Comparison of Immune Reactivity Profiles against Various Environmental Allergens between Adult Patients with Atopic Dermatitis and Patients with Allergic Respiratory Diseases

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To clarify the pathomechanisms underlying the involvement of different organs by atopic dermatitis (AD) and allergic respiratory disease (ARD), we compared the immune reactivities to various environmental allergens between 46 adult patients who suffered only from AD but were without any history of ARD and 41 patients who had only ARD, using a RAST FEIA (radioallergosorbent test/fluoroenzyme immunoassay) and a scarification patch test. We also studied 42 healthy adult subjects in a similar fashion. Total serum IgE antibody levels were found to be far higher in the AD group than in the ARD and healthy control group, and RAST revealed that the AD group was sensitized to far larger numbers of allergens such as food mix, cereal mix, fungus mix and Candida albicans than were the other groups. The ARD group displayed a high incidence in RAST, comparable to that of the AD group, only against Japanese cedar and grass pollen mix antigen. However, the most remarkable difference in the immune reactivity profiles was that the AD group showed a uniquely higher RAST score and a lower incidence of positive patch test reactions to C. albicans antigen than did the ARD group. The reactivities in the ARD group to C. albicans antigen did not differ from those in the control group. Our present data suggest that a more pronounced shift from Th1 to Th2 cells, reactive against various allergens, takes place in AD patients. Key words: serum IgE antibody; patch test; C. albicans.

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The lesions of atopic dermatitis (AD) share several histological characteristics with delayed type contact hypersensitivity provoked in non-atopic subjects (1). Mitchell et al. (2) have demonstrated that patch testing for 48 h with house dust mite antigen in atopic subjects can induce eczematous lesions. These observations suggest that the pathomechanisms of AD lesions and those of positive patch test reactions to environmental allergens are closely related and may represent delayed type contact hypersensitivity. In spite of accumulating evidence suggesting that atopic diseases can be mediated by Th2 type T cells and IgE specifically reactive to environmental allergens, it is still not clear why mucosal reactions are the main features in allergic respiratory disease (ARD), either allergic rhinitis or atopic asthma, while skin manifestations are predominant in AD. To characterize the distinct immune reactivities underlying AD and ARD, we studied patients by comparing their serum total IgE levels, specific IgE antibodies and scarification patch test responses to various environmental allergens.

MATERIALS AND METHODS

Patients and controls

We studied the following three Japanese groups: an AD group consisting of 46 patients with AD who did not have any history of ARD (23 males and 23 females aged from 17 to 34 years, mean 22 years), an ARD group consisting of 41 patients who had only ARD (23 males and 15 females aged from 17 to 39 years, mean 26 years) and a control group consisting of 42 healthy volunteers who did not have any personal or family history of atopic diseases (21 males and 21 females aged from 19 to 38 years, mean age 24 years). We selected adult patients consecutively seen by us. All the patients with AD fulfilled the criteria of Hanifin & Rajka (3). Most of them had moderate to severe lesions according to the criteria of Rajka & Langeland (4). In the ARD group, 34 subjects had allergic rhinitis, 4 had atopic asthma and 3 had both. Patients with allergic rhinitis in the ARD group were so diagnosed by otorhinolaryngologists or were those who repeatedly showed such typical symptoms as sneezing, nasal blockage and discharge only during a restricted season. All patients with atopic asthma were diagnosed by a pneumologist. The absence of skin lesions in the ARD group was confirmed when their skin tests were carried out from autumn to winter.

Detection of antigen-specific and total serum IgE antibodies

Total serum IgE antibodies were measured by fluoroenzyme immunoassay (FEIA, CAP system, Pharmacia, Uppsala, Sweden) and antigen-specific IgE antibodies by RAST FEIA, CAP system (Pharmacia, Uppsala, Sweden). The allergens used were: Dermatophagoides farinae (D. farinae), weed pollen mix (common ragweed, mugwort, marguerite, dandelion and golden rod), grass pollen mix (cocksfoot, sweet vernal grass, Bermuda grass, timothy and common reed), Japanese cedar, food mix (egg white, milk, wheat, peanut and soya bean), cereal mix (wheat, corn, rice, sesame seed and buckwheat), fungus mix (Candida albicans (C. albicans), Penicillium notatum, Cladosporium herbarum, Aspergillus fumigatus, Alternaria tenuis and Helminthosporium intermedium) and C. albicans. RAST scores of 2 or higher were defined as positive. (RAST scores were defined as: score 0 < 0.35 kUA/l, 0.35 ≤ score 1 < 0.7 kUA/l, 0.7 ≤ score 2 < 3.5 kUA/l, 3.5 ≤ score 3 < 17.5 kUA/l, 17.5 ≤ score 4 < 50 kUA/l, 50 ≤ score 5 < 100 kUA/l, 100 kUA/l ≤ score 6.)

