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IgE Antibody to Sweat in Atopic Dermatitis

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Of 45 patients with atopic dermatitis skin-tested with their own sweat, 43 showed positive immediate-type skin reactions to titres between 1 and 256. Of 22 non-atopic patients 18 showed negative reactions. Skin reactivity of the atopic patients to the sweat and house dust did not run parallel. Radioallergosorbent test (RAST) using the sweat collected from a healthy subject detected IgE antibody in 24 atopic patients with a score from 0.5 to 3.5, whereas all the control subjects showed the score 0. This IgE antibody to sweat did not cross-react with the mite extract (Dermatophagoides farinae) or Staphylococcus aureus. These results indicate that atopic patients have specific IgE antibody to sweat. ***Key words: Sweat; IgE antibody; Atopic dermatitis.***

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It is known that patients with atopic dermatitis complain of itching during or after sweating. This phenomenon is one of the diagnostic features of atopic dermatitis (1). In addition, typical eczematous lesions of atopic dermatitis are often observed in the neck, the cubital and popliteal fossae and the other flexures which are the areas of sweat accumulation. It is clinically apparent that sweat has a significant role in the pathogenesis of atopic dermatitis. Sulzberger et al. (2) demonstrated that human sweat could produce whealing and itching when it got into cutaneous tissue, and the reaction was greater in atopic than in non-atopic individuals. They suggested that autologous sweat which was forced into the skin could produce itching in atopic dermatitis. However, the relation between atopic dermatitis and sweat is not fully understood. We investigated type I allergy to sweat in atopic dermatitis, and found that the patients with atopic dermatitis had specific IgE antibody to sweat in the serum.

MATERIALS AND METHODS

Patients

A total of 45 patients with atopic dermatitis (24 males and 21 females, mean age 21.7 years, range 6–50 years) were selected for this study. The diagnosis of atopic dermatitis was based on typical clinical features according to the diagnostic criteria of Hanifin & Rajka (1). Total serum IgE was measured by Phadebas radioimmunosorbent test (RIST) and were expressed as international units per millimeter (IU/ml).

As controls we examined 22 subjects (13 males and 9 females, mean age 25.9 years, range 6–52 years) without atopic history, consisting of 3 normals, 8 with urticaria, 3 with acne vulgaris. 8 with local skin infections.

Preparation of the sweat antigen

The patients with apparent eczematous changes or scratch marks on the back were excluded from the test. Sweat was collected by the "anaerobic" method described by Boysen et al. (3). The patient came to the hospital after taking a bath at home. An approximately 25×30 cm^2 area of the back of each patient was covered with vaseline and wrapped by Saran Wrap, and then the subject was warmed in a small sauna at 45–50°C. About 50–80 ml of clear sweat could be collected within an hour. The collected sweat was immediately sterilized using a Millipore filter and was kept frozen at −80°C until used. To differentiate an allergic reaction from an irritant reaction in skin test, the sweat was dialyzed against phosphate buffered (0.005 M, pH 7.2) saline (PBS) using a Visking tube. Protein concentration of the sweat was estimated by the method of Lowry et al. (4) using bovine serum albumin as standard and was shown to be approximately 0.4 mg/ml before dialysis and approximately 0.2 mg/ml after dialysis against PBS.

Skin test

Sweat from each patient was made in two-fold serial dilutions with saline and the series of the diluted sweat was injected into the normal-appearing skin of the patient's own forearm. A commercially available house dust allergen (Torii Co. 1:1000) was similarly diluted and injected in the opposite forearm. The dialyzed sweat was skin-tested in comparison to the undialyzed sweat in the same way. All subjects stopped taking oral antihistamine for 2–3 days prior to skin testing. The reading of the reaction was done 15 min after injection. A wheal greater than 9×9 mm or erythema greater than 20×20 mm was interpreted as positive. Skin test threshold was expressed as the maximum titre with positive reaction.

