Acta Derm Venereol 2004; 84: 32–36

Taylor & Francis
healthsciences

CLINICAL REPORT

Relationship between Skin Bacterial Colonization and the Occurrence of Allergen-specific and Non-Allergen-specific Antibodies in Sera of Children with Atopic Eczema/Dermatitis Syndrome

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In this study we investigated skin bacterial colonization, allergen-specific IgE and antiphospholipid/antinuclear antibodies in 72 children with atopic eczema/dermatitis syndrome (age 2–17 years). Bacteria were found on the skin in 41 cases and serological allergen-specific IgE positivity in 37. The different forms of antibodies appeared in the ratio 21/72 (33 antibodies in 21 children). The occurrence of antiphospholipid antibodies was significantly higher in the patients than in the controls. There were significantly more allergens in the group with bacterial colonization than in the group without colonization. The SCORAD index showed a significant positive association with the skin colonization. We conclude that there are significant relationships between the occurrence of Staphylococcus aureus colonization and the levels of inhalant allergen-specific IgE in children with atopic eczema/dermatitis syndrome, and between the occurrence of antiphospholipid IgM positivity and atopic eczema/dermatitis syndrome. ***Key words: antiphospholipid antibody; inhalant allergens; bacterial colonization; Staphylococcus aureus.***

(Accepted June 26, 2003.)

Acta Derm Venereol 2004; 84: 32–36.

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Atopic eczema/dermatitis syndrome (AEDS) (1) is a chronic cutaneous inflammatory disease that nearly always begins in childhood and follows a remitting/flaring course that continues throughout life (2–4). An eczematous, severely pruritic disease, AEDS may be exacerbated by several triggering factors, e.g. allergens, irritants, seasonal/climate changes and psychic stress. The disease often moderates with age, but patients suffer from a life-long skin sensitivity to irritants. Atopy predisposes patients to various occupational skin diseases. Most, but not all, individuals with AEDS have a personal or family history of allergic rhinitis or asthma (5), along with increased serum IgE antibodies against inhalant or food allergens (extrinsic type) (6, 7), or against epithelial antigens (8, 9). The role of IgE remains obscure, however. The skin bacterial colonization (as a base of superantigens) and the exogenous allergens play an important role in the progression of the clinical status. Antinuclear (ANA) antibodies have also been observed in an animal model of atopic dermatitis (10). In addition, the impaired skin barrier, sweating function (11) and ion content (12) can contribute to the pathogenesis of AEDS, including IgE-mediated hypersensitivity against sweat antigen (13) or epithelial antigens (9). For a better understanding of the antibody pattern in AEDS (14), we compared the occurrence of allergen-specific IgE and antiphospholipid (APL) antibodies, including anticardiolipin (ACL), and anti-β_2-glucoprotein I (Aβ_2GPI), as well as ANA antibodies in 72 children with AEDS from the aspect of bacterial colonization.

PATIENTS AND METHODS

Seventy-two children (34 boys, 38 girls; mean age 8 years, range 2–17) with atopic eczema/dermatitis syndrome were included in the study; all were suffering from serious or medium–severe forms of the disease. The average SCORAD index (15) was 48.3 (range 24–90). The control group comprised 22 healthy children (10 boys, 12 girls; mean age 8.6 years, range 1.5–14). A tampon was used to take samples from the affected skin for microbiological examination, and the bacteria were isolated on blood agar medium. The number of bacteria was determined using a semiquantitative method. The detection of ANA antibodies was carried out by indirect immunofluorescence on HEp2 cells. ELISA assays were used for measuring anticardiolipin and anti-β_2-glycoprotein I (16). Serum total IgE was detected by laser nephelometry. The allergen-specific IgE was determined by an immunoblot assay (INTEX Basel). Statistical analysis was performed using Fisher's exact test and unpaired *t*-test and the odds ratio was calculated.

