median (range)	221 (43), 4.3 (0.35–74)		
C. albicans, *n* (%), median (range)	232 (45), 3.4 (0.36–100)		
Staphylococcal enterotoxin A (SEA), *n* (%), median (range)	94 (18), 0.92 (0.35–30)		
Staphylococcal enterotoxin B (SEB), *n* (%), median (range)	127 (25), 0.84 (0.35–22)		
Staphylococcal toxic shock syndrome toxin-1 (TSST-1), *n* (%), median (range)	93 (18), 0.97 (0.35–35)		
Dermatophagoides pteronyssinus, *n* (%), median (range)	274 (53), 6.4 (0.37–100)		

[a]Defined as AD patients with sensitization to one or several of the investigated antigens (skin-associated microbial antigens and/or aeroallergens). [b]Defined as AD patients with no sensitization to the investigated antigens. [c]p-values obtained from Fisher's test. [d]p-values obtained from Mann-Whitney rank-sum test. [e]Specific serum IgE ≥0.35 kU/l. [f]IgE values in kU/l.
NS: not significant.

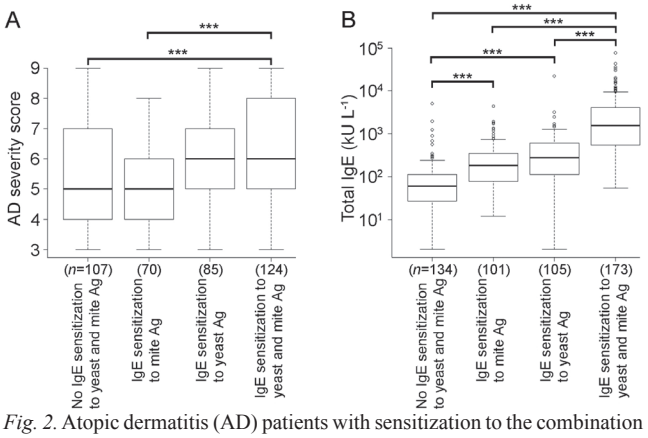

Fig. 2. Atopic dermatitis (AD) patients with sensitization to the combination of yeast and mite allergens exhibit severe AD and high levels of total IgE. (A) Subjects with "IgE sensitization to yeast and mite antigens (Ag)" exhibited more severe eczema ($p<0.001$, Kruskal-Wallis (KW) and Nemenyi-Damico-Wolfe-Dunn (NDWD)) in comparison to patients without IgE sensitization to these antigens or those with IgE sensitization to mite. (B) AD patients with "IgE sensitization to yeast Ag", as well as "IgE sensitization to yeast and mite Ag" exhibited higher total serum IgE ($p=0.001$, KW and NDWD) compared with subjects in the group "No IgE sensitization to mite and yeast Ag". Analyses were done among the subset of IgE-associated AD patients. Sensitization to yeast is classified as specific serum IgE ≥0.35 kU/l to Malassezia or Candida or both. ***$p<0.001$.

RESULTS

Subject characteristics

Patients with IgE-associated eczema scored higher in severity and exhibited higher total IgE levels in comparison with patients with non-IgE-associated AD ($p<0.001$) (Table I). Moreover, statistically significant differences in gender and the frequency of other atopic manifestations (rhinoconjunctivitis and asthma) were found between the two groups (Table I).

Sensitization to yeast and mite antigens and relationship to severity and total serum IgE levels, in patients with IgE-associated atopic dermatitis

To further investigate the importance of sensitization to yeasts and mites in the IgE-associated subset of patients with AD, four groups were constructed, based on specific IgE-levels to *C. albicans*, *M. sympodialis* and *D. pteronyssinus* (herein used as a "reference allergen") (Fig. 2). If the patient had serum-specific IgE ≥0.35 kU/l to either *C. albicans* or *M. sympodialis* antigens, and no sensitization to *D. pteronyssinus* antigen (<0.35 kU/l), they were denoted as "sensitized to yeast antigen". Thus, patients selected to the group denoted as "sensitized to mite antigen" had specific IgE ≥0.35 kU/l to *D. pteronyssinus* and no sensitization to *C. albicans* or *M. sympodialis* antigens (<0.35 kU/l). The two groups of AD patients with and without IgE sensitization to both mite and yeast antigens are also shown (Fig. 2). When comparing the clinical severity of the AD and the total IgE level, the group of patients sensitized to the combination of both yeast and mite antigens exhibited a more severe disease ($p<0.001$, KW and NDWD tests) in comparison with patients sensitized to mite antigen or subjects without sensitization to these two antigens (Fig. 2A). Moreover, subjects with IgE sensitization to yeast antigen, as well as the group with IgE sensitization to the combination of yeast and mite antigens exhibited significantly higher total serum IgE, in comparison with AD patients without IgE sensitization to these two antigens ($p<0.001$, KW and NDWD tests) (Fig. 2B).