Radioallergosorbent test (RAST)

A large amount of sweat was collected from one of us (J. A.) who was healthy and non-atopic. The collected sweat was processed as described and freeze-dried, then solubilized with distilled water, and finally concentrated 2, 10 AND 50 times. These were coupled with cyanogen bromide-activated paper discs at 4°C for 3 days. Part of the 50 times-concentrated sweat was dialyzed against physiological saline. IgE antibody to sweat in the serum was measured using these sweat-coupled discs and Pharmacia RAST kit. The maximum value

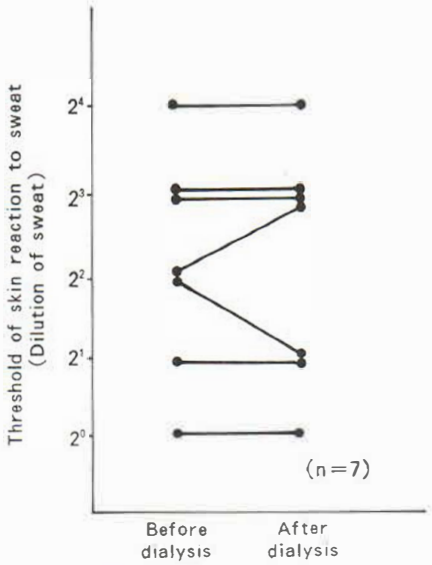

Fig. 1. Comparison of the antigenic activity of the sweat before and after dialysis.

obtained by four antigen preparations was regarded as the IgE antibody titer of the patient.

RAST-inhibition test

To examine cross-reactivity of the antibody to sweat, RAST inhibition test was performed using the mite extract and Staphylococcus aureus in a case who showed the RAST score (2.0). Since proper amounts of these two antigens to use for antibody absorption were not known, the assumption was made that 0.1 mg of the mite extract (Dermatophagoides farinae) or formalin-fixed Staphylococcus aureus (protein A negative Wood 46 strain) was equivalent to 1 mg sweat protein. The sweat was prepared in 1000, 100, 10, 1 and 0.1 μg protein/ml saline, and the mite extract and Staphylococcus aureus were prepared in 100, 10, 1, 0.1 and 0.01 μg weight/ml saline, respectively. Aliquots of the serum (0.08 ml each) were incubated at 37°C for 30 min with the same volumes of the five concentrations of the three antigens mentioned above in parallell with the same volumes of saline solution as the control. After incubation IgE antibody to sweat in each sample was measured by RAST using the discs coupled with 50 times concentrated and dialyzed sweat.

RESULTS

Skin test thresholds to own sweat in the patients with atopic dermatitis and in the normal controls

Forty-three of 45 atopic patients showed positive immediate-type skin reactions to sweat at titres between 1 and 256 (geographic mean of 43 positives; titre 11.8), whereas only 4 of 22 control subjects (3 with urticaria and 1 with folliculitis) showed positive reaction at titres between 2 and 8 (geographic mean of 4 positive cases; titre 6. This difference is statistically significant (chi square test, $p<0.01$). All subjects injected with sweat complained of piercing sensation during infusion of the sweat into the skin at the sweat concentration between titres 1 and 4. The sensation stopped immediately after infusion.

The effect of dialysis on the antigenic activity of the sweat

The antigenic activity of the sweat before and after dialysis as demonstrated by skin test thresholds showed no difference when examined in 7 patients (Fig. 1).

Comparison of the skin reactivities of the patients with atopic dermatitis to sweat and house dust

Fig. 2 demonstrates the relation between the skin reactivities to sweat and house dust of 45 patients with atopic dermatitis. The skin test thresholds to house dust were titres 1 000 to 64000 (mean of 36 positive cases; titre 8 300). The skin test thresholds to the two antigens did not run parallel ($r=0.2247$).

The relation of the serum IgE levels and the skin test thresholds to sweat in the patients with atopic dermatitis

Fig. 3 shows the relation between geographically plotted serum IgE level and the skin test threshold to

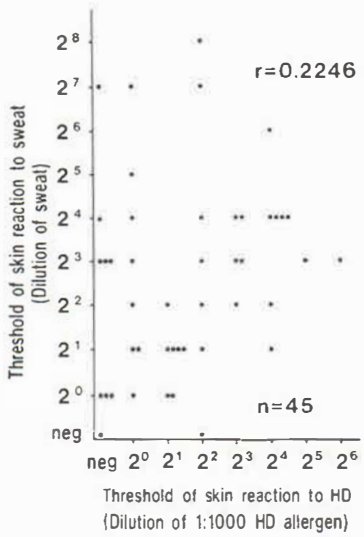

Fig. 2. The relation between the skin test reactivities to sweat and house dust in atopic dermatitis. No significant correlation was observed between the two.

