

INVESTIGATIVE REPORT

IgE-mediated Hypersensitivity Against Human Sweat Antigen in Patients with Atopic Dermatitis

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Sweating aggravates itch in atopic dermatitis, but the mechanism is unclear. In this study, we examined the involvement of type I hypersensitivity in the aggravation of atopic dermatitis by sweating. Skin tests with autologous sweat were positive in 56 of 66 patients (84.4%) with atopic dermatitis, but only in 3 of 27 healthy volunteers (11.1%). Sweat samples from both patients and healthy volunteers induced varying degrees of histamine release from basophils of patients with atopic dermatitis. However, the histamine release was impaired by removal of IgE on the basophils. Incubation of basophils with myeloma IgE before sensitization with serum of patients blocked the ability to release histamine-induced sweat. IgE antibody against antigen(s) in sweat may be present in serum of patients with atopic dermatitis. Key words: histamine; basophils; skin test; autoallergy.

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Atopic dermatitis is a chronic inflammatory skin disease that frequently occurs in patients with a personal or family history of atopy (1). Histological analysis of the lesions shows an increase of the mast cell population accompanied by an infiltration of inflammatory cells, coupled with the dominant expression of Th2-type cytokines by the lymphocytes, particularly in acute-phase lesions (1, 2). Challenge by the house dust-mite antigen on the skin of patients with atopic dermatitis *in vivo* and human skin slices sensitized by sera of the patients *in vitro* induces the release of pro-inflammatory mediators and cytokines, such as histamine, arachidonic acid metabolites and tumour necrosis factor- α (3, 4). Elimination and/or avoidance of antigens for IgE from the environment of patients has therefore been suggested as one of the important treatments of patients with atopic dermatitis (1). Moreover, cleaning the skin surface after a shower, or after taking

a bath, is largely beneficial to patients (1, 5), and suggests the presence of aggravating factors on the skin surface. On the other hand, immediate-type skin reactions to human dander extracts (6, 7) and human sweat (8, 9) have been reported in patients with atopic dermatitis. Adachi & Aoki (9) showed the binding activity of IgE antibody against sweat concentrate collected from a healthy volunteer in patients with atopic dermatitis. More recently, Valenta et al. (10) and Natter et al. (11) have identified several intracellular autoantigens for IgE antibodies in the sera of patients with severe skin manifestations of atopic dermatitis. However, a recombinant protein antigen, Hom s 1, reported as the most frequently recognized autoantigen by serum IgE of patients with atopic dermatitis, merely induced mild erythematous skin reactions in patients with Hom s 1-specific IgE antibodies. Moreover, no significant correlation was observed between the binding activity of serum IgE to the sweat antigen and skin reactions to autologous sweat samples in the study by Adachi & Aoki (9). It is therefore unclear whether such IgE and autoantigens are involved in the pathogenesis of atopic dermatitis or not, especially via the mechanism of type I hypersensitivity. In this study, we show a high incidence of positive skin reactions to autologous sweat in patients with atopic dermatitis regardless of their total serum IgE concentrations, and demonstrate that basophils of the patients release histamine in response to sweat antigen(s) by a mechanism mediated by specific IgE.

MATERIALS AND METHODS

Subjects

The following subjects were included in the study: 66 patients (34 men and 32 women; 13–37 years of age, mean \pm SD 24.7 \pm 5.2) with atopic dermatitis; 7 patients (5 men and 2 women; 20–35 years of age, mean \pm SD 24.6 \pm 4.8) with allergic rhinitis without atopic dermatitis, urticaria or asthma; and 27 healthy volunteers (15 men and 12 women; 20–42 years of age, mean \pm SD 27.8 \pm 6.7) without allergic symptoms. The protocol of the study was approved by the ethics committee of Hiroshima University, Faculty of Medicine. All patients and volunteers were informed of the aim and protocol and agreed to participate in the study. Twenty-five of the 66 patients with atopic dermatitis had complications of allergic rhinitis and another 15 had complications of asthma. Twelve of these suffered from both

asthma and allergic rhinitis. Histamine H₁-receptor antagonists were discontinued for at least 3 days prior to the study. Disease severity of each patient was evaluated according to the grading of atopic dermatitis by Rajka & Langeland (12). Total serum IgE concentrations of the patients were measured using a fluoroenzyme immunoassay kit (UniCap[®]; Pharmacia-Upjohn, Tokyo, Japan).

