

Immediate Wheal Reactivity to Autologous Sweat in Atopic Dermatitis Is Associated with Clinical Severity, Serum Total and Specific IgE and Sweat Tryptase Activity

Tiina Ilves^{a, d} Anu Virolainen^e Ilkka Tapani Harvima^{a–c}

^aDepartment of Dermatology, University of Eastern Finland, ^bKuopio University Hospital, KYS, and

^cCancer Center of Eastern Finland, Kuopio, ^dDepartment of Dermatology, Mikkeli Central Hospital, Mikkeli, and

^ePori Private Medical Center (Porin Lääkäritalo Ltd.), Pori, Finland

Key Words

Autologous sweat · Atopic dermatitis · Tryptase · IgE · *Malassezia*

Abstract

Background: Sweating can worsen atopic dermatitis (AD). The purpose of this work was to study the associations between reactivity to autologous sweat and the clinical severity of AD as well as investigate the possible wheal-inducing factors of sweat. **Methods:** Intracutaneous skin tests with autologous sweat were performed on 50 AD patients and 24 control subjects. In skin biopsies, tryptase and PAR-2 were enzyme and immunohistochemically stained. The associations between skin test reactivity and sweat histamine concentration, tryptase or chymase activity levels, tryptase or PAR-2 expression and AD clinical severity or IgE levels were investigated. **Results:** The wheal reactions in the intracutaneous tests with autologous sweat were positive, weakly positive and negative in 38, 34 and 28% of the AD patients, respectively, and in 4, 46 and 50% of the healthy controls, respectively ($p = 0.008$). In AD, the wheal reaction was associated significantly with clinical severity, serum total and specific IgE levels and sweat tryptase activity, but not with sweat histamine and chymase.

In nonlesional AD skin, the percentage of PAR-2⁺ mast cells (MCs) or the number of tryptase⁺ MCs did not differ significantly between the intracutaneous test reactivity groups.

Conclusion: Reactivity to autologous sweat correlates with the clinical severity of AD, and tryptase may be one of the factors involved in the sweat-induced wheal.

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Introduction

Atopic dermatitis (AD) is a chronic skin disease with well-known triggering factors, including chemical and mechanical irritants, allergens, microbes, low humidity and sweating. Sweating is a physiological process necessary for thermoregulation in hot conditions. However, AD patients often complain about itching and worsening of their skin symptoms after sweating. Moreover, some predilection sites in AD, such as the face, neck and cubital and popliteal fossae indicate the important role of sweating in aggravation of the disease [1]. There are few reports

T.I. and A.V. share first authorship.

about the possible mechanisms via which sweating contributes to the pathogenesis of AD. In pruritic AD skin, epidermal-barrier disruption and intense scratching allow the components of sweat to penetrate deep into the skin and come into contact with the lowermost epidermal keratinocytes and even the uppermost dermal cells. Recently, sweat cytokines IL-1 and IL-31 were shown to activate epidermal keratinocytes [2]. In addition, the sweat of AD patients can contain high levels of total IgE and specific IgE against inhalant allergens that reflect the corresponding serum IgE levels. It is suggested that the specific IgE antibodies could participate in allergen-trapping in the skin [3]. Immediate-type skin reactions to autologous sweat in AD patients have been shown by some authors [4, 5]. Hide et al. [3] found that basophils from many AD patients, but not from healthy controls, released histamine in response to sweat samples from AD patients, allergic rhinitis patients and healthy controls, suggesting that human sweat contains antigen(s) to which atopic patients can react. Interestingly, the later efforts of the study group to purify the antigen and analyze its proteins led to the recent identification of a protein from *Malassezia globosa*, MGL_1304 [1].

