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Original article

Epicutaneous aeroallergen sensitization in atopic dermatitis infants – determining the role of epidermal barrier impairment

Background: Sensitization to atopens is an early phenomenon that overlaps with the onset of atopic dermatitis (AD) in infancy. Early epidermal barrier impairment may facilitate the epicutaneous penetration of atopens.
Objective: To correlate transepidermal water loss (TEWL) and aeroallergen sensitization in infants with AD.
Methods: In this cross-sectional study we enrolled 59 AD children and 30 controls aged 3–12 months. Transepidermal water loss in uninvolved skin, specific immunoglobulin E, atopy patch test (APT) and skin prick tests were performed with respect to seven aeroallergens, i.e., *Dermatophagoides pteronyssinus*, *D. farinae*, cat, dog, birch pollen, ambrosia, and cockroach. Environmental conditions were assessed by a questionnaire, and the house dust mite (HDM) concentration was determined in dust samples.
Results: Eighty-nine percent of AD infants had a positive APT *vs* one out of eleven controls. AD infants had a significantly higher mean TEWL than controls (27.4 *vs* 11.1 g/m^2/h, $P < 0001$). Children with two or more positive APT had higher TEWL than the others (31.1 *vs* 19.0 g/m^2/h, $P < 0.025$). No correlation was found between indoor APT results and exposure to HDM, cats, and dogs at home.
Conclusions: This study confirms the high prevalence of delayed sensitization to indoor and outdoor aeroallergens in AD infants, and shows that the higher the TEWL, the higher the prevalence of sensitization to aeroallergens. These data are in favor of a major role of a constitutive epidermal barrier impairment in determining early atopen sensitization in infants with AD.

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Atopic dermatitis (AD) is one of the most common pediatric diseases which considerably affects the quality of life of children and their families, and its incidence has increased over recent decades (1). AD presents clinically as a chronic relapsing dermatitis, which is initiated in a large part of the pediatric population by intrinsic defects in the uppermost layer of the epidermis, the stratum corneum (2). Epicutaneous sensitization is a possible major route of penetration for allergens and antigens that may pertain to later mucosal allergic disorders, as already suggested in animal models (3). At the clinical level, skin symptoms are the earliest manifestations of the atopic status. Moreover, given that initial skin lesions are more prominent on air-exposed areas and that sensitization to aeroallergens occurs very early in life, infantile AD may reflect the allergen penetration phase (4). Amongst the aeroallergens involved in the sensitization of AD patients, house dust mite (HDM), pet dander, and pollens from trees and plants are the most prevalent (5).

Transepidermal water loss (TEWL) represents the outward diffusion of water through skin and its measurements are used to gauge skin barrier function (6). Readings increase when the integrity of the stratum corneum barrier is compromised, and they correlate with percutaneous absorption (7). Few studies have characterized either aeroallergen sensitization (8) or TEWL (9) in young AD children, and to the best of our knowledge, no study has simultaneously evaluated epidermal barrier impairment measured using a tewameter and the risk of aeroallergen sensitization in infants with AD.

Because sensitization to atopens is an early phenomenon that overlaps with the onset of AD in infancy, we hypothesized that early epidermal barrier impairment may facilitate the epicutaneous penetration of atopens (4). More specifically, the aim of our study was to establish whether TEWL correlates with aeroallergen sensitization in AD infants.

Abbreviations: AD, atopic dermatitis; APT, atopy patch test; HDM, house dust mite; RAST, radioallergosorbent test; SPT, skin prick test; TEWL, transepidermal water loss.