Sweat collection

Sweat was collected using the methods described by Adachi & Aoki (9), with a slight modification. The skin of the subject's trunk was wiped twice with a cotton swab immersed in 50% isopropyl alcohol. The trunk of the subject was covered with 2–3 layers of wrapping film (Saran Wrap[®]; Asahi-Kasei, Tokyo, Japan), the edges sealed with adhesive tape. After running for 15 to 30 min, the film was removed from the subjects' skin and the sweat on the area that was covered was collected using an injection syringe (Terumo, SS01T2613S, Tokyo Japan). The sweat samples were filtered (0.22 µm filter) (Millipore, SLGV013SL, Bedford, MA) and used immediately for skin testing of the patients or stored at –20°C until use for analysis *in vitro*.

Gel chromatography

One and a half millilitres of filtered sweat samples of three patients with atopic dermatitis was lyophilized, reconstituted with saline and part or all of the samples were applied to an HPLC system. The samples were fractionated in 500 µl saline/fraction by TSK-GEL G2000 column (TOSOH, Tokyo, Japan) at a flow rate of 1 ml/min.

Skin test

In skin tests, 20 µl of crude or fractionated autologous sweat samples, prepared as above, was injected intradermally into the volar forearm skin (crude samples) or back skin (fractionated samples) of the subjects. After 15 min, the diameters of the erythema and wheal were measured. The reactions were assessed as "positive" if the diameter of sweat-induced wheal was equal to or larger than 8 mm, or that of the flare equal to or larger than 20 mm.

Histamine release assay

Peripheral blood leucocytes containing about 1% basophils were obtained from patients with atopic dermatitis and from healthy volunteers, as described by Grattan et al. (13), as well as histamine release assay, except that the final reaction volume was reduced to 100 µl/tube. Sweat samples and goat anti-human IgE antibody (Seikagaku Co. Tokyo, Japan) were diluted 10 times and 3,000 times, respectively, if not indicated. A mouse IgG2b monoclonal antibody (CRA-1), which recognizes an epitope on the high affinity IgE receptor (Fc_εRI) without the interference of IgE binding (Kyokuto Pharmaceutical Indust. Co. Ltd, Ibaraki, Japan) (14), was used as a positive control for the assays at 50 ng/ml. The amounts of histamine were measured by the HPLC system, as described by Koro et al. (3).

Removal of IgE and passive resensitization of basophils

IgE antibodies on the surface of basophils were removed by treatment with 10 mM lactic acid (pH 3.9) as described by Hide et al. (15), with the exception that the saline used for washing cells and lactic acid preparation was supplemented

with 0.03% human serum albumin (Sigma, A1653, Tokyo, Japan). For passive sensitization, the acid-treated basophils were pre-incubated in 2 ml Ca²⁺-free, 4 mM EDTA buffer solution (13, 15) in the presence or absence of 10 µg/ml myeloma IgE (Chemicon, Temecola, CA) at 37°C for 30 min, followed by the addition of 2 ml serum of patients with atopic dermatitis (AD1, see below) at 37°C for 60 min (13, 15). After sensitization, the cells were washed three times with buffer and exposed to the challenge stimuli, as described above.

Statistics

Data were summarized as mean ± SEM. To calculate significance levels between groups, two-way ANOVA with Bonferroni's post test was performed using GraphPad InStat version 3.01 for Windows 95/NT (GraphPad Software, San Diego, CA). Analyses of a contingency table using the Fisher exact test and of linear regression were also performed using the same program.