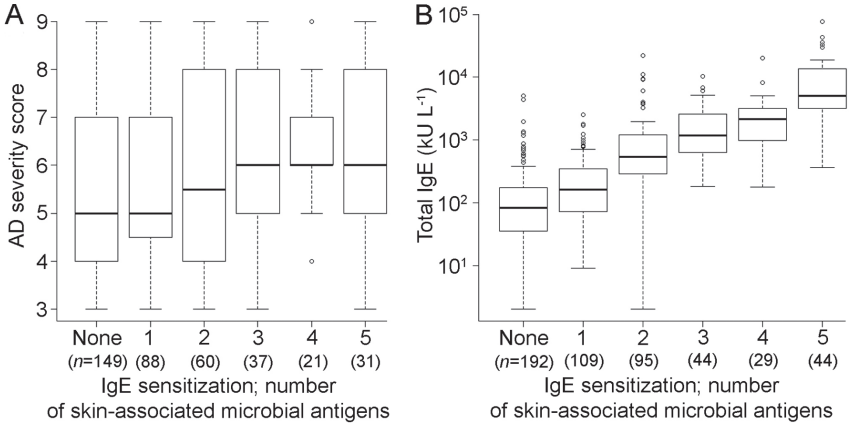

Fig. 3. Impact of sensitization to several microbial antigens (yeast and *S. aureus*) for eczema severity and total serum immunoglobulin E (IgE) levels in patients with IgE-associated atopic dermatitis (AD). (A) AD severity and (B) level of total IgE increased with the number of microbial antigens to which the AD patients exhibited IgE sensitization ($p<0.001$, for both tests, linear-by-linear (LBL)). IgE sensitization (≥0.35 kU/l) to skin-associated antigens of *Malassezia*, *Candida* and/or *S. aureus* (Staphylococcal enterotoxin A and B, toxic shock syndrome toxin-1) were assessed in the subset of IgE-associated AD patients. Data were analysed using the LBL test. The line within the box represents the median values of AD severity score and total IgE in A and B, respectively, of patients sensitized to none or 1–5 of the investigated microbial antigens. The boundary of the box indicates the 25th and 75th percentiles. Error bars indicate the 90th and 10th percentiles.

Impact of sensitization to several microbial antigens (yeast and S. aureus *antigens) for eczema severity and total IgE in patients with IgE-associated atopic dermatitis*

Subjects in the IgE-associated group of patients with AD were selected into groups depending on the number of microbial antigens to which the patient was sensitized (no sensitization to microbial antigens or sensitization to 1–5 antigens from *M. sympodialis*, *C. albicans* and *S. aureus* enterotoxins SEA, SEB or TSST-1). The severity of AD and the level of total IgE increased with the number of microbial antigens to which the patients were sensitized ($p<0.001$, in both LBL tests) (Fig. 3). To investigate whether a similar sensitization pattern as above could

be observed for inhalant allergens, we analyzed data from patients with IgE-associated AD demonstrating positive or negative skin prick tests to pollen, dust mite and animal dander allergens as individual groups. No significant difference in AD severity was found with respect to the extent of sensitization to the tested aeroallergens. However, the level of total IgE correlated with the number of aeroallergens to which the patients were sensitized ($p < 0.001$, $n = 250$, LBL test).