The mechanisms of pruritus in AD are complex and involve many factors. Proteinases, especially tryptase, 1 of the major enzymes of mast cells (MCs), may induce itch through proteinase-activated receptor 2 (PAR-2), and promote neurogenic inflammation [6–8]. PAR-2-like immunoreactivity was found to be greater in lesional than in nonlesional AD skin [9]. As PAR-2 has been detected on MCs [10], it is possible that tryptase can cause the release of other MC mediators, including histamine. The role of another major serine proteinase of skin MCs, chymase, is unknown. Even though the role of MC-derived histamine in AD has been under debate for many years, recent studies have revealed that histamine worsens the epidermal barrier by decreasing filaggrin expression and regulates the functions of Th1, Th2 and Th17 cells. Additionally, histamine H4 receptor antagonists significantly reduce itching in AD [11].

Another group of pruritogenic molecules is the neuropeptides. Of these, substance P and vasoactive intestinal peptide (VIP) are related to AD itch [12]. Neuropeptides may lower the itch threshold of cutaneous nerve fibers, and serum or plasma VIP levels have been found to correlate with itch severity in intrinsic AD [12]. VIP can activate human MCs, resulting in both histamine release and the production of proinflammatory mediators [13].

The presence and role of tryptase, chymase and histamine in the sweat of AD patients are not known. There-

fore, the primary purpose of this work was to study the association of positive skin test reactions to autologous sweat with the sweat levels of tryptase, chymase, histamine and VIP in AD patients compared with those in healthy controls. In addition, the skin sweat reactivity was correlated to clinical AD severity and serum total and specific IgE levels.

Material and Methods

Study Subjects

Fifty subjects with AD (37 women and 13 men with a mean age of 38 years old, range: 21–64 years) volunteered for the study. The patients were recruited from the outpatient clinic of the Department of Dermatology, Kuopio University Hospital. The general entry criteria were: an age of ≥ 18 years, a diagnosis of AD using standard criteria [14] and a willingness to comply with the protocol. Exclusion criteria were: severe AD, active autoimmune diseases, cancer, renal or liver diseases and pregnancy. The subjects had not received any potent systemic therapy (e.g. corticosteroids, cyclosporine, cytostatic drugs and biologics) or any UV light treatment during the preceding month. On the site of the skin test or biopsy, no potent local treatment (e.g. potent topical corticosteroids and calcineurin inhibitors) had been used during the preceding month. The Ethics Committee of Kuopio University Hospital, Kuopio, Finland, approved the protocol. Participants signed an informed consent prior to study entry. Study subjects were divided into 4 subgroups according to their current clinical status: no symptoms, almost symptomless (hardly noticeable redness in a small area), mild (mild erythema, papulation and excoriation) and moderate symptoms (moderate erythema, papulation and excoriation). Patients with severe disease were excluded because the sweat sample was collected from the large skin area of the back, and because of epicutaneous testing. The control group consisted of 24 healthy subjects (21 women and 3 men with a mean age 42 years, range 21–68 years). All the participants were questioned about symptoms due to sweating. Three groups were defined (no itching, itching and worsening of skin symptoms). Additionally, mean total serum IgE levels and specific IgE levels against dust mixture (including *Betula verrucosa* t3, *Phleum pratense* g6, *Artemisia vulgaris* w6, cat dander e1, horse dander e3, dog dander e5, *Dermatophagoides pteronyssinus* d1 and *Cladosporium herbarum* m2) and *Malassezia* spp. (m70) were measured using the ImmunoCAP method (Pharmacia Diagnostics AB, Uppsala, Sweden).

Sweat Collection

Samples of autologous sweat were collected from all study subjects using a previously described method [4]. Briefly, after alcohol cleaning, the healthy-looking skin of almost the entire upper and middle back of study subjects was covered with a wrapping film for 15–30 min, and sweating was induced by pedaling on an exercise bicycle. The sweat was then collected with a syringe. The sweat samples were immediately used for skin testing after filtering through a 0.22- μ m Millex-GV filter (Merck-Millipore, Darmstadt, Germany). For mediator analyses, a portion of each sweat sample was stored at -22°C .

