

Cutaneous late phase reaction in adult atopic dermatitis patients with high serum IgE antibody to *Dermatophagoides farinae*: correlation with IL-5 production by allergen-stimulated peripheral blood mononuclear cells

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Received 22 November 2001; received in revised form 23 January 2002; accepted 24 January 2002

Abstract

It is known that, in patients of allergic asthma and rhinitis, the late-phase reaction (LPR) occurs 6–12 h after allergen challenge, but there are few reports concerning cytokine production in the cutaneous LPR in atopic dermatitis (AD). We report here the results of our study on the relationship between the cutaneous LPR and the production of cytokines such as IL-4, IL-5, IL-2 and IFN- γ by peripheral blood mononuclear cells (PBMC) of AD patients. We selected 29 pure AD patients with no history of atopic airway diseases who showed high serum IgE antibody against *Dermatophagoides farinae* and performed skin prick testing with three different antigens and observed the resultant cutaneous reactions in 23 of the AD patients. Furthermore, we measured the cytokine production by the cultured PBMC under the stimulation of the antigens and compared it with the results of the skin tests. 13 (57%) of these 23 AD patients demonstrated positive LPR in response to *D. farinae*, and the mean concentration of IL-5 produced by PBMC was higher in these LPR-positive AD patients compared to the LPR-negative ones. Additionally, we noticed that there was a positive correlation between the mean diameter of the erythema of LPR and the level of IL-5 production by PBMC in the LPR-positive patients. We suggest that there are at least two groups in AD patients, i.e. LPR-positive and LPR-negative ones. The observation of LPR can be an important and practical way to classify AD patients into subgroups, which may enable us to regard IL-5 or eosinophils as a target for treatment. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Atopic dermatitis; Late-phase reaction; Interleukin-5

1. Introduction

There have been several criteria [1–3] proposed for the diagnosis of atopic dermatitis (AD). They are based on the clinical symptoms, distribution

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of the eruptions, and the duration of the illness. Even in AD patients with similar signs and symptoms, there are considerable variations in their skin reactivity to environmental allergens as well as in their immunoreactivity [4]. Thus, some AD patients have high serum IgE antibody levels, while others show low levels [5]. There are also differences in the kinds of triggering factors, such as allergic and non-allergic ones [6–10], which may be related to the development and persistence of AD. Even among AD patients with high titers of serum IgE antibodies, the kinds and numbers of allergens involved in the onset of AD are different [4] and they can also change with time [11]. Among various allergens, a high incidence of skin reactivity has been demonstrated against a house dust mite allergen because it is the most prevalent one in our daily lives. Moreover, the types of the underlying allergic reactions vary as well: some patients show IMRs, while others show delayed type hypersensitivity responses to environmental allergens [5,8]. Imayama et al. [5] reported that they could classify AD patients into four subgroups by their IgE levels and atopic patch tests to mite allergen. Thus, AD patients

even diagnosed by the aforementioned criteria may have remarkably different backgrounds.

It has been demonstrated that a group of AD patients shows the late-phase reaction (LPR) to allergens, which is histopathologically characterized by eosinophil infiltration [12,13]. However, our preliminary studies with prick testing revealed that not all the AD patients with positive immediate reaction (IMR) to aeroallergens demonstrated the LPR. Based on these observations, in this study we tried to determine the relation of the LPR to IMR by employing a prick test. Moreover, by evaluating the cytokine profile produced by allergen-stimulated peripheral blood mononuclear cells (PBMC) obtained from AD patients with high radioallergosorbent test (RAST) scores against a *Dermatophagoides farinae* allergen, we investigated the relationship between the LPR and the patterns of cytokines released by allergen-stimulated PBMC based on comparisons between the patients with LPR and those without it.

2. Material and methods

2.1. Patients and control subjects

We selected 29 adult AD patients (17 males and 12 females) aged 25 ± 7 years (mean $\pm$ S.D.) with high scores (> 3) of specific IgE antibody against antigen *D. farinae* who, however, did not have any history of other atopic airway diseases. The severity of the skin lesions in all our AD patients was evaluated with the criteria proposed by Rajka and Langeland [2]. Oral medications such as anti-histaminics and anti-allergic agents were discontinued 3 days before the skin and in vitro tests. We also studied 13 randomly selected age- and sex-matched healthy controls (HC) aged 26 ± 4 years (mean $\pm$ S.D.). The RAST scores of these subjects are shown in Tables 1 and 2. Informed consent was obtained before the study.