Serum levels of IL-18 in patients with atopic dermatitis

Serum IL-18 levels were determined in randomly selected samples of patients in the IgE-associated AD group that presented total IgE > 500 kU/l, allergic symptoms of rhinoconjunctivitis and asthma. As a counterpart, patients with non-IgE-associated AD (total IgE < 100 kU/l, no asthmatic symptoms) were used for comparison. A significant difference was found in IL-18 levels between these two groups of patients (Fig. 4). Furthermore, a correlation was found between serum IL-18 level (median 413 pg/ml, range 168–1,471 pg/ml, $n = 38$) and serum-specific IgE levels to *S. aureus* enterotoxin B (median 0.43 kU/l, range 0–7.9 kU/l, $n = 38$) among patients in the IgE-associated subset ($r_s = 0.42$, $p = 0.012$, $n = 38$), and a correlation between total serum IgE level (median 2835 kU/l, range 540–77,800 kU/l, $n = 38$) and serum IL-18 level ($r_s = 0.37$, $p = 0.02$, $n = 38$).

DISCUSSION

Environmental allergens have been implicated as aggravating factors in AD, especially in IgE-associated (extrinsic) AD (26). Microorganisms are reported to be involved in the pathogenesis of AD, not only by modulating serum IgE levels, but also by influencing several disease susceptibility genes and interacting with the cutaneous innate immune response (8). Moreover, several studies have demonstrated the occurrence of IgE sensitization to microbial antigens in AD (27–29). Most of the patients in this study showed an IgE-associated phenotype, and a minority had the type without allergen-specific AD (Table I), which is often referred to as intrinsic AD (30, 31). The frequency of intrinsic AD in the literature is quite variable (31), and the definition is not always consistent from author to author (30). Several factors, such as the methods used, definitions of AD forms, and the design of a study may govern the reported distribution of intrinsic and extrinsic AD (30, 32). Hence, the frequency of non-IgE-associated and IgE-associated AD in this cohort study may differ from figures reported in epidemiological studies elsewhere. However, this separation of intrinsic and extrinsic AD seems to be of importance because of the differences in the pathophysiology between them (30, 31). It is therefore important also to include allergen-specific testing for microorganisms, as some patients may not be sensitized to food or aeroallergens detected by routine allergen panel tests, but may show reactivity to allergens derived from the yeast *Malassezia* (20, 32).

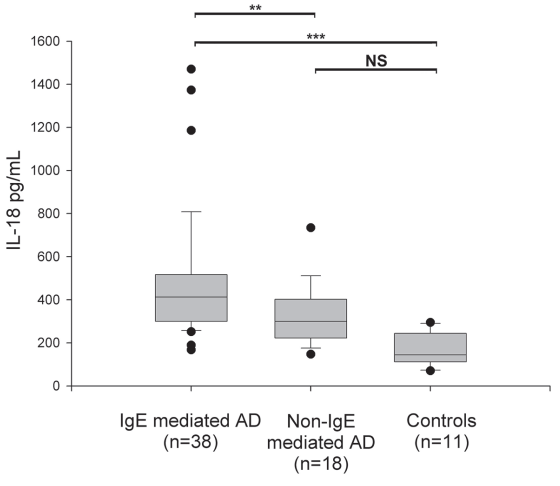

Fig. 4. Serum interleukin (IL)-18 levels in patients with atopic dermatitis (AD) with IgE-associated disease (subjects with total IgE of > 500 kU/l, allergic symptoms of rhinoconjunctivitis and asthma) in comparison with patients with non-IgE-associated AD (AD patients with total IgE of < 100 kU/l and no asthmatic symptoms). Data were analysed using Kruskal-Wallis test and Nemenyi-Damico-Wolfe-Dunn *post-hoc* test. $^{**}p < 0.01$ and $^{***}p < 0.001$. NS: not significant.

Allergen-specific IgE to yeasts in this study was assessed with the ImmunoCap™ assay using the m70 antigen (*M. sympodialis*) and m5 antigen (*C. albicans*). The m70 antigen was earlier linked to *Pityrosporum orbiculare*, then, after taxonomic revision, altered to *M. furfur*, and more recently, after development of new methods based on culture and molecular biology, found to represent antigens from *M. sympodialis* (33). However, IgE reactivity to other *Malassezia* species may be overlooked if only m70 is assessed (34).