Skin Tests

The anterior forearm skin of each study participant was pricked with autologous sweat. Prick test reactivity was confirmed with 10 mg/ml histamine dihydrochloride (ALK-Abelló, Hørsholm, Denmark) as a positive control, and 0.9% sodium chloride, 10% glycerol in PBS and Soluprick Negative control solution (ALK-Abelló) as negative controls. After 15 min, the test reactions were registered as positive (a wheal diameter ≥ 3 mm), weakly positive (a wheal diameter < 3 mm, but greater than the diameter of the wheals induced by negative controls) or negative (no wheal or the wheal diameter smaller than the diameter of the wheals induced by negative controls).

In intracutaneous (i.c.) tests, 20 μ l of filtered autologous sweat samples or different concentrations of histamine (100, 333, 1,000 and 10,000 nmol/l in Ringer solution, and 0.9 mmol/l in 0.9% sodium chloride solution) were injected into the volar forearm skin of study subjects. For control purposes, 5.4 mmol/l histamine dihydrochloride and 0.9% sodium chloride solutions were used. The immediate wheal reaction and the reaction after 15 min were measured. The reactions were assessed as positive (the final wheal diameter was at least 2 times larger than immediately after injection and at least a half of the diameter induced by histamine solution), weakly positive (the wheal diameter increased less than 2 times) or negative (the wheal did not increase or the wheal diameter was smaller than the diameter of the wheal induced by a negative control).

Epicutaneous tests were performed on the back skin of 47 AD patients and 8 healthy controls. Before testing, the squamous layer was damaged by tape stripping the test area with an adhesive tape 3 times [15]. Then Finn chamber patches with 0.9% sodium chloride, 10% glycerol in PBS or an autologous sweat sample were applied for 48 h. The epicutaneous test reactions were registered 48 h after the removal of test patches.

Sweat Histamine, Tryptase, Chymase and VIP Measurement

The concentration of histamine in autologous sweat was analyzed with the radioenzyme assay as described in previous literature [16]. The results are expressed as nmol/l of sample solution. Sweat VIP concentration was analyzed with an enzyme immunoassay kit (Phoenix Pharmaceuticals, Karlsruhe, Germany). The results are expressed as ng/ml.

The enzyme activities of tryptase and chymase in autologous sweat were analyzed using 0.2 mM Z-Gly-Pro-Arg-p-nitroanilide and Suc-Ala-Ala-Pro-Phe-p-nitroanilide (Sigma-Aldrich, Schnelldorf, Germany), respectively, as described previously [17]. The results were calculated as U/l of sample solution ($U = \mu\text{mol/min}$).

Skin Biopsies

After local anesthesia (1% lidocaine with adrenalin), 4-mm punch biopsies were collected from the lesional skin of 8 AD patients and nonlesional skin of 22 AD patients as well as from the skin of 6 healthy subjects. The specimens were immediately embedded in OCT compound (Sakura Finetek, Torrance, Calif., USA) and then frozen for the preparation of 5- μ m-thick cryosections.

Sequential Double-Staining of PAR-2 on MCs

The method has been described previously [18]. First, the enzyme histochemical staining of tryptase⁺ MCs was performed using 1 mM Z-Gly-Pro-Arg-4-methoxy-2-naphthylamide as the substrate (Bachem, Bubendorf, Switzerland), 0.5 mg/ml Fast Garnet GBC salt as the chromogen (Sigma-Aldrich), 0.5 mg/ml α_1 -

proteinase inhibitor (Sigma-Aldrich) and 100 mM Tris-HCl buffer, pH 7.6. After that, photographs were taken from at least 10 random sites of the upper dermis per skin sample. The dye was then dissolved by 15% Tween 20 incubation overnight, and the same cryosections were stained immunohistochemically using anti-PAR-2 rabbit polyclonal antibody (20 μ g/ml, Novus Biologicals, Littleton, Colo., USA) and a Vectastain Elite ABC kit (Vector Laboratories, Burlingame, Calif., USA). The stainings were controlled by unrelated immunoglobulin. The previously photographed sites were rephotographed. By comparing the photographs simultaneously, tryptase⁺ cells with PAR-2 immunoreactivity were counted, and their percentage was calculated.