Methods

Subjects

This cross-sectional study, conducted from May 2002 to June 2004, enrolled children with AD according to the UK working party criteria (10). Patients were aged 3 to 12 months and had a Scoring Atopic Dermatitis (SCORAD) index (11) greater than 10. Exclusion criteria included the administration of systemic immunosuppressive or steroid therapy during the previous 2 months, or antihistamine treatment within 7 days prior to inclusion. During the same period, a control group of 30 children aged 3–12 months was recruited with the following exclusion criteria: personal history of atopic diseases, any skin disease, fever, and steroid or immunosuppressive therapy within the last 2 months. All control subjects answered the standardized questionnaire and accepted the TEWL measure, but only a minority of the parents (11 children) consented to allergy testing and were patch/prick/immunoglobulin E (IgE) tested. Two years after enrollment, AD children underwent a follow-up visit with clinical examination, TEWL and allergy testing. The study was approved by the Regional Ethics Committee.

Procedures

After obtaining written consent, a complete history was recorded using a standardized questionnaire, and a thorough clinical examination was carried out by the same investigators. The environment of the children was assessed using the following items: rural or urban housing, pets at home, presence of a fitted carpet, or cuddly toys in the children's room. The severity of AD was assessed using the SCORAD index, a score that records the percentage of involved skin, the intensity of the lesions, i.e., erythema, papules, crusts, lichenification and xerosis, and subjective but essential parameters such as pruritus and sleep disturbance (11). The following tests were performed: TEWL ($g/m^2/h$) in noninvolved skin, serum specific IgE (sIgE), atopy patch test (APT) and skin prick tests (SPT) using a panel including seven aeroallergens. Using a semi-quantitative technique, the HDM concentration was measured in a dust sample collected from the children's room by their parents according to a standardized procedure. Topical steroid therapy was discontinued at least 3 days prior to allergy testing. TEWL and skin testing were not performed during summer (June–September).

Transepidermal water loss measurement

Transepidermal water loss measurements ($g/m^2/h$) were performed using an evaporimeter (Tewameter T210, Monaderm®; Monaco) according to published guidelines (12). In particular, measurements were performed in a windowless room maintained at constant temperature, far from the heating system. For an adequate reading, children had to remain still during the whole procedure, and the parents were requested to refrain from applying a skin moisturizer during the previous 24 h. The tewameter probe was applied to the medial aspect of the uninvolved volar forearm and held in place for 2 min until a steady TEWL value was obtained.

Assessment of aeroallergen sensitization

We performed allergy skin tests and sIgE to seven common aeroallergens, i.e., *Dermatophagoides pteronyssinus*, *D. farinae*, cat, dog, birch pollen, ambrosia, and cockroach (*Blatella germanica*).

Atopy patch tests. Atopy patch tests were performed with extracts containing 200 index of reactivity/g of allergen in a petrolatum vehicle (Stallergènes, Anthony, France). Atopy patch tests were performed on the back on clinically uninvolved back skin for 48 h in 10 mm Finn Chambers® (Epitest Ltd Oy, Tuusala, Finland). A patch test with the vehicle served as negative control. Readings were taken at 72 h and graded according to the international criteria revised by the European Task Force on Atopic Dermatitis (13). To avoid nonspecific reactions, APT were considered positive from + + (i.e., erythema, papules, and/or vesicles) onwards. Atopy patch tests were applied and read by the same trained investigator throughout the procedure.

Skin prick tests. Skin prick tests were performed on uninvolved skin at the flexor part of the right forearm with standardized allergen extracts (Stallergènes) for the seven aeroallergens mentioned above, and for a panel of four common food allergens (cow's milk, egg, peanut, and wheat). Histamine phosphate and physiological saline solution were used as positive and negative controls, respectively. The cutaneous response was scored 15 min after challenge as the wheal diameter, and SPT were regarded as positive if the wheal diameter was at least 3 mm and superior or equal to the positive control.

Specific IgE. Serum samples were obtained at enrollment. Total IgE and specific IgE antibodies to HDM, cat, dog, birch pollen, ambrosia and cockroach were determined using the CAP-RAST FEIA (Pharmacia Diagnostics, Uppsala, Sweden). Sensitization was defined as a concentration of IgE of 0.35 kU/l or greater.