Approximately 50% of subjects with IgE-associated AD in this study were sensitized to *M. sympodialis* or *C. albicans* antigens and 25% to SEB (Table I). Furthermore, subjects with sensitization to the combination of both yeast and mite antigens showed significantly higher total IgE levels and a more severe disease in comparison with subjects without sensitization to these two antigens (Fig. 2). *D. pteronyssinus* antigen, in this study, is used as a "reference allergen", because it is thought to be one of the major allergens contributing to AD (35, 36). However, our results indicate that yeast antigens are also of crucial importance. These results could be explained by the fact that yeasts are continuously present in the stratum corneum and outer parts of the sebaceous glands, while the skin is exposed to mites more superficially, mainly whilst in bed at night. Furthermore, the contribution of mites in various study populations may depend on the climate, e.g. latitude and relative humidity. In contrast to the results for IgE sensitization to microbial allergens,

we were not able to show that reactivity to an increasing number of airborne allergens affects disease severity. Nevertheless, it is notable that the level of total IgE correlated with increasing skin prick test positive reactions to aeroallergens (pollen, animal dander and dust mite). Airborne allergens, such as antigens from dust mite, are additional factors predisposing to allergic diseases, and a common pattern in atopic patients seems to be reactivity to many allergens (21). Sensitization to *Candida* allergens in AD has been related to positive nasopharyngeal and gastrointestinal cultures (10), but *Candida* may also colonize the skin in AD (37). Thus, sensitization to *Candida* via the epidermis is a possibility, supported by studies showing that sensitization to *Candida* is more related to AD than to respiratory allergic diseases (21). In accordance with results presented elsewhere (38), our results demonstrate that sensitization to both yeasts and mites leads to even higher total IgE levels, as well as more severe disease (Fig. 2).

Patients with AD may have a constitutionally dysfunctional skin barrier, an altered cytokine profile, and defects in the innate immunity (5, 39, 40). This facilitates microbial colonization of the skin, resulting in high frequencies of infection, aggravation of skin inflammation, and more pronounced impairment of the skin barrier. Toxins and surface antigens from microorganisms will then penetrate this impaired barrier more easily and sensitization may occur. This may retain the immunologically active cells in the skin and perpetuate of the eczema. The high serum levels of specific IgE to microbial antigens in patients with AD probably reflect the extent of damage to the epidermal barrier, which allows allergens to penetrate and gain access to the adaptive immune system. Moreover, it is reported that self-antigens contribute to the exacerbation of AD and that cross-reactivity of environmental allergens, such as allergens from *Malassezia*, with structurally related self-antigens may play a role in auto-reactivity in subsets of patients with AD (41, 42). Obviously, we present no direct proof that sensitization to microbial antigens underlies the pathogenesis of severe AD, and it is possible that the observed specific IgE to microbial allergens may merely be an epiphenomenon of severe AD. The direct causative roles need further investigation. Nevertheless, the results presented here indicate that sensitization to microbial antigens is of clinical importance, and this conclusion is compatible with results presented elsewhere (6, 27, 38).

In several open, controlled studies, both topical and systemic antifungal treatment have been used to reduce the severity of skin symptoms of yeast-sensitized AD patients, especially patients with head and neck dermatitis. Unfortunately, treatment studies have been relatively short-term and have not fully taken into consideration that the aim of antifungal treatment is not primarily to cure an infection but to reduce the colonization. Thus, in the early phase of treatment, disruption of the yeast cells will cause a release of fungal antigens, which may up-load antigen-presenting cells and pathogen-recognition receptors and facilitate both the adaptive immune response and non-specific skin inflammation. Therefore it seems reasonable that antifungal treatment has a delayed impact on serum IgE and yeast-specific IgE levels with no change after one month, but a significant reduction in IgE levels after three months (43). However, clinical improvement may be observed before a decrease in the laboratory parameters (44, 45). Long-term systemic antifungal treatment has been successful in selected patients with chronic severe AD (46) and is advocated in recalcitrant AD in *Malassezia*-sensitized patients (47). Our results clearly indicate that microbial sensitization should be analysed in order selectively to reduce the continuous burden of microbial allergens penetrating the disrupted skin barrier.