Statistical Analysis

Statistical analyses were performed using SPSS for Macintosh (SPSS, Chicago, Ill., USA). The differences in numeric variables between the i.c. test reactivity groups were evaluated with the Kruskal-Wallis test. The Mann-Whitney U test was performed to compare the differences in sweat histamine and tryptase concentrations between the AD and control groups. The associations between i.c. test reactivity and clinical status or symptoms caused by sweating were tested by the χ^2 test using Monte Carlo probability (10,000 samples). Linear associations between parameters were tested using the Spearman correlation coefficients.

Results

Only Intracutaneous Tests with Autologous Sweat Showed Skin Reactivity

Epicutaneous tests with autologous sweat were negative in all studied subjects. The skin prick test with autologous sweat was positive in only 1 and weakly positive in 3 AD patients. The i.c. tests for reactivity to autologous sweat were positive in 38%, weakly positive in 34% and negative in 28% of the AD patients (table 1). Only 1 (4%) control subject reacted positively to autologous sweat in the i.c. test. A weakly positive reaction was seen in 46% and a negative reaction in 50% of the control subjects. The difference between AD patients and controls was statistically significant ($\chi^2 = 9.7$, d.f. = 2, $p = 0.008$).

Intracutaneous Test Reactivity to Sweat Correlated with Clinical Severity

The severity of eczema was moderate in 10 (20%) and mild in 12 (24%) AD patients. Fifteen (30%) patients were almost symptomless and 13 (26%) were symptomless at the time of the study. A significant difference in clinical status was found between the groups with regard to i.c. test reactivity to autologous sweat ($\chi^2 = 13.7$, d.f. = 6, $p = 0.029$; table 1). Moreover, a linear trend ($p = 0.009$) in the χ^2 test, and a moderately positive correlation ($r_s = 0.37$, $p = 0.008$) between disease severity and i.c. test reactivity in the Spearman correlation test were seen.

Table 1. Clinical parameters of different groups according to reactivity to autologous sweat in the i.c. tests

Autologous sweat i.c. test result	Clinical severity groups				Symptoms due to sweating			Mean serum IgE, kU/l	Mean <i>Malassezia</i> spp. IgE, kU/l	Mean dust mixture IgE, kU/l	Mean sweat histamine concentration, nmol/l	Mean sweat tryptase activity, U/l
	1	2	3	4	1	2	3					
<i>AD patients</i>												
Positive (n = 19)	2	5	7	5	3	9	7	1,830.1±2,107.1	11.0±15.0	27.8±31.6	87.5±139.1	0.24±0.34
Weakly positive (n = 17)	4	7	1	5	5	8	4	507.4±1,077.7	1.9±4	12.2±19.7	82.8±167.8	0.09±0.07
Negative (n = 14)	7	3	4	0	7	6	1	236.5±540.6	0.2±0.7	7.1±16.5	60.2±102.6	0.09±0.07
p value ^a	0.029				n.s.			<0.001	<0.001	0.026	n.s.	0.04
<i>Controls</i>												
Positive (n = 1)					0	1	0	29.7	0	0	8.0	0
Weakly positive (n = 11)					9	2	0	165.5±245.1	0	0.1±0.25	172.8±455.9	0.10±0.09
Negative (n = 12)					12	0	0	16.6±12.0	0	0	74.3±49.1	0.09±0.11
p value ^a					n.s.			n.s.			n.s.	n.s.
p value ^b	0.008								<0.001	<0.001	n.s.	n.s.

Clinical severity groups: 1 = symptomless, 2 = almost symptomless, 3 = mild eczema, 4 = moderate eczema. Symptoms due to sweating: 1 = no itching, 2 = itching, 3 = itching and worsening of skin symptoms. n.s. = Not significant.

^a Indicates differences between groups according to reactivity to autologous sweat in i.c. tests.

^b Indicates differences between AD and control groups.