Assessment of HDM concentration at home

Parents were asked to collect dust by vacuuming the mattress and the fitted carpet of their child's bedroom, according to a published standardized protocol (14). The HDM concentration in dust samples was determined by the same investigator using the Acarex test®, (Karapharm, Marseille, France) a semi-quantitative guanine test. Results were graded into four classes as follows: absence or very low (< 2 μg/g), low (2–10 μg/g), moderate (10–100 μg/g), or high concentration of HDM (> 100 μg/g).

Data analysis

The Student's *t*-test was used to compare the mean values of the TEWL measurements and the SCORAD index. We used the chi-squared test to assess the associations between qualitative variables. Data without a normal distribution were assessed using the nonparametric statistical Wilcoxon test. Differences were considered significant for $P < 0.05$. SAS software was used for all statistical analyses.

Results

Fifty-nine children with AD aged (mean ± SD) 7.3 ± 2.4 months (sex ratio = 0.37) were included in the study. According to the SCORAD index, AD was moderate in 64%, mild in 17%, and severe in 19%. The control group comprised 30 children (17 males) aged 7.0 ± 2.4 months. All of them answered the standardized questionnaire and accepted the TEWL measurement, but

only a minority of the parents (11 children) consented to allergy testing and were patch/prick/IgE tested. The general characteristics of the population studied are shown in Table 1.

Eighty-nine percent of infants with AD had at least one positive APT to the seven aeroallergens tested, 16% had at least one positive SPT, and 30% had at least one positive radioallergosorbent test (RAST) directed against one of the seven aeroallergens tested. The percentage of (++) APT-positive reactions ranged from 76% (*D. pteronyssinus)* to 37% (*B. germanica)* (Table 2). In controls, 10 out of 11 had negative APT and total IgE < 15 kIU/l; the control infant with positive APT (to HDM) had total IgE = 202 kIU/l without specific IgE to the seven allergens tested.

With a mean TEWL of 27.4 g/m^2/h compared with 11.1 g/m^2/h in control infants, infants with AD had a significantly greater TEWL ($P < 0001$) (Fig. 1). Children with two or more positive APT had a greater TEWL (31.13 g/m^2/h) compared to those with no or only one positive APT (19.03 g/m^2/h, $P < 0.05$) (Table 3). Moreover, SCORAD mean values were 33.8 and 38.8 in children with a TEWL below and above 30, respectively.

House dust mite concentration in carpets and mattresses was <2, 2–10, 10–100, and >100 μg/g in 16%, 39%, 31%, and 14% of cases, respectively. No association was found between HDM APT results and exposure to HDM, or between cat or dog APT and exposure to cat or dog at home (data not shown).

Table 1. Demographic and AD characteristics of patients included in the study

Characteristic	Mean ± SD or *n* (%)	
	AD (*n* = 59)	Controls (*n* = 30)
Age, months	7.3 ± 2.4	7.0 ± 2.8
Male	43 (**72.9**)	17 (**56.6**)
Female	16 (**27.1**)	13 (**43.4**)
Parental history of atopy	47 (**79**)	12 (**40**)
Parental history of asthma	7 (**12**)	4 (**13**)
Palmar hyperlinearity	10 (**17**)	0 (**0**)
Personal history of recurrent wheezing	15 (**25**)	5 (**16**)
Housing		
Rural	28 (**47.5**)	13 (**43.4**)
Urban	31 (**52.5**)	17 (**56.6**)
Fitted carpet in the child's bedroom	48 (**81**)	25 (**83**)
Pets (cat and/or dog) at home	33 (**56**)	18 (**60**)
Severity of AD		
Mild (SCORAD <20)	10 (**17**)	NA
Moderate (SCORAD 20–50)	38 (**64**)	NA
Severe (SCORAD >50)	11 (**19**)	NA
Hypereosinophilia (eosinophils >700/mm^3)	40/51 (**78**)	0/11 (**0**)
Total sIgE >15 kU/l	29/53 (**55**)	1/11 (**9**)
Food sensitization	SPT (%) / sIgE (%)	0/11 (**0**)
Hen's egg	28/50 (**56**) / 29/49 (**47**)	
Peanut	6/49 (**12**) / 8/49 (**16**)	
Cow's milk	6/49 (**12**) / 12/48 (**12**)	
Wheat	6/49 (**12**) / 6/47 (**12**)	

SD, standard deviation; AD, atopic dermatitis; SCORAD, Scoring Atopic Dermatitis; SPT, skin prick test; sIgE, specific immunoglobulin E; NA, not applicable. Important values are expressed in bold.