RESULTS

Skin tests for autologous sweat samples and serum IgE concentrations

Of 66 patients with atopic dermatitis, 56 (84.8%) showed positive skin reactions to their own sweat, whereas only 3 out of 27 individuals (11.1%) in healthy controls showed the positive reactions ($p < 0.0001$; Table I). In patients with allergic rhinitis there was also a strong association with the positive skin reactions. There was no association with disease severity of atopic dermatitis. Moreover, there was no statistical difference in serum IgE concentrations between patients showing positive skin tests and those with negative reactions, while serum IgE concentration was positively correlated with disease severity ($p < 0.01$, data not shown). Wheal sizes in the skin tests were not apparently correlated with the disease severity (data not shown), but marginally correlated with serum IgE concentrations ($p < 0.05$) (Fig. 1).

Histamine release from basophils of patients with atopic dermatitis by sweat samples of various subjects

Histamine release activity in the sweat samples of various subjects was studied using the leucocytes of a

Table I. Skin reactions to autologous sweat

Disease	n	Positive skin reaction n (%)
Atopic dermatitis	66	56 (84.8)**
Mild	17	14 (82.4)**
Moderate	20	18 (90.0)**
Severe	26	21 (80.8)**
Undefined	3	3 (100)*
Allergic rhinitis	7	5 (71.4)*
Healthy controls	27	3 (11.1)

* $p < 0.005$, ** $p < 0.0001$ (patients versus controls, Fisher's exact test)

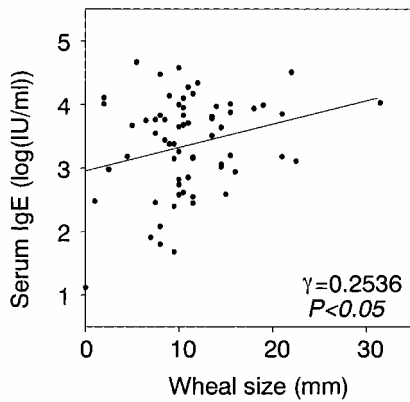


Fig. 1. The relationship between skin reactions to autologous sweat and serum IgE concentrations. Serum IgE concentrations of patients with atopic dermatitis were correlated weakly with wheal sizes in skin tests.

patient with atopic dermatitis (AD1). Twelve out of 23 (43.5%) sweat samples collected from patients with atopic dermatitis, 2 out of 7 (28.5) samples from patients with allergic rhinitis and 8 out of 17 (47.1%) samples collected from healthy controls induced more than 4%, up to 76.0% net release of histamine. There was no apparent difference in the mean or the variance of the histamine release activities between the sweat of patients with atopic dermatitis, those of patients with allergic rhinitis, and healthy controls. Moreover, five sweat samples of healthy individuals who did not show positive skin reactions to the skin test induced more

than 6% histamine release from leucocytes of patients with atopic dermatitis.

No correlation was found between histamine-releasing activities in sweat samples and serum IgE concentrations of the sweat donors (data not shown). This suggests that human sweat contains varying amounts of factor(s) that induce histamine release from both skin mast cells and basophils of patients with atopic dermatitis, and that the presence of the factor(s) itself is not necessarily related to the disease.

Comparison of sweat-induced histamine release from basophils of patients with atopic dermatitis and those of healthy volunteers

We tested the above-mentioned hypothesis by incubating the sweat samples with basophils of two other patients with atopic dermatitis (AD2, AD3) and those of two healthy controls (Cont1, Cont2). As shown in Fig. 2A, none of the sweat samples induced histamine release from the basophils of healthy controls, whereas most of the samples induced histamine release from the basophils of donors with atopic dermatitis. The degrees of histamine release from basophils of AD1 and those of AD2 were correlated (Fig. 2B). These results further confirm that the disease specificity of the histamine release and the skin reactions evoked by sweat samples are due to a difference in the cellular reactivity rather than that of the sweat factor(s).