IL-18 may be of importance in infection-associated murine models of AD, and IL-18 is secreted from murine keratinocytes in response to Protein A from *S. aureus* (48, 49). The chronic stage of AD is characterized by production of IL-12 and IL-18 (40). In this study we found higher serum levels of IL-18 in a subset of patients with IgE-associated AD, in comparison with patients with non-IgE-associated disease (Fig. 4). It is of interest that human keratinocytes produce IL-18 (50) and IL-18 is secreted from dendritic cells upon stimulation by *Malassezia* (51). Moreover, associations have been reported between high levels of IL-18 in serum and stratum corneum, severity of AD, and skin colonization by *S. aureus* in patients with AD (15, 16, 52). In our material we found a correlation between the serum IL-18 level and the serum-specific IgE levels to *S. aureus* enterotoxin B. This has to be further investigated in a larger patient group, but may be an additional indication of the importance of microbial antigens in the pathogenesis of AD.

In conclusion, we have demonstrated the occurrence of microbial sensitization in severe AD, which calls for new and more detailed treatment strategies. These strategies should be based on monitoring sensitization to all antigens, including mites and microbes (*Malassezia*, *Candida* and *S. aureus*). Through the reduction or elimination of microbial trigger factors at the skin surface, in combination with anti-inflammatory and barrier-repair therapy, it may be possible to decrease the immune and inflammatory response and considerably improve the clinical course of AD.

ACKNOWLEDGEMENTS

This work was supported by grants from The Swedish Government Support for Clinical Research (ALF), Capio Research Foundation, Welander and Finsen Research Foundations and the Royal Physiographic Society of Lund.

The authors declare no conflicts of interest.