2.2. Prick tests

We used three antigens, i.e. *D. farinae*, *Alternaria* and *Candida albicans* obtained from Torii Pharmaceutical Co, Ltd., Japan. *Alternaria* and

Table 1
Results of RAST score in 29 AD patients with high scores (> 3) of specific IgE antibody against antigen *D. farinae*

RAST score	<i>Alternaria</i> (%)	<i>C. albicans</i> (%)
Not evaluated	17/29 (59)	0/29 (0)
0	2/29 (7)	5/29 (17)
1	1/29 (3)	1/29 (3)
2	4/29 (14)	3/29 (10)
> 3	5/29 (17)	20/29 (70)

Table 2
Results of RAST score in 13 HCs

RAST score	<i>D. farinae</i> (%)	<i>Alternaria</i> (%)	<i>C. albicans</i> (%)
Not evaluated	4/13 (31)	5/13 (38)	4/13 (31)
0	6/13 (46)	7/13 (54)	8/13 (61)
1	1/13 (8)	0/13 (0)	0/13 (0)
2	2/13 (15)	1/13 (8)	0/13 (0)
> 3	0/13 (0)	0/13 (0)	1/13 (8)

C. albicans were chosen as controls of environmental allergens, to which the subjects were not specifically hypersensitive according to their RAST scores. We placed a droplet of each allergen on the skin of the middle portion of the volar forearm and pricked the skin with a 27-gauge needle. The prick tested sites were examined four times, i.e. after 15 min, 8 h, 24 h, and 48 h. We measured the longer and shorter diameters of developing wheals at 15 min, and erythema at 8, 24, and 48 h. We evaluated the IMR and LPR as positive when the subjects showed wheals, which were equal to or greater than 9 mm at 15 min, and erythema equal to or greater than 5 mm in diameter at 8 h [14–16]. In our preliminary study we confirmed the reproducibility of this method to evaluate these skin reactions.

2.3. Collection of PBMC and their culture with each allergen

We collected 25 ml of heparinized venous blood from each subject. PBMC were separated by a density gradient method using Ficoll–Paque and suspended at a density of 2×10^6 cell/ml in RPMI 1640 supplemented with 10% fetal calf serum, L-glutamine (5 mM) and antibiotics containing penicillin, streptomycin, and fungizone (Sigma, St. Louis, USA). One milliliter of PBMC suspension was added to each well of 24-well plates. They were cultured in the presence of one of the following allergens: *D. farinae*, *Alternaria*, and *C. albicans* at a final concentration of 10 µg/ml (all were gifts from Torii Pharmaceutical Co, Ltd., Japan). After 3-day-culture, we collected the supernatants and assayed them for the level of Th1-cytokines, interleukin 2 (IL-2) and interferon gamma (IFN-γ), and Th2-cytokines, IL-4 and IL-5 by using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Inc. Minneapolis, USA). Cytokine measurements were performed in duplicate. The minimum detectable doses of IL-2, IFN-γ, IL-4, and IL-5 were 7, 3, 0.13, and 3 pg/ml, respectively.

2.4. Statistical analysis

For statistical analyses, we used analysis of variance (ANOVA) plus Scheffe's *F* test and Spearman's tests.

3. Result

3.1. Profile of cytokines produced by allergen-stimulated PBMC

At first we assayed the Th1- and Th2-cytokine levels in the supernatants obtained from 3-day cultures of PBMC from 29 AD patients with high IgE scores and 13 HC in the presence of each allergen, comparing the results obtained by the stimulation with the different allergens. We also examined the differences between the AD patients and HC.

As for Th1 cytokines, the production of IL-2 or IFN-γ showed a statistically significant increase ($P < 0.01$) in the presence of *C. albicans* in both the AD patients and HC as compared with those without any allergen (Figs. 1 and 2). On the other hand, we did not find any changes in their production in response to either *Alternaria* or *D. farinae* allergens, to which both the AD patients and HC displayed only moderate IL-2 and IFN-γ production. However, a tendency was noted that the production was lower in the AD patients than in HC, although there was no statistical difference except for that of the IFN-γ production in response to *Alternaria* allergen ($P < 0.05$) (Figs. 1 and 2).