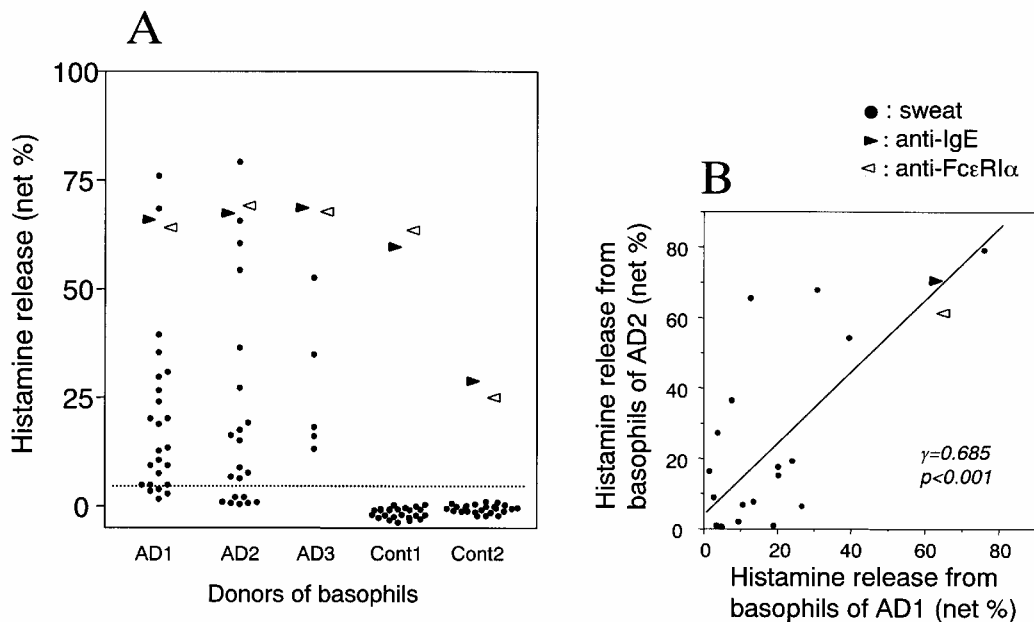


Fig. 2. Histamine release from basophils of various donors in response to sweat samples. A. Sweat samples derived from patients with atopic dermatitis, patients with allergic rhinitis and healthy volunteers were incubated with basophils of five different donors; three patients with atopic dermatitis (AD1, AD2, AD3) and two healthy volunteers (Cont1, Cont2). B. Histamine release from basophils of AD1 and of AD2 were correlated ($p<0.001$, $\gamma=0.685$). Histamine release by anti-IgE antibody and by anti-Fc ϵ RI antibody are depicted by filled triangles and open triangles, respectively.

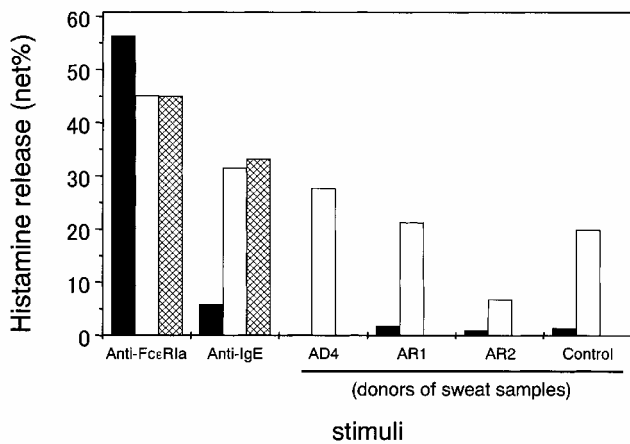


Fig. 3. Effect of passive sensitization of basophils by serum of a patient with atopic dermatitis and blocking by myeloma IgE. Basophils of a non-allergic healthy volunteer (Cont1) were treated with lactic acid to remove endogenous IgE (filled bar) and passively sensitized with serum of a patient with atopic dermatitis (AD2) in the absence (open bar) or presence (hatched bar) of an excess amount of myeloma IgE as described in the Materials and Methods section. Basophils that received each treatment were incubated with sweat samples of the patients with atopic dermatitis (AD4), patients with allergic rhinitis (AR1, AR2) or a healthy control (control). The amounts of histamine release were measured as described in the Materials and Methods section.

Dose-response of histamine release from basophils by sweat samples of patients with atopic dermatitis and healthy volunteers

Sweat samples of two patients with atopic dermatitis and one healthy control were diluted to varying degrees and incubated with basophils of AD1. All samples tested induced histamine release in a dose-dependent manner with slopes that were similar to or less steep than those by anti-IgE antibody. The maximal release of histamine (83.1%) was induced by a sweat sample obtained from a patient with atopic dermatitis at three times dilution (data not shown). These results suggest that the histamine release was induced by a receptor-mediated mechanism, possibly with multiple kinetic interactions.