REFERENCES

1. Leung DY, Bieber T. Atopic dermatitis. Lancet 2003; 361: 151–160.
2. Cork MJ, Danby SG, Vasilopoulos Y, Hadgraft J, Lane ME, Moustafa M, et al. Epidermal barrier dysfunction in atopic dermatitis. J Invest Dermatol 2009; 129: 1892–1908.
3. Voegeli R, Rawlings AV, Breternitz M, Doppler S, Schreier T, Fluhr JW. Increased stratum corneum serine protease activity in acute eczematous atopic skin. Br J Dermatol 2009; 161: 70–77.
4. Brown SJ, McLean WH. Eczema genetics: current state of knowledge and future goals. J Invest Dermatol 2009; 129: 543–552.
5. De Benedetto A, Agnihothri R, McGirt LY, Bankova LG, Beck LA. Atopic dermatitis: a disease caused by innate immune defects? J Invest Dermatol 2009; 129: 14–30.
6. Werfel T. The role of leukocytes, keratinocytes, and allergen-specific IgE in the development of atopic dermatitis. J Invest Dermatol 2009; 129: 1878–1891.
7. Biedermann T. Dissecting the role of infections in atopic dermatitis. Acta Derm Venereol 2006; 86: 99–109.
8. Baker BS. The role of microorganisms in atopic dermatitis. Clin Exp Immunol 2006; 144: 1–9.
9. Faergemann J. Atopic dermatitis and fungi. Clin Microbiol Rev 2002; 15: 545–563.
10. Savolainen J, Lammintausta K, Kalimo K, Viander M. Candida albicans and atopic dermatitis. Clin Exp Allergy 1993; 23: 332–339.
11. Travers JB, Norris DA, Leung DY. The keratinocyte as a target for staphylococcal bacterial toxins. J Investig Dermatol Symp Proc 2001; 6: 225–230.
12. Tanaka T, Tsutsui H, Yoshimoto T, Kotani M, Matsumoto M, Fujita A, et al. Interleukin-18 is elevated in the sera from patients with atopic dermatitis and from atopic dermatitis model mice, NC/Nga. Int Arch Allergy Immunol 2001; 125: 236–240.
13. Ando M, Shima M. Serum interleukins 12 and 18 and immunoglobulin E concentrations and allergic symptoms in Japanese schoolchildren. J Investig Allergol Clin Immunol 2007; 17: 14–19.
14. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 regulates both Th1 and Th2 responses. Annu Rev Immunol 2001; 19: 423–474.
15. Inoue Y, Aihara M, Kirino M, Harada I, Komori-Yamaguchi J, Yamaguchi Y, et al. Interleukin-18 is elevated in the horny layer in patients with atopic dermatitis and is associated with Staphylococcus aureus colonization. Br J Dermatol 2011; 164: 560–567.
16. Trzeciak M, Glen J, Bandurski T, Sokolowska-Wojdylo M, Wilkowska A, Roszkiewicz J. Relationship between serum levels of interleukin-18, IgE and disease severity in patients with atopic dermatitis. Clin Exp Dermatol 2011; 36: 728–732.
17. Herz U, Bunikowski R, Mielke M, Renz H. Contribution of bacterial superantigens to atopic dermatitis. Int Arch Allergy Immunol 1999; 118: 240–241.
18. Johansson C, Sandström MH, Bartosik J, Särnhult T, Christiansen J, Zargari A, et al. Atopy patch test reactions to Malassezia allergens differentiate subgroups of atopic dermatitis patients. Br J Dermatol 2003; 148: 479–488.
19. Gaitanis G, Magiatis P, Hantschke M, Bassukas ID, Velegraki A. The Malassezia genus in skin and systemic diseases. Clin Microbiol Rev 2012; 25: 106–141.
20. Casagrande BF, Fluckiger S, Linder MT, Johansson C, Scheynius A, Crameri R, et al. Sensitization to the yeast Malassezia sympodialis is specific for extrinsic and intrinsic atopic eczema. J Invest Dermatol 2006; 126: 2414–2421.
21. Chang FY, Lee JH, Yang YH, Yu HH, Wang LC, Lin YT, et al. Analysis of the serum levels of fungi-specific immunoglobulin E in patients with allergic diseases. Int Arch Allergy Immunol 2011; 154: 49–56.
22. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol 1980; Suppl 92: 44–47.
23. Rajka G, Langeland T. Grading of the severity of atopic dermatitis. Acta Derm Venereol 1989; Suppl 144: 13–14.
24. RDevelopmentCoreTeam. R: a language and environment for statistical computing. In. Vienna, Austria: R Foundation for Statistical Computing; 2009.
25. Hothorn T, Hornik K, Van de Wiel MA, Zeileis A. A Lego system for conditional inference. American Statistician 2006; 60: 257–263.
26. Tokura Y. Extrinsic and intrinsic types of atopic dermatitis. J Dermatol Sci 2010; 58: 1–7.
27. Reefer AJ, Satinover SM, Wilson BB, Woodfolk JA. The relevance of microbial allergens to the IgE antibody repertoire in atopic and nonatopic eczema. J Allergy Clin Immunol 2007; 120: 156–163.
28. Novak N, Allam JP, Bieber T. Allergic hyperreactivity to microbial components: a trigger factor of îintrinsicî atopic dermatitis? J Allergy Clin Immunol 2003; 112: 215–216.
29. Schnopp C, Grosch J, Ring J, Ollert M, Mempel M. Microbial allergen-specific IgE is not suitable to identify the intrinsic form of atopic eczema in children. J Allergy Clin Immunol 2008; 121: 267–268 e1; author reply 268.
30. Roguedas-Contios AM, Misery L. What is intrinsic atopic dermatitis? Clin Rev Allergy Immunol 2011; 41: 233–236.
31. Schmid-Grendelmeier P, Simon D, Simon HU, Akdis CA, Wuthrich B. Epidemiology, clinical features, and immunology of the ”intrinsic” (non-IgE-mediated) type of atopic dermatitis (constitutional dermatitis). Allergy 2001; 56: 841–849.
32. Brenninkmeijer EE, Spuls PI, Legierse CM, Lindeboom R, Smitt JH, Bos JD. Clinical differences between atopic and atopiform dermatitis. J Am Acad Dermatol 2008; 58: 407–414.
33. Scheynius A, Johansson C, Buentke E, Zargari A, Linder MT. Atopic eczema/dermatitis syndrome and Malassezia. Int Arch Allergy Immunol 2002; 127: 161–169.
34. Sandström Falk MH, Tengvall Linder M, Johansson C, Bartosik J, Bäck O, Särnhult T, et al. The prevalence of Malassezia yeasts in patients with atopic dermatitis, seborrhoeic dermatitis and healthy controls. Acta Derm Venereol 2005; 85: 17–23.
35. Chapman MD, Rowntree S, Mitchell EB, Di Prisco de Fuenmajor MC, Platts-Mills TA. Quantitative assessments of IgG and IgE antibodies to inhalant allergens in patients with atopic dermatitis. J Allergy Clin Immunol 1983; 72: 27–33.
36. Nakamura T, Hirasawa Y, Takai T, Mitsuishi K, Okuda M, Kato T, et al. Reduction of skin barrier function by proteolytic activity of a recombinant house dust mite major allergen Der f 1. J Invest Dermatol 2006; 126: 2719–2723.
37. Arzumanyan VG, Magarshak OO, Semenov BF. Yeast fungi in patients with allergic diseases: species variety and sensitivity to antifungal drugs. Bull Exp Biol Med 2000; 129: 601–604.
38. 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: 1273–1279.
39. Lin YT, Wang CT, Chiang BL. Role of bacterial pathogens