As for Th2-cytokines, IL-4 and IL-5, their production by the PBMC from the AD patients in response to *C. albicans* and *D. farinae* was significantly increased ($P < 0.05$) when compared to that produced without an allergen (Figs. 3 and 4). IL-4 production by the PBMC stimulated with *D. farinae* tended to be higher in the AD patients than that found in HC, although there was no statistical difference (Fig. 3). On the other hand, IL-5 production by the PBMC in response to *D. farinae* was significantly higher in the AD patients ($P < 0.05$) than in HC (Fig. 4). Such a significantly high level was not noted with *C. albicans* in the AD patients.

3.2. Profile of balance of Th1/Th2 cytokine production in AD and HC

We also examined the balance of the production of Th1-cytokine, IFN-γ, and Th2-cytokine, IL-4,

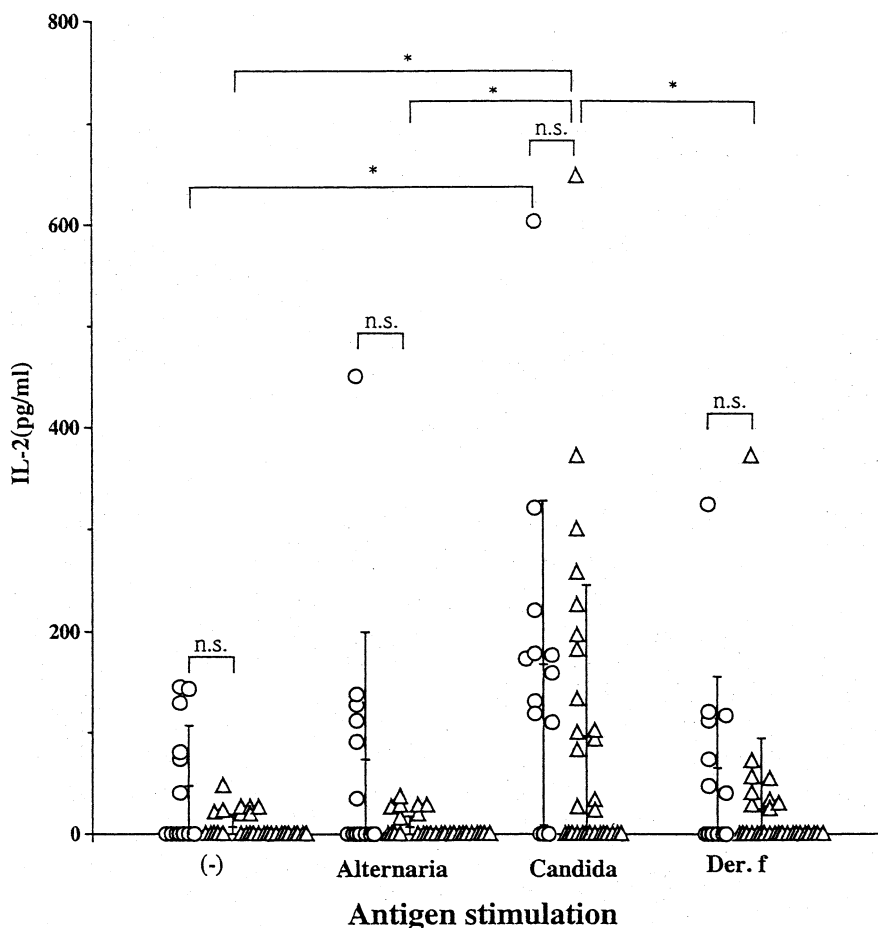


Fig. 1. IL-2 production by allergen-stimulated PBMC in AD patients and HC. Bars show means and standard deviations. There was an increment of the IL-2 production in response to *C. albicans* antigen by comparison with those in response to other allergens. Δ , AD ($n = 29$); $\circ$, HC ($n = 13$). * $P < 0.05$; n.s., not significant.

against each allergen. The cytokine production by PBMC in response to *D. farinae* showed a definite Th2 dominance in the AD patients in contrast to the Th1 dominance noted in the control subjects (Fig. 5A). A similar pattern of response was found with *Alternaria* (Fig. 5B), but not with *C. albicans* (data not shown). There were no statistically significant correlation between IFN- γ and IL-5 or between IFN- γ and IL-4.