Itching and worsening of skin symptoms due to sweating was declared by 12 (24%) and itching by only 23 (46%) AD patients. Fifteen patients (30%) did not report any symptoms from sweating. Three control subjects (12.5%) complained about slight itching, but the remaining 21 (87.5%) did not experience any symptoms from sweating. The symptoms caused by sweating did not differ significantly between the groups with regard to the i.c. test reactivity to autologous sweat (table 1). However, a linear trend in the χ^2 test ($p = 0.015$) and a moderate correlation in the Spearman test were detected ($r_s = 0.35$, $p = 0.01$).

Intracutaneous Test Reactivity to Sweat Was Associated with Total and Specific IgE Levels

Mean total serum IgE was $934.2 \pm 1,612.8$ kU/l in AD patients and 85.4 ± 178.5 in control subjects. In the patients, but not in the control group, significant differences in IgE levels were found between the groups with regard to i.c. test reactivity to autologous sweat (medians 80.1, 110.5 and 1,047.0 kU/l, from negative to positive test reactivity, respectively; $p < 0.001$; table 1: mean levels). Furthermore, total IgE levels correlated positively with i.c. test wheal sizes ($r_s = 0.66$, $p < 0.001$) and disease severity ($r_s = 0.46$, $p = 0.001$) in the AD group.

Specific IgE antibodies against dust mixture were found in 39 (80%; mean 16.5 ± 25.2 kU/l) AD patients. Additionally, specific IgE antibodies against *Malassezia* spp. were measured because *Malassezia* antigens in the sweat have been shown to be associated with the reactiv-

ity to autologous sweat [1]. IgE antibodies against *Malassezia* spp. were found in 24 (44%; mean 4.9 ± 10.6 kU/l) AD patients (table 1). One of the control subjects had a low IgE antibody level to dust mixture (0.84 kU/l), but no elevated specific IgE levels against *Malassezia* spp. were seen in the control group. In the AD patients, significant differences were found in *Malassezia* spp.-specific and dust mixture-specific IgE levels between the i.c. test reactivity groups ($p < 0.001$ and $p = 0.026$, respectively), between the clinical severity groups ($p = 0.001$ and $p = 0.006$, respectively) and between the groups with regard to the severity of symptoms due to sweating ($p = 0.001$ and $p = 0.021$, respectively).

No Associations between Sweat Histamine or VIP and Reactivity to Autologous Sweat

In total, 63 (85.1%) study subjects (40 AD patients and 23 controls) had detectable amounts of histamine in their sweat. The mean sweat histamine concentration was 78.1 ± 138.6 nmol/l in the AD group and 116.7 ± 307.4 nmol/l in the control group. The difference was not statistically significant. The mean sweat histamine concentration did not differ significantly between the groups with regard to i.c. test reactivity, either in AD patients or control subjects (table 1). Furthermore, the histamine concentration in sweat did not correlate significantly with the wheal size in sweat i.c. tests. Additionally, i.c. tests with different concentrations of histamine were performed on 5 AD patients and 3 controls to determine the concentration

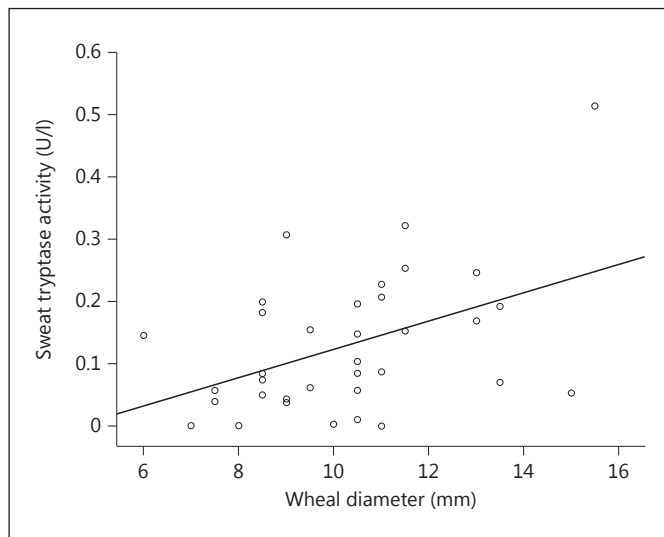


Fig. 1. Relationship between sweat tryptase activity levels and the i.c. test wheal diameters.