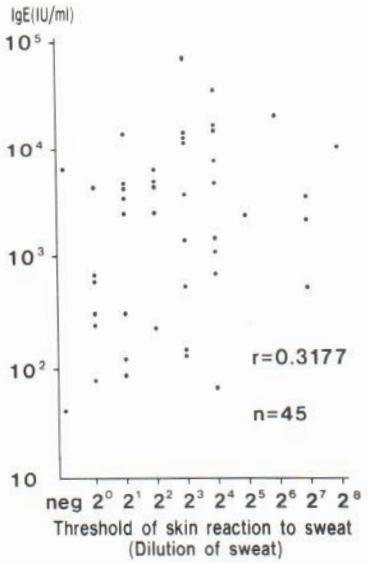

Fig. 3. The relation between serum IgE levels and the skin test thresholds to sweat in atopic dermatitis.

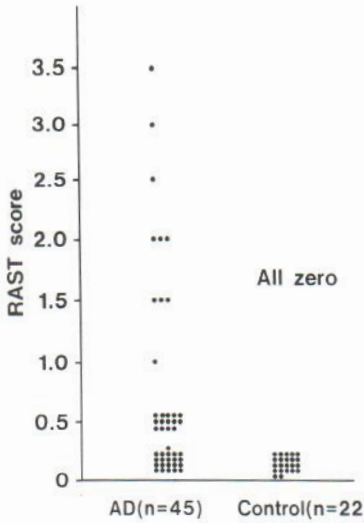

Fig. 5. IgE antibody titers to the sweat in patients with atopic dermatitis and controls.

sweat in each of 45 patients with atopic dermatitis. A statistically significant correlation was detected between the two ($r=0.3177$, $p<0.05$).

IgE antibody to sweat

Various RAST scores were obtained in the same serum sample by using different preparations of sweat antigens. Generally the higher the antigen concentrations the higher the scores and also dialyzed antigen gave higher scores (Fig. 4). Ten patients showed RAST scores from 1.0 to 3.5, 14 gave the score 0.5 and 21 gave the score 0. All 22 control subjects showed RAST score 0. The distribution of RAST scores shown in Fig. 5 seems to indicate that the RAST score 1.0 or higher may be a reliable marker for the presence of IgE antibody to the sweat. The data in Fig. 6 demonstrate no correlation in atopic dermatitis

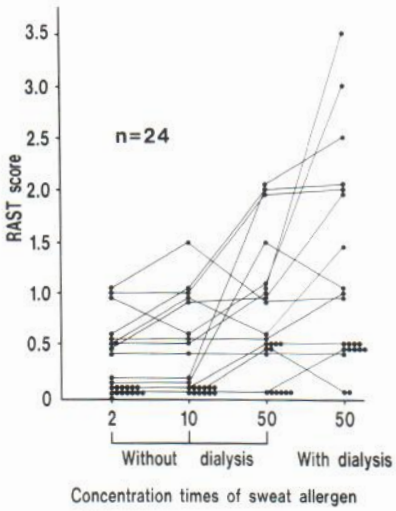

Fig. 4. RAST scores to the sweat detected by different sweat concentrations coupled to the disc.

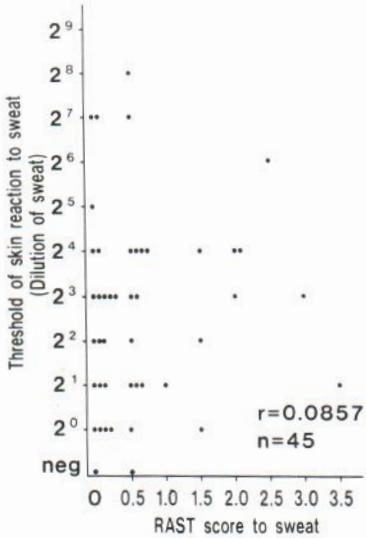

Fig. 6. The relation between the RAST scores to the sweat and the skin test thresholds to the sweat in patients with atopic dermatitis. No significant correlation was observed between the two.