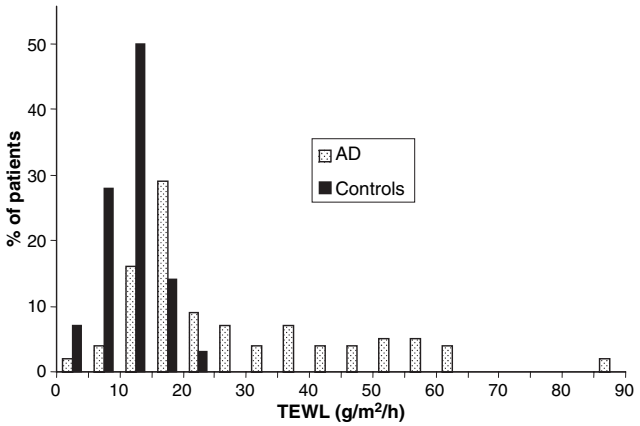

Figure 1. Distribution of transepidermal water loss (TEWL) readings in atopic dermatitis (AD) and control groups. The majority of AD patients (79%) had a TEWL above 15 g/m^2/h, while TEWL was less than 15 g/m^2/h in 83% of controls.

Table 2. APT, SPT and specific IgE results in the AD group (*n* = 59), and in the subset of patients from 3 to 6 months (*n* = 23) and from 7 to 12 months (*n* = 36)

Allergen	*D. pteronyssinus*	*D. farinae*	Dog	Cat	Birch pollen	Cockroach	Ambrosia
Positive APT							
Total	45 (**76**)	34 (**58**)	27 (**46**)	25 (**42**)	26 (**44**)	22 (**37**)	33 (**56**)
3–6 months	17 (74)	12 (52)	11 (48)	12 (52)	12 (52)	13 (56)	11 (48)
7–12 months	28 (78)	22 (61)	16 (44)	13 (36)	14 (39)	9 (25)	22 (61)
Positive SPT							
Total	2	3	1	3	1	3	1
3–6 months	0	0	0	1	0	0	0
7–12 months	2	3	1	2	1	3	1
Specific IgE							
Total	6 (**12**)	4 (**7**)	8 (**16**)	5 (**10**)	0	1	0
3–6 months	0	0	3	2	0	0	0
7–12 months	6	4	5	3	0	1	0

Values are expressed as *n* (%). APT, atopy patch test; SPT, skin prick test; IgE, immunoglobulin E. Important values are expressed in bold.

Table 3. Correlation between TEWL and aeroallergen sensitization

	0 or 1 positive APT	2 or more positive APT	
Mean TEWL (g/m^2/h)	19.031	31.134	$P = 0.0256$
SD	10.7	19.0	
95% CI	12.8–25.2	25.2–37.9	

TEWL, transepidermal water loss; APT, atopy patch test; SD, standard deviation; CI, confidence interval.

Total IgE was greater than 2 kIU/l in 53 cases, with a mean of 240 kIU/l (range 4–4768). Regarding sensitization to food products in the AD group, we found positive SPT and sIgE to hen's eggs in 56% and 47% of patients, respectively, and to peanuts, cow's milk, and wheat in 12%.

Two years after enrollment, 30 children from the AD group, aged (mean ± SD) 33 ± 2.8 months (sex ratio 0.42) underwent a follow-up visit. According to the SCORAD index, AD was mild in 80%, moderate in 13.5%, and severe in 6.5%. Mean TEWL was 17.3 ± 9.6 g/m^2/h compared with 30.4 ± 20.6 g/m^2/h in this subset of patients at enrollment. Thirteen cases (43%) of physician-diagnosed asthma were identified. Mean initial TEWL values in the asthma group (31 g/m^2/h, $n = 13$) and in the nonasthma group (28.7 g/m^2/h, $n = 17$) were not significantly different. Atopy patch tests, SPT and sIgE were positive in 60%, 63% and 76.5% of children, respectively (Table 4). Significant differences were found when comparing the results with those obtained at baseline, especially in SPT and sIgE results (Fig. 2).