3.3. Immediate reaction (IMR) and late-phase reaction (LPR) at prick test sites

We could perform skin tests in 23 out of the 29

AD patients with high RAST scores to *D. farinae* from whom the reactivities of PBMC were studied. All the 23 AD patients showed positive IMR 15 min after pricking with *D. farinae*, while they did not show any IMR against *Alternaria* or *C. albicans* (Table 3). The immediate wheal and erythematous reactions to *D. farinae* disappeared in a few hours, leaving ill-defined erythema equal to or greater than 5 mm in diameter at the same prick test sites up until 24–48 h in 13 out of the 23 AD patients, whereas none of them showed such LPR to either *Alternaria* or *C. albicans*. There was no difference in the specific IgE antibody levels between the LPR-positive and -negative patients.

We also performed the same skin tests in 10 out of the 13 HC. One of them in whom there was no IL-5 production by his PBMC in response to *D. farinae* showed positive-LPR against *D. farinae*. Unfortunately, his RAST score was not evaluated. Including him, 4 of the 10 HC showed positive IMR against *D. farinae* though their RAST scores were zero (Table 4).

3.4. High level of IL-5 production by PBMC in response to *D. farinae* in LPR-positive AD patients

Since the previous data showed a marked incre-

ment of IL-5 production by the PBMC of the AD patients in response to *D. farinae*, we next determined whether there was any correlation between positive LPR skin tests and IL-5 production by PBMC in response to *D. farinae*. The results showed that IL-5 production was significantly higher in the LPR-positive AD patients (mean $\pm$ S.D. = 58.3 ± 67.5 pg/ml) than in the LPR-negative AD patients (mean $\pm$ S.D. = 7.9 ± 13.6 pg/ml) ($P < 0.05$), indicating a close relationship between LPR and IL-5 production by PBMC in response to *D. farinae* (Fig. 6A). However, there was no significant difference in IL-4 production between the LPR-positive and LPR-negative AD patients.

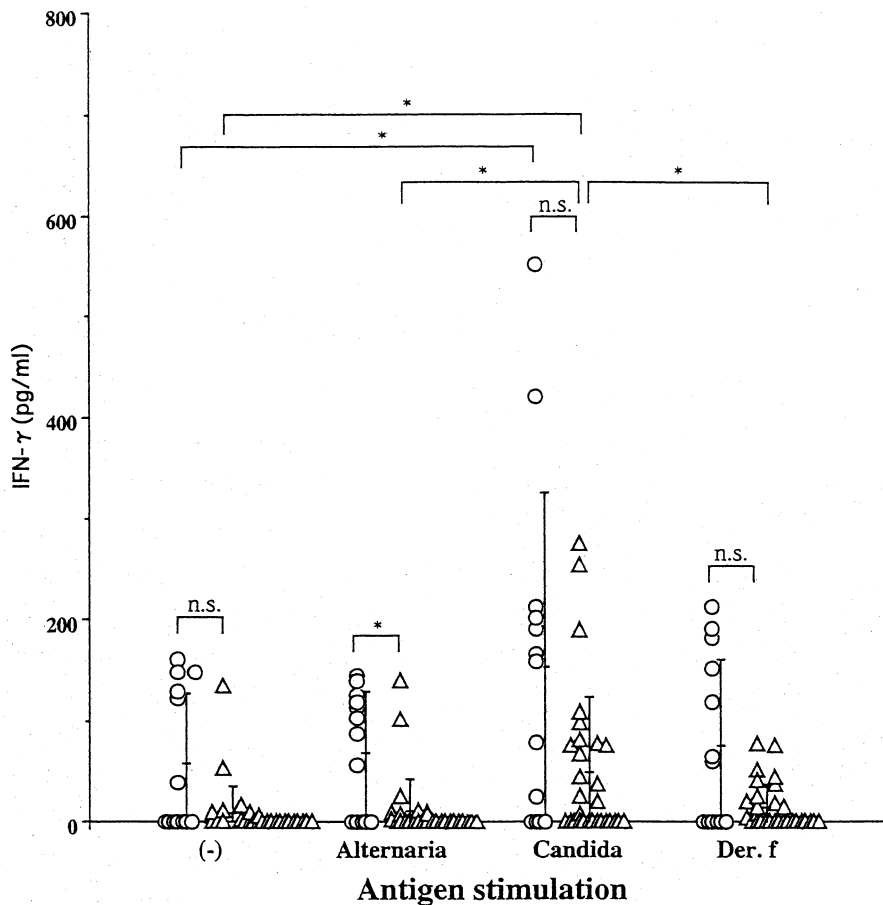


Fig. 2. IFN- γ production by allergen-stimulated PBMC in AD patients and HC. Bars show means and standard deviations. There was an increment of the IFN- γ production in response to a *C. albicans* antigen by comparison with those in response to other allergens or no allergen. The data showed a tendency of a decrease in IFN- γ production in AD patients. Δ , AD ($n = 29$); $\circ$, HC ($n = 13$). * $P < 0.05$; n.s., not significant.