 ISSN 0001-5555
DOI: 10.1080/00015550310015824

Table I. *The occurrence of non-allergen specific antinuclear (ANA) and antiphospholipid (APL) IgG and IgM antibodies in children with atopic eczema/dermatitis syndrome (AEDS) and healthy controls*

Antibody positivity	AEDS (*n*:72)	Healthy controls (*n*:22)
ANA IgG	10 (14%) (9 speckled, 1 nucleolar)	2 (1 speckled, 1 nucleolar)
APL IgG and/or IgM	*18 (25%)**	1
Anticardiolipin IgM	12 (16%)	1
Anticardiolipin IgG	2 (2.7%)	0
Anti-β2-glycoprotein I IgM	6 (8.3%)	0
Anti-β2-glycoprotein I IgG	3 (4.1%)	0
Total number of patients with non-allergen specific (ANA, APL) IgG and/or IgM antibodies	21 (29.3%)	2

*$p = 0.0378$.

RESULTS

Occurrence of skin bacterial positivity (colonization or infection)

Bacteria were detectable on the skin surface in 41 of 72 patients with AEDS (57%); Staphylococcus aureus in the great majority of cases (40/41) and Streptococcus pyogenes in 3 cases. Double positivity was found in 2 patients. The symptoms of pyoderma were visible in 5 cases.

Occurrence of non-allergen-specific (ANA, antiphospholipid) IgG and IgM antibodies

Elevated levels of ACL IgM appeared in 12 patients, IgG in 2, $A\beta_2GPI$ IgM in 6, IgG in 3 and ANA in 10/72 (Table I). The occurrence of the 2 APL antibodies was significantly higher in patients with AEDS (18/72) than in controls (1/22), ($P = 0.0378$). The odds ratio was 7 in this case. On the other hand, the occurrence of ANA positivity did not indicate any significant association with AEDS.

Occurrence and types of allergen-specific IgE

We found 37 children with some type of allergen-specific IgE positivity (51%). In the 72 patients with AEDS, the most frequent allergens were inhalant types: rye pollen (34.7%), mites (22.2%), weeds (22.2), grasses (20.8%), cat and/or dog epithelium (20.8%), trees (12.5%). The occurrence of corn (8.3%), soy (8.3%) and tomato (6.9%) positivity was less common. We did not consider positivity of less than 6% (Table II).

Correlation between non-allergen-specific antibodies, total and allergen-specific IgE

There was no statistically significant difference between the occurrence of non-allergen-specific (ANA, APL) antibodies in patients with normal ($11/31 = 35\%$) and high ($10/41 = 24\%$) serum total IgE. However, the appearance of non-allergen-specific antibodies was significantly higher in children with AEDS presenting normal serum levels of IgE than in the healthy controls ($p = 0.0169$, odds ratio 10.5) (Table III). It was not a surprise that there were great differences in total and allergen-specific IgE levels between patients and controls. Whereas elevated serum total IgE was seen in 41/72 (57%) of the children with AEDS only 9.1% of the controls showed the feature ($p = 0.0001$, odds ratio 13.226). The number of patients with allergen-specific IgE positivity (suffering from the extrinsic type of AEDS) was 37 of 72 (51%) versus 0/22 among controls ($p = 0.0001$, odds ratio 44.5) (Table III).

Occurrence of ANA, antiphospholipid and allergen-specific IgE positivity in children with and without existing skin bacterial colonization

There was no significant relation between in the appearance of APL or ANA antibodies and the presence of bacterial colonization in AEDS children. On the other hand, the total number of allergen-specific IgE positive in children with extrinsic AEDS ($n = 37$) was significantly higher in those with bacterial colonization (92/20) than in those without bacterial colonization (36/17), ($p = 0.0474$, odds ratio 2.17). In particular, rye-specific IgE positivity was significantly higher in the colonized group (17/41) than in the non-colonized

Table II. *Occurrence and types of allergen-specific IgE in children with extrinsic (37/72 cases) type of atopic eczema/ dermatitis syndrome*

Types of allergen	No. of positive cases (%)
Rye	*24 (34.7)*
Dermatophagoides pteron, D.farin.	*16 (22.2)*
Weeds	*16 (22.2)*
Grasses	*14 (20.8)*
Cat and/or dog	*14 (20.8)*
Trees	8 (12.5)
Corn	6 (8.3)
Soy	6 (8.3)
Tomato	5 (6.9)
Wheat	4 (5.5)
Nuts	4 (5.5)
Orange	4 (5.5)
Mould	3 (4.0)
Milk	2 (3.1)
Egg	1 (1.5)
Fish	1 (1.5)