needed for a positive test reaction. A positive reaction to 10,000 nmol/l histamine was detected in 1 AD patient and to 0.9 mmol/l histamine in all tested subjects. There was a weakly positive reaction to 100 nmol/l histamine in 1 AD patient, to 333 nmol/l in 1 patient and 1 control, to 1,000 nmol/l in 2 controls and to 10,000 nmol/l in 7/8 of the tested subjects. As 72% of the study subjects showed histamine concentrations of <100 nmol/l in their sweat, the i.c. test reactivity to autologous sweat cannot be explained by histamine in sweat.

Similarly to histamine, VIP seems not to induce reactivity to autologous sweat as low sweat VIP concentrations were found only in 2 AD patients (0.44–0.51 ng/ml) and 3 controls (0.06–0.14 ng/ml). One of the patients had a positive i.c. test reaction to autologous sweat and a weakly positive reaction was seen in 2 controls.

Sweat Tryptase Activity Was Associated with Reactivity to Autologous Sweat and the Clinical Severity of AD

As much as 92% ($n = 46$) of the AD patients and 79% ($n = 19$) of the control subjects had detectable levels of tryptase activity in their sweat. The mean level of tryptase activity in the sweat was 0.147 ± 0.224 U/l in the AD group and 0.087 ± 0.096 U/l in the control group (table 1). No statistically significant difference was seen between the 2 groups. However, within the AD group, the mean sweat tryptase activity differed between the groups of weakly positive and positive i.c. test reactivity (medians 0.061 and 0.168, respectively; $p = 0.02$), and almost sig-

nificantly between the groups of negative and positive i.c. test reactivity (medians 0.095 and 0.168 U/l, respectively; $p = 0.05$). Moreover, a positive correlation was found between tryptase activity levels in the sweat and the i.c. test wheal sizes ($r_s = 0.37$, $p = 0.01$; fig. 1). A positive correlation was also detected between sweat tryptase activity and the clinical severity of AD (medians 0.057, 0.104, 0.125 and 0.150 U/l in the groups no symptoms, almost no symptoms, mild eczema and moderate eczema, respectively, $r_s = 0.37$, $p = 0.009$). However, the tryptase activity levels in the sweat did not correlate with clinical symptoms caused by sweating either in AD patients (medians 0.070, 0.104 and 0.106 U/l in the groups no symptoms, itching and itching and worsening of symptoms, respectively) or in the control subjects (median 0.065 and 0.053 U/l in the groups no symptoms and itching, respectively). Interestingly, sweat tryptase activity in AD patients correlated moderately with *Malassezia* spp.-specific ($r_s = 0.3$, $p = 0.037$), but not to dust mixture-specific IgE levels. Chymase activity was not detected in sweat samples.

No Correlation between Skin Tryptase⁺ and PAR-2⁺ MCs and Reactivity to Autologous Sweat

As sweat tryptase activity correlated with the positive i.c. test reactivity to autologous sweat, the role of PAR-2 on skin MCs was investigated. First, tryptase⁺ MCs were counted. The number of tryptase⁺ MCs was significantly lower in the control group (54 ± 13 cells/mm²) than in either nonlesional (83 ± 26 cells/mm²; $p = 0.04$) or lesional (109 ± 44 cells/mm²; $p = 0.002$) AD skin. No statistically significant difference was seen between lesional and nonlesional AD skin. The percentage of tryptase⁺ MCs showing PAR-2 was $52 \pm 6\%$ in AD lesions, $38 \pm 12\%$ in nonlesional AD skin and $31 \pm 12\%$ in the skin of control subjects. The percentage of PAR-2⁺ MCs was significantly greater in AD lesions than in nonlesional AD skin ($p = 0.005$) or healthy skin ($p = 0.005$). No significant difference was seen between healthy and nonlesional AD skin.