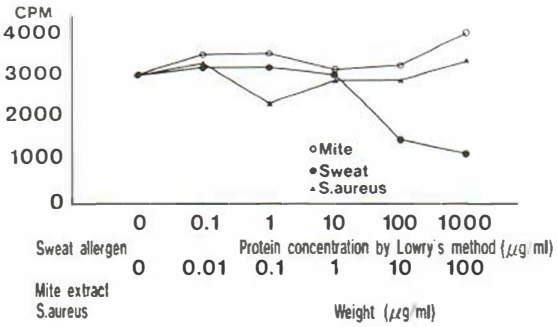

Fig. 7. The results of RAST-inhibition test. Mite extract and S. aureus did not reduce the conjugation of IgE antibody to the disc coupled with sweat.

between the skin test thresholds and the RAST scores ($r=0.0857$).

Specificity of the antibody to sweat

Amounts of IgE antibody to sweat were decreased by adding sweat to the serum in a dose dependent fashion, whereas they were not changed by adding the mite extract or Staphylococcus aureus (Fig. 7).

DISCUSSION

It was in 1953 that Sulzberger et al. (2) demonstrated that the sweat of the patients with atopic dermatitis induced wheal and flare reactions when injected into the patients' own skin. He hypothesized that sweat retention often observed in the skin of atopic dermatitis (dry skin) might cause sweat leakage into the skin and give irritant effects to the skin (5). Since then the relation of atopic dermatitis and sweat has continued to be the subject of investigation. Cotton et al. (6) studied the sweat of normal and atopic subjects by physical and chemical methods but could not detect any differences between the two. Förström et al. (7) found that human sweat had higher IgE values in atopic patients than in normals. Wilkinson et al. (8) detected anti-sweat precipitins (IgG) in the serum of atopic dermatitis, but they were also detected in hospital staff and in many skin disorders. Up to now, no one has attempted to demonstrate IgE antibody to own sweat in any disease conditions, including atopic dermatitis. There are possibly two reasons for this. First, sweat was thought to have nonspecific irritant effects to the skin that might make the analysis of immunological reactions difficult. In this investigation a piercing sensation was actually experienced in all subjects during infusion of the sweat into the skin. However, sweat induced a typical wheal and flare reaction in some subjects and not in the others. Therefore, the type I allergic reaction to the sweat seems to be independent from the irritant effects of the sweat. Secondly, sweat is usually contaminated with many substances, such as horny cells, skin surface bacteria and contactants (house dust and mites). To minimize contaminations with impure substances, we adopted Boysen et al. 's "anaerobic" method for sweat collection which is considered to be the only available method at present.

However, there is still a possibility that it may be contaminated with other antigenic substances. Patients with atopic dermatitis are often allergic to house dust (9, 10), mite (11), human dander (12, 13), or Staphylococcus aureus (14, 15), and therefore the antibody to sweat must be shown not to cross-react with these antigenic substances. Moreover, the patients with atopic dermatitis are colonized with Staphylococcus aureus on both involved and uninvolved skin (14). We compared the skin reactivities of atopic patients to the sweat and the house dust, but found statistically insignificant correlation between the two. Therefore, we do not think that sweat has serious cross-reactivity with house dust. We used RAST-inhibition test to examine cross-reactivity with mite and Staphylococcus aureus, and no cross-reactivity was demonstrated. Only the test with human dander was left. Although Berrens & Guikers (16) demonstrated that atopic dermatitis had IgE antibody to human dander, it is a complex mixture of cornified epidermal cells, sebum, sweat and numerous microorganisms. Cross-reactivity between sweat and human dander has been reported (17), but Sulzberger et al. (2) observed that the skin test with scale extract showed different skin reactions from sweat. Silpananta & Wilkinson (18) described that the characterization of main antigenic components of sweat differed from that of the human dander. From these data and since we did not know a method to collect pure dander that did not contain sweat, we omitted examination of cross-reactivity between sweat and dander. The sweat obtained by Boysen et al. 's method contained practically no horny materials. We believe that the results of our investigation can be regarded as evidence for the presence of IgE antibody to sweat. The incidence of positive IgE antibody to sweat (RAST score >0.5, 22.2%) was much less than positive skin tests (95.6%). However, this is similar to the results reported on mite allergy in chronic urticaria by Yamamoto et al. (19), who showed that histamine

release from leukocytes could be observed well before the detection of IgE antibody.

Skin test thresholds to sweat were correlated with serum total IgE levels in the atopic dermatitis patients we studied, Stone et al. (20) have shown a relation between various clinical data and IgE levels.

Major proteins of normal human sweat are albumin and alpha 1-antitrypsin (21), and more than 400 polypeptide components (22). Analysis of sweat antigens is left for future investigations.

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