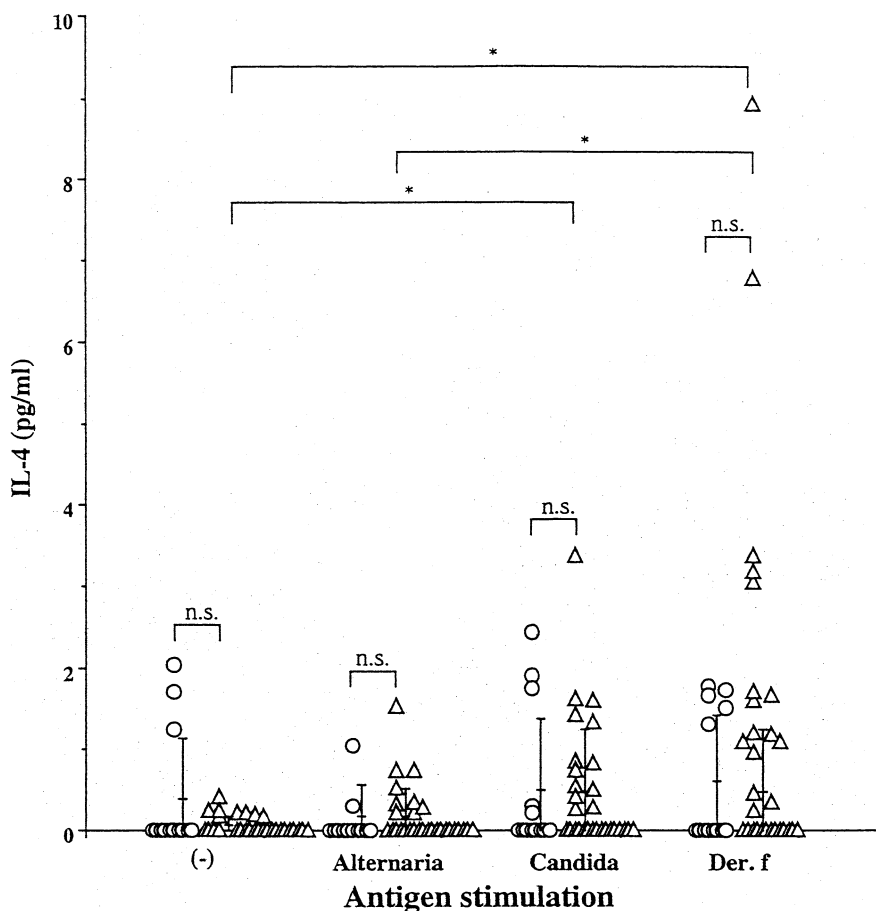


Fig. 3. IL-4 production by allergen-stimulated PBMC in AD patients and HC. Bars show means and standard deviations. There was an increase in IL-4 production in response to both *C. albicans* and *D. fariniae* antigen, when compared with that in response to *Alternaria* or no allergen. However, no statistically significant difference in IL-4 production with either allergen was found between AD patients and HC. Δ , AD ($n = 29$); $\circ$, HC ($n = 13$). * $P < 0.05$; n.s., not significant.

We further examined a correlation between the magnitude of LPR and the levels of IL-5 produced by *D. fariniae*-stimulated PBMC. There was a positive correlation between the mean diameter of the erythema of LPR and the extent of the IL-5 production by PBMC in the AD patients ($P < 0.05$), indicating that the larger the diameter of the erythema of LPR, the higher the level of IL-5 (Fig. 6B). However, the number of circulating eosinophils did not correlate with the levels of IL-5 production (data not shown).

The clinical severity of AD skin lesions seemed to be somewhat greater in those with positive

LPR than in those without it, but the correlation was not clear enough. The clinical features of the LPR-positive AD patients tended to show more diffuse erythrodermic skin changes than those of the LPR-negative patients.