The nonlesional AD skin group was divided according to the i.c. test reactivity to autologous sweat into positive ($n = 9$), weakly positive ($n = 8$) and negative ($n = 5$) groups. There were no significant differences found in the percentage of PAR-2⁺ MCs or in the number of tryptase⁺ MCs between the i.c. test reactivity groups. In addition, there were no differences observed in these cell numbers after division of the nonlesional AD skin group into the clinical severity group and the group severity of symptoms due to sweating. There were no significant correlations between the number of tryptase⁺ or PAR-2⁺ MCs and any of the specific IgE levels.

Discussion

A marked clinical finding of this study is that the immediate-type reactivity of AD patients to autologous sweat was induced, but practically only in the i.c. test. It is possible that the concentration of an antigen or tryptase in the sweat is not high enough to induce wheals in prick tests. In addition, no delayed-type reactivity was detected in the epicutaneous scratch-patch test. A similar result was published previously [19]. These results suggest that autologous sweat cannot effectively induce skin wheals and inflammation in dry or slightly excoriated atopic skin.

As much as 19/50 of AD patients reacted positively to autologous sweat in i.c. tests, while only 1/24 of controls revealed a similar positive reaction. The positive i.c. test result could be considered to be specific for AD whereas the weakly positive i.c. test in 17/50 of the AD patients and 11/24 controls could reflect a nonspecific reaction to some yet unknown component in sweat. The result, to some extent, parallels the finding of Hide et al. [3], who showed a positive i.c. test reaction to autologous sweat in 84% of AD patients, but in only 11% of controls. One explanation for this difference between the studies could be that the interpretation of test positivity was not the same. We also found clearly higher percentages in both groups when weakly positive reactions were included.

In this study, the highest mean IgE levels were found in AD patients with positive test reactions to autologous sweat and the lowest in the group with no i.c. test reactivity. In addition, total serum IgE levels correlated strongly and significantly with i.c. test wheal sizes. The results partially confirm the findings of a previous work where a significant correlation between i.c. test wheal sizes and serum IgE levels was found but no difference was seen in serum IgE levels between patients with positive reactions and those with negative reactions [4]. Moreover, in contrast to that study, we also observed a positive correlation between total serum IgE levels and the clinical severity of AD. Patients with more severe AD reacted positively to autologous sweat more often than patients with mild or no symptoms. Additionally, sweat seemed to induce more itching and a worsening of AD skin symptoms in patients who reacted to sweat in the i.c. tests than those with no test reactivity.

In addition to total IgE, specific IgE levels against dust mixture and *Malassezia* spp. were also significantly higher in patients who reacted positively to autologous sweat in i.c. tests compared to those with weakly positive or negative reactions. Moreover, patients with specific antibod-

ies had more severe disease and symptoms due to sweating. Therefore, the reactivity to autologous sweat can simply reflect the severity of AD. There are also some reports about the association between the sensitization to *Malassezia* spp. and the clinical severity of AD, especially in the subgroup of head and neck dermatitis [20–23], although not all studies have confirmed this result [24]. On the other hand, it is possible that AD patients react to *Malassezia* antigens in their sweat. A Japanese study highlighted the importance of *M. globosa* by identifying its protein MGL_1304 as the major factor of sweat-induced histamine release from the basophils of AD patients. [1]. Our study suggests a possible role of other *Malassezia* spp. in inducing reactivity to autologous sweat. Firstly, the sweat collected from the large area of healthy-looking back skin in this study may have contained a mixture of *Malassezia* antigens that induce the IgE-mediated activation of MCs after i.c. injection. Secondly, i.c. test reactivity correlated with specific IgE levels against *Malassezia* mix m70.