Effect of IgE removal and passive sensitization of basophils with the serum of a patient with atopic dermatitis

To study whether the histamine release was mediated by IgE or not, we removed the cell surface IgE of the leucocyte mixture with lactic acid treatment and passively sensitized the cells with the serum of a patient with atopic dermatitis or with myeloma IgE. The sweat-induced histamine release from basophils of two patients with atopic dermatitis (AD2 and AD4) was abolished by the removal of cell surface IgE (data not shown). In contrast, the basophils of a healthy control (Cont1), which were not reactive to the sweat samples,

released histamine after sensitization by the sera of the patient (AD2). Moreover, such an effect of the sensitization was totally abolished by preincubation of the basophils with myeloma IgE. The histamine release induced by anti-IgE antibody was not affected by the preincubation with myeloma IgE (Fig. 3). These results demonstrated that histamine release from basophils was dependent on specific IgE in the serum of the patient with atopic dermatitis.

Gel chromatography of sweat samples and skin reactions

For estimation of the molecular weight of the sweat antigen(s), sweat samples of three patients with atopic

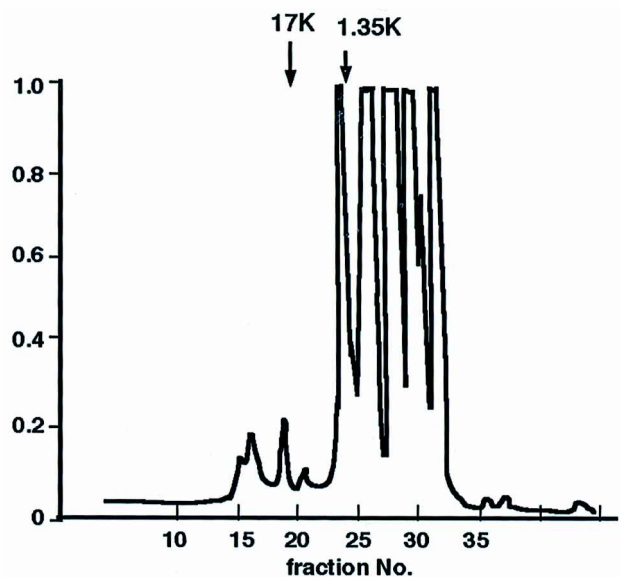


Fig. 4. Gel chromatography (top) of a sweat sample of a patient with atopic dermatitis and the skin test reactions (bottom). A concentrated sweat sample obtained from a patient with atopic dermatitis (AD5) was fractionated by gel filtration as described in the Materials and Methods sections. Aliquots of 20 μ l out of 500 μ l in each fraction were injected into the back skin of the donor (positive fraction no. 19–24 are indicated). Similar results were obtained by two other patients with atopic dermatitis and fractions of their sweat samples.

dermatitis were fractionated by gel filtration and applied for skin testing of the donor patients. All patients reacted to the fractions of their own sweat eluted between molecular markers of 17 kDa and 1.35 kDa (Fig. 4).

DISCUSSION

In this study, we have demonstrated that many patients with atopic dermatitis are sensitive to autologous sweat antigen(s) and release histamine in response to these antigen(s) through the mechanism of type I hypersensitivity. The high sensitivity (84.4%) and specificity (86.6%) of the skin test by autologous sweat samples for patients with atopic dermatitis and healthy volunteers suggest a strong association of this hypersensitivity with atopic dermatitis, in agreement with the previous study reported by Adachi & Aoki. (9). While serum IgE concentrations were correlated positively with disease severity, there was no difference of either wheal diameters or sensitivity of the skin test between patients with different degrees of disease severity. Moreover, five out of seven (71.4%) patients with allergic rhinitis also showed positive skin reactions to their sweat, suggesting the involvement of the sweat allergy in a wide range of atopic diseases. Furthermore, whereas the wheal size of the patients with atopic dermatitis was weakly correlated with serum IgE concentrations, there was no difference between the total serum IgE concentrations of patients with a positive skin test and those of patients with a negative skin test. The reason why patients with allergic rhinitis also showed positive reactions to their sweat is not clear. However, both atopic dermatitis and allergic rhinitis may be categorized as "atopic diseases", which is predisposed for the development of IgE against a variety of antigens. Taken together, the immediate skin hypersensitivity against sweat may be a common feature of atopic diseases regardless of the disease severity and targeted organs with clinical manifestations. On the other hand, the basophils of a patient with atopic dermatitis released various amounts of histamine in response to sweat samples collected from patients with atopic dermatitis, patients with allergic rhinitis or healthy volunteers. These results suggest that sweat from the general human population contains various amounts of antigen(s) with a potential to release histamine from patients with allergic diseases.