in atopic dermatitis. Clin Rev Allergy Immunol 2007; 33: 167–177.
40. Bieber T. Atopic dermatitis. N Engl J Med 2008; 358: 1483–1494.
41. Schmid-Grendelmeier P, Fluckiger S, Disch R, Trautmann A, Wuthrich B, Blaser K, et al. IgE-mediated and T cell-mediated autoimmunity against manganese superoxide dismutase in atopic dermatitis. J Allergy Clin Immunol 2005; 115: 1068–1075.
42. Zeller S, Glaser AG, Vilhelmsson M, Rhyner C, Crameri R. Immunoglobulin-E-mediated reactivity to self antigens: a controversial issue. Int Arch Allergy Immunol 2008; 145: 87–93.
43. Bäck O, Bartosik J. Systemic ketoconazole for yeast allergic patients with atopic dermatitis. J Eur Acad Dermatol Venereol 2001; 15: 34–38.
44. Lintu P, Savolainen J, Kortekangas-Savolainen O, Kalimo K. Systemic ketoconazole is an effective treatment of atopic dermatitis with IgE-mediated hypersensitivity to yeasts. Allergy 2001; 56: 512–517.
45. Takechi M. Minimum effective dosage in the treatment of chronic atopic dermatitis with itraconazole. J Int Med Res 2005; 33: 273–283.
46. Kolmer HL, Taketomi EA, Hazen KC, Hughs E, Wilson BB, Platts-Mills TA. Effect of combined antibacterial and antifungal treatment in severe atopic dermatitis. J Allergy Clin Immunol 1996; 98: 702–707.
47. Darabi K, Hostetler SG, Bechtel MA, Zirwas M. The role of Malassezia in atopic dermatitis affecting the head and neck of adults. J Am Acad Dermatol 2009; 60: 125–136.
48. Nakano H, Tsutsui H, Terada M, Yasuda K, Matsui K, Yumikura-Futatsugi S, et al. Persistent secretion of IL-18 in the skin contributes to IgE response in mice. Int Immunol 2003; 15: 611–621.
49. Terada M, Tsutsui H, Imai Y, Yasuda K, Mizutani H, Yamanishi K, et al. Contribution of IL-18 to atopic-dermatitis-like skin inflammation induced by Staphylococcus aureus product in mice. Proc Natl Acad Sci U S A 2006; 103: 8816–8821.
50. Mee JB, Alam Y, Groves RW. Human keratinocytes constitutively produce but do not process interleukin-18. Br J Dermatol 2000; 143: 330–336.
51. Buentke E, Heffler LC, Wallin RP, Lofman C, Ljunggren HG, Scheynius A. The allergenic yeast Malassezia furfur induces maturation of human dendritic cells. Clin Exp Allergy 2001; 31: 1583–1593.
52. Ikezawa Z, Komori J, Ikezawa Y, Inoue Y, Kirino M, Katsuyama M, et al. A role of Staphyococcus aureus, interleukin-18, nerve growth factor and semaphorin 3A, an axon guidance molecule, in pathogenesis and treatment of atopic dermatitis. Allergy Asthma Immunol Res 2010; 2: 235–246.