One limitation of the study was the measurement of specific IgE antibody levels to *Malassezia* spp. by using the m70 antigen. The allergen source for m70 antigen is *M. sympodialis* and it can also detect other *Malassezia* spp. [25, 26]. However, some patients, especially those with antibodies to *M. globosa* and *M. pachydermatis*, may have a negative m70 ImmunoCAP test result [26]. As the most frequent *Malassezia* spp. on human skin are *M. sympodialis*, *M. restricta* and *M. globosa* [27, 28], the test may not find all patients with relevant sensitization to *Malassezia* spp. However, the correlation of the i.c. test reactivity with both total IgE and specific IgE antibodies against not only *Malassezia* spp. but also against a mixture of different dust allergens suggests that *Malassezia* spp. may not be the only causative factor, even though *Malassezia* antigens can play a role in the sweat-induced reaction. A probability also exists that positive reactions to sweat reflect antigenic reactivity to human manganese superoxide dismutase enzyme. This cross-reacts with Mala s 11 from *M. sympodialis*, and it is elevated in severe AD [29, 30].

Almost all AD patients and many control subjects displayed tryptase activity in their sweat. Surprisingly, the levels of sweat tryptase activity did not differ significantly between AD patients and control subjects. In comparison, tryptase activity is increased in the lesional dermis and the serum of AD patients [31, 32]. Nevertheless, the possible role of sweat tryptase in AD was indicated by the finding that sweat tryptase activity was greater in the group with positive i.c. test reactions to autologous sweat than in the groups with weakly positive or negative test

reactions. Furthermore, sweat tryptase activity was associated with more severe AD, although not with symptoms due to sweating. The number of tryptase⁺ MCs in AD skin was not associated with i.c. test reactivity. It is possible that MCs typically found around skin appendages are in a more activated state in patients with more severe AD, and consequently tryptase-heparin proteoglycan complexes are released and diffuse into the sweat. One mechanism for constant low MC activation and mediator secretion can be piecemeal degranulation [33]. Histamine is known to be diffused and metabolized rapidly and chymase is subject to inactivation by protease inhibitors [17], and so their levels may not parallel tryptase levels. Interestingly, a moderate correlation was seen between sweat tryptase activity and *Malassezia* spp. but not dust mixture-specific IgE antibody levels. In a previous study, the treatment of IgE-sensitized bone marrow-derived MCs with *M. sympodialis* extract induced degranulation [34], but the association between skin MC activation and sweat tryptase activity remains to be elucidated. Many tryptase effects are mediated through PAR-2 [6, 7]. PAR-2 agonists, when injected into the dermis, are able to activate the receptor on MCs, resulting in their activation and mediator release [9]. However, in our study, even though the percentage of PAR-2⁺ MCs was higher in lesional skin than in nonlesional skin, suggesting the plasticity and activation of MCs in the inflamed skin, this percentage in nonlesional AD skin was not associated with i.c. test reactivity to autologous sweat, clinical AD severity or the severity of symptoms due to sweating.

The majority of sweat samples contained histamine in both the AD and control groups. However, the concentrations were low and, according to additional i.c. testing with a dilution series of histamine solutions, probably not high enough to elicit wheal responses. Moreover, there

were no differences in histamine concentrations between the groups with regard to i.c. test reactivity to autologous sweat. The measurable concentrations of VIP, another possible pruritogenic molecule, were found only in a minority of study subjects. These subjects had variable results in the i.c. tests with autologous sweat. In addition, chymase activity was not detected in any sweat sample, a result which is in agreement with results showing no chymase activity in skin suction blister fluids [35]. Therefore, neither histamine nor VIP or chymase seems to play a role in sweat-induced reactivity, a result that further emphasizes the finding of the association of sweat tryptase activity with a positive i.c. test reaction in AD.

In conclusion, this study demonstrated that the positive skin reactivity of AD patients to autologous sweat is correlated with: (1) the clinical severity of AD, (2) total and specific IgE levels and (3) sweat tryptase activity. The level of sweat tryptase activity correlated with the clinical severity of AD. Further studies are warranted for clarifying the possible role of sweat tryptase in promoting the wheal reaction.

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Disclosure Statement

The authors have no conflict of interest.

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