Table 4. APT, SPT and specific IgE results in the AD group at the 2-year follow-up visit (V2) compared to those at baseline (V1) ($n = 30$)

	D. pteronyssinus	*D. farinae*	Dog	Cat	Birch pollen	Cockroach	Ambrosia
APT							
V2	20 (**66.5**)	12 (**40**)	3 (**10**)	0 (**0**)	5 (**17**)	2 (**6.5**)	0 (**0**)
V1	21 (70)	18 (60)	9 (30)	9 (30)	15 (50)	7 (23)	9 (30)
SPT							
V2	14 (**46.5**)	17 (**56.5**)	2 (**6.5**)	6 (**20**)	3 (**10**)	0 (**0**)	0 (**0**)
V1	1 (3)	0 (0)	0 (0)	3 (10)	0 (0)	0 (0)	0 (0)
sIgE							
V2	22 (**73**)	19 (**63**)	9 (**30**)	9 (**30**)	9 (**30**)	6 (**20**)	7 (**23**)
V1	5 (16)	3 (10)	7 (23)	4 (13)	0 (0)	0 (0)	0 (0)

Values are expressed as *n* (%). APT, atopy patch test; SPT, skin prick test; sIgE, serum immunoglobulin E. Important values are expressed in bold.

Discussion

Very few studies examining skin parameters have been conducted in AD infants prior to the age of 1 year. In the present study, such parameters were assessed at a very early stage of AD. Despite the limited sample size, our study confirms the high prevalence of positive APT to indoor and outdoor aeroallergens in young children with AD (5, 15), while SPT and RAST were mostly negative (Fig. 2). This is also in accordance with studies that showed more frequently positive APT in the younger age group, i.e., before the age of 2 or 3 years, compared with older children (16). Patch testing is a useful tool to assess sensitization to allergens, i.e. aeroallergens, contact and food allergens, and it has been shown that positive SPT or sIgE are not a prerequisite for a positive APT response (17). Based on the history of aeroallergen-triggered AD flares, APT proved to have higher specificity than SPT and sIgE (1). In this study, owing to the young age of our patients and the absence of gold standards, we were not able to determine the sensitivity and specificity of the different tests with regard to the patients' history, as is recommended in older patients (1). However, to reduce reading bias, especially irritation phenomena, we considered APT as positive only results from + + onwards.

Transepidermal water loss is the outward diffusion of water through skin (18). One could consider TEWL mainly as a marker for the inside–outside barrier, but the majority of studies investigating TEWL and percutaneous absorption found a quantitative correlation (7), leading to consider TEWL also as a marker for the outside–inside barrier. Our study shows that uninvolved skin of infants with AD differs from that of control

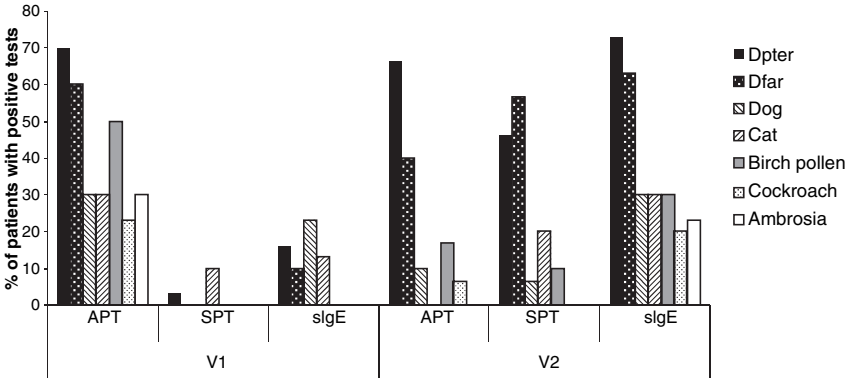

Figure 2. Atopy patch test, skin prick test and immunoglobulin E results at baseline (V1) compared with the results at the 2-year follow-up visit.