We have demonstrated that the histamine release induced by sweat samples was mediated by specific IgE antibodies by the following observations. First, the histamine release from basophils of patients with atopic dermatitis was abolished by the treatment of the basophils with lactic acid, which removes IgE from the cellular surface. Secondly, the passive sensitization of basophils of a healthy control with sera of the patients

made them reactive to the sweat. Finally, this sensitization was completely blocked by the preincubation of the basophils with an excessive amount of purified myeloma IgE. The skin reactions of three patients to fractions of their own sweat samples prepared by gel chromatography suggest that the molecular weight of the antigen(s) is between 1.35 kDa and 17 kDa. This is apparently smaller than the majority of the endogenous components reported before as to be auto-antigens for IgE (10, 11), including *staphylococcal enterotoxin A* (SEA) (27.8 kd) and *staphylococcal enterotoxin B* (SEB) (28.4 kDa) (16), which might be contained in the sweat samples. Moreover, a dot blot study of these fractions did not show any apparent binding of serum IgE in sera of these patients (data not shown). Thus, the histamine-releasing antigen(s) is present in only small concentrations in sweat and appears to be different from previously reported antigens for IgE.

The origin of the antigen(s), whether it was produced in sweat glands, degraded from keratinocytes, leaked from plasma or even a contaminant of the environment is not clear. At least 9 out of 64 individuals who showed a positive skin reaction to their own sweat showed no specific serum IgE against the house dust-mite antigen (data not shown). Moreover, at least three of them showed a negative skin reaction to the highest concentration (300 AU/ml) of a standard house dust-mite antigen preparation (Hollister-Stier, Bayer, WA) (data not shown), negating the involvement of house dust-mite antigens. Fungi and bacteria colonize the skin surface of many patients with atopic dermatitis (17). However, the histamine release study with basophils did not reveal any difference between sweat samples collected from patients and those from healthy volunteers, in terms of the amounts of antigens. Moreover, the incidence of the patients' positive skin reactions to their own sweat seems too high for a simple contamination of *Malassezia furfur* antigens, or staphylococcal antigens (SEA and SEB), against which about 40–65% (18) or 34% (19) of patients with atopic dermatitis were reported to be sensitized. Finally, the histamine-releasing activity for basophils was also detected in sweat samples that were collected from two individuals without skin lesions after thorough cleaning of the body with hot water, towel and soap (TT, ms in prep.). We therefore suggest that the antigen(s) could be an endogenously produced substance rather than an exogenous substance. The simple contamination of a leaked substance of the plasma is unlikely to explain our findings, because intradermal injection of the sera of the patients with atopic dermatitis did not induce wheal-and-flare reactions (data not shown). Thus the sweat antigen(s) is likely to be produced in the skin itself, and possibly in the sweat glands.

Valenta et al. (10) and Natter et al. (11) have

demonstrated the presence of auto-IgE against the endogenous intracellular antigens in patients with severe atopic dermatitis. The sweat factor(s) described in our study is probably different from the antigens reported by these authors because of the high histamine-releasing activity, a smaller size of the molecule(s) and the induction of skin reactions in patients with atopic dermatitis in a wide range of disease activities. However, our results do support the concept of "auto-allergy" proposed by Valenta et al. (20). Identification of the sweat antigen(s), which is currently in progress in our laboratory, should provide a better understanding of the pathogenesis of atopic dermatitis and a rationale for improved skin care for the patients.

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