Table III. *Occurrence of non-allergen-specific antibodies and allergen-specific IgE in relation to total IgE levels in sera of children with atopic eczema/dermatitis syndrome (AEDS) and healthy controls*

Laboratory findings	AEDS ($n=72$) (%)	Healthy controls ($n=22$) (%)
Elevated serum total IgE	41 (57%)**	2 (9.1%)
Allergen-specific IgE positivity	37 (51%)**	0 (0%)
Non-allergen-specific antibodies (ANA and/or APL) together with:		
normal serum level of IgE	11/31 (35%)*	1/20 (5%)
high serum levels of IgE	11/41 (24%)	1/2 (50%)
Antiphospholipid antibodies together with:		
absence of allergen-specific IgE	7/35 (20%)	1/22 (5%)
presence of allergen-specific IgE	9/37 (27%)	0/0 (0%)

*$p=0.0169$, **$p=0.0001$.
APL: antiphospholipid.

group (7/31), ($p=0.0184$, odds ratio 4.25). These data suggest that the existence of bacterial colonization on the skin could predispose the patients with AEDS to polysensibilization, mainly to inhalant allergens (Table IV).

Linkage between bacterial colonization, ANA, antiphospholipid, allergen-specific IgE positivity and SCORAD index

The average SCORAD index was 48.3 in the 72 children with AEDS, but there was no difference in the SCORAD index of patients with bacterial colonization (53.2), ANA (52.9), ACL (52.3), APL (49.9), combined ANA and/or APL (50.2) and allergen-specific IgE positivity. On the other hand, there was a strong association between a SCORAD index higher than 48.3 and the existence of bacterial colonization ($p=0.0007$, odds ratio 6.5) (Table V).

DISCUSSION

Although there have been many previous studies focusing on the role of skin colonizing bacteria, exogenous allergens, cytokines, other local and systemic findings (7, 17–23), this is the first to analyse the occurrence of antiphospholipid and antinuclear antibodies and their potential connection with skin bacterial colonization in children with AEDS. Our results show that APL IgM (mainly anticardiolipin) and ANA IgG are the two types of antibodies that

Table IV. *The occurrence of antinuclear antibodies (ANA) antiphospholipid (APL) and allergen-specific IgE positivity in children with atopic eczema/dermatitis syndrome with and without skin bacterial colonization*

Antibody positivity	Patients with bacterial colonization (n:41) n (%)	Patients without bacterial colonization (n:31) n (%)
Non-allergic types:		
ANA	8 (19.5%)	2 (6.4%)
Anticardiolipin	9 (22%)	4 (13%)
ANA + anticardiolipin	5 (12.2%)	2 (6.4%)
Antiphospholipid (anticardiolipin and/or anti-β2-gycoproteinI)	12 (29.2%)	6 (19%)
Number of patients with non-allergen-specific antibodies (ANA and/or ACL and/or $A\beta_2GPI$)	15 (36.5%)	6 (19.2%)
Total number of non-allergen-specific antibodies	22/15	11/6
Allergen specific IgE:		
Rye	17 (41.5%)*	7 (22.6%)
Mite	12 (29.3%)	4 (13%)
Weed	11 (26.8%)	5 (16.1%)
Pet (cat, dog)	11 (26.8%)	3 (9.7%)
Grass	10 (24.4%)	4 (13%)
Tree	5 (12.2%)	3 (9.7%)
Corn	4 (9.7%)	2 (6.4%)
Soy	3 (7.3%)	3 (9.7%)
Tomato	3 (7.3%)	2 (6.4%)
Number of patients with allergen-specific IgE	20 (48.8%)	17 (54.8%)
Total number of the allergen-specific IgE antibodies	92/20**	36/17

*$p=0.0184$, **$p=0.0474$.