Skin tests

All subjects were prohibited from taking antihistamines, systemic steroids or applying topical steroids on the volar aspects of the forearms for more than 1 week before the skin testing. Chamber scarification patch tests and immediate prick tests were performed but only the results of the chamber scarification tests are reported here. We observed a correlation between
the results of RAST and those of immediate prick tests to environmental allergens generally, though the incidence was slightly lower in the latter (5). Thus we present here the data of specific IgE antibodies only by those of RAST. Chamber scarification patch tests were carried out as described by Frosch & Kligman (6), with minor modifications. We occluded the sites for 48 h, then judged the skin reactions 72 h after application of the patches according to the ICDRG (International Contact Dermatitis Research Group) criteria of patch tests (7). The skin tests were performed as explained in detail elsewhere (5).

The skin tests were approved by the ethics committee of Tohoku University School of Medicine. All the subjects gave informed consent before the examinations.

Allergens
All antigens used in the skin test were purchased from Torii Co, Tokyo, Japan. We used the same batch of allergens on all patient groups. The allergens of cockfoot, ragweed, Japanese cedar, egg white and D. farinae were prepared by extracting them in 50% glycerin and 5% NaCl aqueous solutions (8). The allergens of C. albicans and Penicillium notatum were freeze-dried material from respective culture fluids with 50% glycerin. The concentrations of allergen provided by the manufacturer were: pollens, 1:20 weight by volume in 50% glycerin; fungus and D. farinae, 1:100; and other allergens, 1:10; protein nitrogen unit: Japanese cedar, 3,100 U/ml; ragweed, 27,300 U/ml; cockfoot, 22,500 U/ml; C. albicans, 6,860 U/ml; Penicillium notatum, 18,900 U/ml; D. farinae, 12,270 U/ml; and egg white, 73,790 U/ml. Each allergen was diluted 1:1 with control solution (50% glycerin, 5% NaCl) (Torii Co), to which we added phenol to obtain a final concentration of 0.5%.

Statistical analysis
Differences were analyzed by using the \( \chi^2 \) test, Fisher’s exact probability test or the Mann-Whitney test. Fisher’s exact probability test was used when subjects in the divided groups were fewer than 5. For the other cases the \( \chi^2 \) test was used.

RESULTS
Total IgE
Both the AD and ARD groups showed a significantly higher IgE titer than the control group. Especially, the mean IgE titer of 1,745.2 kU/l for the AD group was much higher than those of the ARD and control groups (p < 0.0001 for AD versus ARD: Mann-Whitney test). The mean titer of 402.8 kU/l for the ARD group was significantly higher than 160.5 kU/l for the control group (p < 0.01).

Antigen-specific IgE antibodies in the serum (RAST)
As for RAST scores to weed pollen mix and mite allergens (D. farinae), the cumulative frequency distribution showed a fairly high level for the AD group (p < 0.05, Mann-Whitney test), whereas only a small difference was found between the AD and ARD groups for grass pollen mix and Japanese cedar (Fig. 1). Although the control group also had IgE antibodies to mites and pollens, their titer was much lower than those of the AD and ARD subjects. On the other hand, the RAST scores of the AD group to fungus antigens such as fungus mix and C. albicans and food antigens such as food mix and cereal mix were significantly higher than those of the ARD subjects (p < 0.0001 for fungus mix, p < 0.0001 for C. albicans; p < 0.01 for food mix and p < 0.01 for cereal mix).

Multi-allergen sensitization
We examined the number of allergens to which each patient had a positive RAST score by counting the number of positive RAST scores to the following 6 allergens: D. farinae, weed pollen mix, grass pollen mix, Japanese cedar, food mix and fungus mix. The patients with AD tended to react to larger numbers of allergens than those with ARD; 37% of the AD patients reacted to 4 or more allergens, whereas only 13% of the ARD patients did so (p < 0.05, chi-square test).

Patch test reaction
Both the AD and control groups revealed a similarly high incidence of positive reactions to C. albicans: 90% and 84%, respectively. In contrast, the AD group showed only a 34% positive incidence (p < 0.001 for AD versus ARD, \( \chi^2 \) test). Both the AD and ARD groups reacted to D. farinae allergen, but the incidence of the ARD group (39%) was higher than that of the AD group (18%), being comparable to that of the control group (35%). As for pollens such as ragweed and cockfoot, the AD and ARD groups showed a similar incidence of positive reactions (ragweed: 13% versus 15%, cockfoot: 23% versus 17%), whereas to Japanese cedar, a major allergen for Japanese patients with allergic rhinitis, the ARD group showed a significantly higher incidence of positive reactions (29%) than the AD (5%) (p < 0.01) and control groups (3%). The AD group had a somewhat higher incidence of positive reactions to egg white (10%) than the ARD (0%) and control groups (3%), though the incidence in all 3 groups was generally low. Likewise, we could not find any specific tendency in patch test reactions to Penicillium notatum allergen in the atopic patients (AD: 5%; ARD: 7%, control: 0%).

Correlation between RAST and patch test
We compared the reactivity patterns of RAST and patch testing in the patients with AD and in those with ARD against D. farinae, Japanese cedar, food and C. albicans allergens. For the RAST scores of food mix allergen, which contained egg white, we selected the egg white allergen patch test. The reaction pattern to antigens other than C. albicans was rather similar in these groups. However, there was a striking difference in the reactivity to C. albicans allergen between the two groups (Fig. 2), namely, the percentage of those with positive RAST and negative patch tests was higher in the AD group, while the percentage of those with negative RAST and positive patch tests was strikingly higher in the ARD group (0.0001, chi-square test).