subjects by showing significantly greater TEWL readings. Moreover, our results in infants tend to confirm the positive correlation between AD severity and epidermal barrier impairment (TEWL) previously observed in older children by Seidenari et al. (9). As gender differed slightly in the AD and control groups, a possible bias could be considered in our TEWL results (19). Indeed, in adults only a slight tendency toward higher TEWL values has been shown in males compared to females (20) and confirmed in our study (11.3 in males *vs* 10.9 in females), which it is not sufficient to explain the significant results we found when comparing AD and control patients for TEWL readings.

More interesting was the finding that the greater the TEWL, the higher the prevalence of sensitization to aeroallergens, which points to a possible causative role of epidermal barrier impairment in the sensitization to atopens in AD infants. It may also explain the lesional pattern, as suggested a century ago by Hall, as an indication that infantile AD may be, at least in some patients, the clinical manifestation of an atopen penetration syndrome (4). To explain this defective barrier function, genetically determined skin defects should be considered (21): there is now strong evidence that variants of the epidermal barrier protein filaggrin may lead to constitutional epidermal barrier impairment and predispose to AD and later to asthma (2, 22). Other epidermal barrier proteins, i.e., protease KLK7 and protease inhibitor SPINK 5, may also be involved, but in a recent study from our group investigating simultaneously filaggrin, SPINK 5 and KLK7 mutations or polymorphisms in a French AD cohort, a significant association was found only for the most common European population filaggrin mutations (23). For others, who tend to follow the inside–outside hypothesis, such a defective barrier may be not primary but secondary to CLA+ T-cell activation and cytokine secretion leading to subclinical inflammation (24). It may also be speculated that environmental factors such as exposure to airborne volatile organic compounds (25) or to proteolytic allergen (Der p1), exaggerated use of detergents for skin hygiene, and infectious agents or toxins (26), can aggravate some constitutional predisposition to damage the epidermal barrier.

In the subset of 30 AD children who were reassessed 2 years later, the number of positive APT markedly decreased while the number of positive APT and sIgE increased (Fig. 2). This is in agreement with previous observations showing that older children and adults with atopic eczema are more likely to demonstrate positive SPT and sIgE than positive APT to the same aeroallergens (27), and SPT have been shown to be more frequently positive in children than in infants (28, 29). Our data confirm a time-dependent switch from delayed reactions to immediate/early reactions in childhood, and a minor role for specific IgE-dependent mechanisms at the onset of AD (4). This hypothesis suggesting that IgE sensitization is not a necessary prerequisite for the development of AD skin features is now advocated (30, 31). Thus, a distinction between extrinsic and intrinsic forms of AD is irrelevant in very young children.

In contrast to several studies conducted in children with AD and/or asthma (32, 33), our study failed to show a strong correlation between atopen exposure and sensitization to HDM or cat/dog dander. Indeed, even in very young children, a cat or a dog at home does not increase the risk of sensitization to cat or dog dander. Likewise, living in a place with a high concentration of HDM does not correlate with a higher prevalence of HDM sensitization. Because HDM and pet dander are very ubiquitous in our environment, sensitization may occur as easily at home as in nondomiciliary settings. The influence of home atopen exposure on subsequent sensitization to the same allergens or on clinical AD flaring in patients is still controversial (34). However, it has recently been shown that such correlations may be specific to some allergens, e.g., Der f1 or Fel d1 but not Der p1 (35).

Together with genetic findings (reviewed in 31), our clinical study suggests that defective barrier functions are likely to play a very significant role in the pathogenesis of AD. A constitutively abnormal stratum corneum barrier may facilitate contact between atopens and the actors of the innate and adaptative immune system, leading to sensitization.

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