Table V. *Occurrence of bacterial colonization, non-allergen-specific (ANA or APL) and allergen-specific antibodies in children with atopic eczema/dermatitis syndrome having a SCORAD index higher or lower than average (mean: 48.3)*

Patients with	SCORAD >48.3 ($n=31$) (%)	SCORAD <48.3 ($n=41$) (%)
Bacterial colonization ($n=41$)	25 (60.9)*	16 (39.1)*
ANA positivity ($n=10$)	5 (50%)	5 (50%)
APL positivity ($n=18$)	6 (33.3%)	12 (66.7%)
ANA and/or APL positivity ($n=21$)	9 (42.9)	12 (57.1%)
Allergen-specific IgE positivity ($n=37$)	18 (48.6)	19 (51.4%)

*$p=0.0007$.
APL: antiphospholipid; ANA: antinuclear antibodies.

show a more frequent occurrence in the AEDS children than in the controls. Furthermore, allergen-specific IgE against rye, mites, weeds, pets and grasses, appeared with higher rates in these patients than in the healthy controls. We assume that some lipid components of these inhalant allergens might provoke the production of APL antibodies, known to be often elevated in microbial infections (24, 25).

It is of special interest that in patients with bacterial colonization the total number of allergen-specific antibodies was significantly higher than in those without pyogenic skin bacteria. The mechanism of this unusual polysensitization toward inhalant allergens could be the facilitated antigen penetration through the impaired skin epithelium in children with an extrinsic type of atopic dermatitis. On the other hand, the occurrence of non-allergic, APL or ANA antibodies was only slightly elevated in the group of atopic patients with colonization compared to those without bacteria. Patients with a SCORAD index higher than 48.3 showed significant association with bacterial colonization. It is therefore concluded that the presence of pyogenic bacteria on the skin of patients with AEDS could contribute not only to the severity of disease, but also to the polysensitization toward some inhalant allergens, especially in patients with high total IgE, representing the extrinsic type of the disease. On the other hand, the appearance of antibodies toward APL and ANA seemed to be independent of atopic diathesis, and more related to the intrinsic type of AEDS. Perhaps these types of antibodies are produced against the phospholipid and nucleoprotein components of inhalant and bacterial antigens attached to a genetically impaired skin of patients with AEDS (4, 26), i.e. identical to those that provoke the diverse allergen-specific IgE response in the atopic subgroup of AEDS (22, 23). In this phenomenon, the lack of Th2 switch could also be an explanation of a dominant IgM/IgG antibody production and only a few IgE sensitizations (4). It is also possible, however, that the APL antibodies are produced against some still unidentified internal antigens and hence represent an indirect sign of the intrinsic character of AEDS.

According to our data, the high frequencies of inhalant allergen-specific IgE and APL/ANA antibodies can be markers of an abberant antigen (allergen) uptake by patients with AEDS, but they cannot be primary causative factors in the induction of the disease. According to the types of antibodies, the patients with AEDS can be divided into two subgroups: group A – atopic patients with elevated total serum IgE or allergen-specific IgE toward a great number of inhalant allergens, and with a rare occurrence of APL/ANA antibodies; group B – non-atopic patients with normal level of total serum IgE, and with more frequent occurrence of APL/ANA antibodies. However, the genetically impaired skin barrier is probably the crucial factor in the pathogenesis of AEDS (4, 11, 12, 26). In addition, secondary damage to the skin caused by pyogenic bacteria can probably modify the barrier function of epithelium further. Finally, the primary or secondary disturbance of immunoregulation cannot be neglected (4). From a clinical point of view, we recommend that the presence or absence of bacterial colonization on the skin should be determined, and the various inhalant allergen-specific IgE levels characterized in patients with AEDS. Analysis of ALP and ANA antibodies does not seem to be of major importance. Although such measurement may suggest the intrinsic subtype of the disease, it has to be stressed that APL antibodies can occur in low frequency also in patients with the extrinsic type of AEDS.

ACKNOWLEDGEMENTS

We acknowledge the technical assistance of all our co-workers at the Regional Immunological Laboratory of the 3rd Department of Internal Medicine, University of Debrecen, and at the Pediatric Health Centre in Borsod-A-Z County Teaching Hospital and the Bacteriology Laboratory of Borsod-A-Z County's National Institute of Public Health Service. The work was supported by grants from the Hungarian Society for Allergology and Clinical Immunology.

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