DISCUSSION
Many reports have described the exacerbation of atopic diseases after exposure to various environmental allergens, especially mite and pollen antigens (9-11). From our present study of serum IgE antibodies and skin reactivities of AD and ARD patients, however, we could not find any distinguishing characteristics in the immunological reactivities to these allergens between the two groups that determine the anatomic site of involvement by allergic reactions, except for a marked IgE production against large numbers of environmental allergens in the AD group, together with a uniquely higher RAST score.
RAST SCORE

Fig. 1. Cumulative frequency distribution of the RAST scores in the AD, ARD and control groups.

![RAST Score Graph](image)

RAST

![Patch Test Graph](image)

Fig. 2. The reactive pattern of the AD group and ARD group against *C. albicans* allergen observed with RAST and patch tests. Those with RAST scores of 2 or higher were grouped into RAST(+).

and a lower incidence of positive patch test reactions to *C. albicans* antigen than the ARD group.

The most remarkable differences in the immune reactivities against environmental allergens in the present study were those against *C. albicans* allergen. All healthy individuals except infants show positive patch test reactions to *C. albicans* antigens (12). The control group did not show any positive patch test reaction to one of the air-borne fungi, *Penicillium notatum*. *C. albicans* is a resident microorganism so ubiquitous, even in the gastrointestinal tract, that even healthy individuals might be sensitized easily. Recently, we have reported that patients with AD showed significantly decreased contact sensitivity to *C. albicans*, despite the fact that, generally, both IgE-mediated skin hypersensitivity and delayed type hypersensitivity to various environmental antigens are pronounced in such patients (5). We have observed reduced proliferative responses of peripheral blood mononuclear cells to *C. albicans* in AD patients (13). In the present study, increased RAST scores, associated with decreased patch test reactions to *C. albicans* allergen, appeared to be specific only to the AD group. We did not find any such differences in the immunological reactivity to *C. albicans* antigen between the ARD group and the control group.

In experimental dermatophytosis, another kind of superficial fungus infection, Jones et al. (14) reported that the development of immediate type hypersensitivity reaction to trichophytin, the fungal allergen, replaced delayed trichophytin reactivity in subjects with atopic background, who also at first developed delayed hypersensitivity as non-atopies. Based on these data, it is likely that repeated exposures to the allergens of the ubiquitous, resident microorganism, *C. albicans*, through the barrier-damaged skin in AD patients, are responsible for the development of this unique dissociation of immediate and delayed hypersensitivity reactions. We further speculate that the more atopic constitution noted in eczematous patients is probably accompanied by a more pronounced shift from Th1 to Th2 cells, which would explain the higher RAST score and lower incidence of patch test reactions to *C. albicans* in comparison with the ARD patients.

The present results suggest a role of other factors that determine the difference in the localization of inflammation between AD and ARD. Interestingly, Dohi et al. (15) demonstrated that, although the titer of IgE specific to house dust mite antigens was much lower in patients with ARD than in patients with AD, the concentrations of allergens that induce an immediate asthmatic response were much lower in the former than in the latter. Furthermore, only patients with
ARD showed the late asthmatic response. These data strongly suggest the significance of local reactivity in determining the development of ARD.

As a factor possibly responsible for the enhancement of local reactivity we may consider the difference in the homing receptors of T cells between AD and ARD. Picker et al. (16, 17) have reported the presence of skin-homing T lymphocytes. These T cells have a unique homing receptor, called cutaneous lymphocyte-associated (CLA) antigen, which can interact with E-selectin. Rossiter et al. (18) demonstrated that Th2 helper T cell clones specific to house dust mite antigen, derived from epicutaneously challenged skin, expressed CLA antigen, while those derived from peripheral blood did not express it. Therefore, it is conceivable that Th2 cells specific to mite or pollen antigens in patients with AD and patients with ARD differ in the expression of CLA antigen, and that only Th2 cells expressing CLA antigen, which may be present only in AD patients, can induce allergic reactions in the skin (19). In addition, it is possible that a decrease in Th1 type T cells with CLA antigen in AD patients might be important for the chronicity of severe dermatitis, an issue that needs to be studied further.

Finally, it is well known that patients with AD have functional defects in the stratum corneum. Even in just xerotic uninvolved skin, the stratum corneum of patients with AD tends to show increased permeability as well as a decreased water-holding capacity, which induces a proclivity to cracking (20). In contrast, the skin of ARD was reported to show no abnormal functional changes either under baseline conditions or after exposure to sodium lauryl sulfate (21). Therefore, environmental allergens can easily penetrate into the skin through the compromised stratum corneum in patients with AD. Indeed, there are several papers reporting that patients with AD reacted more strongly than those with ARD when patch-testing of pollens was performed without scratching (9, 22, 23). Moreover, such atopy patch tests with Aeroallergens in patients with AD were reported to induce the disturbance of the stratum corneum barrier function much earlier, compared to the disturbance induced by contact allergens in patients with contact dermatitis (24).

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