4. Discussion

Our present report is the first systematic study that provides data on the LPR observed in AD patients. Our data demonstrated that the AD

patients with high RAST scores against *D. farinae* could be classified into two groups, the LPR-positive and -negative groups and that the former showed a significantly higher production of IL-5 by allergen-stimulated PBMC, suggesting that IL-5 may be related to their unique skin reactivity. Although *C. albicans* also stimulated the production of IL-5 by PBMC, it was not so high as noted with *D. farinae*.

In most of the patients with extracutaneous atopic diseases, LPR is preceded by IMR that appears within 30 min after challenge with the relevant allergens. LPR has been reported to be

involved in late asthma attacks [25] and nasal obstruction [26], which usually appear 6–12 h after allergen challenge. As in these atopic respiratory diseases, we found that LPR could also be found in AD patients, as reported before [12,13]. A recent study reported, however, that mast cell-deficient mice developed LPR without exhibiting IPR, while the magnitude of ear swelling and local eosinophilia was significantly lower than in normal congenic mice [27]. This indicates that IgE-mediated mast cell activation is not absolutely responsible for LPR, but that it is required for its sufficient expression.

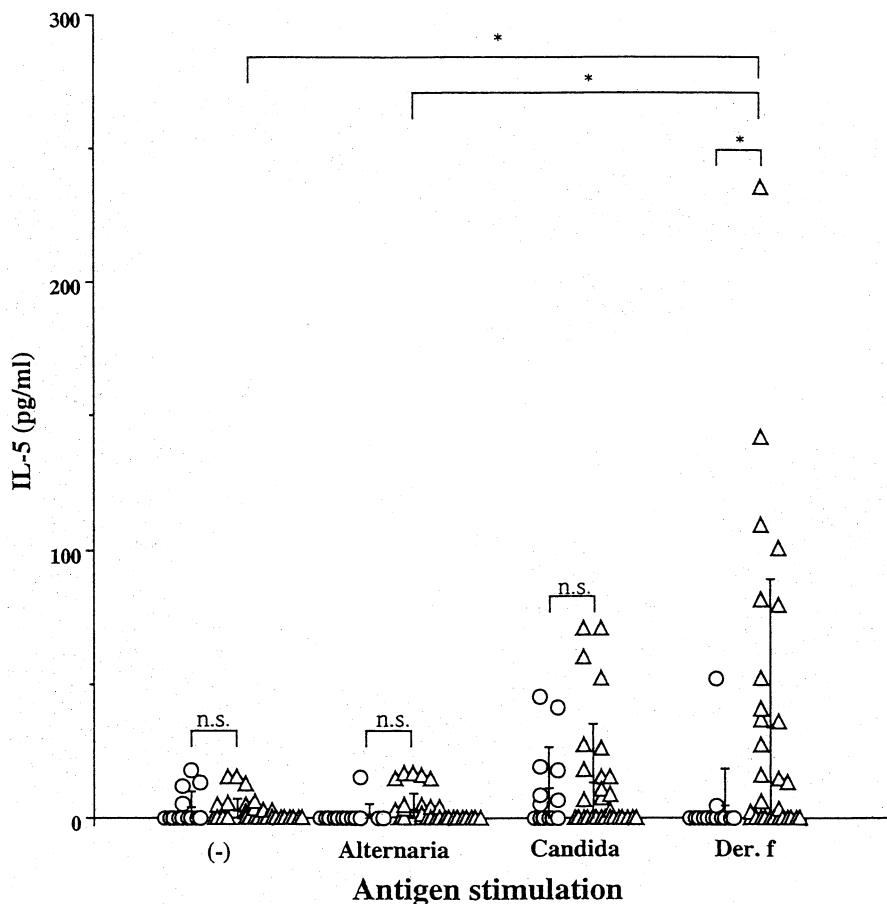


Fig. 4. IL-5 production by allergen-stimulated PBMC in AD patients and HC. Bars show means and standard deviations. There was an increase in IL-5 production in response to either *C. albicans* or *D. farinae* when compared with that in response to *Alternaria* or no allergen. Interestingly, IL-5 production in response to *D. farinae* in AD was higher than that found in HC. However, no statistically significant difference in IL-5 production with *Alternaria* and *C. albicans* was found between patients with AD and HC. △, AD ($n = 29$); ○, HC ($n = 13$). * $P < 0.05$; n.s., not significant.

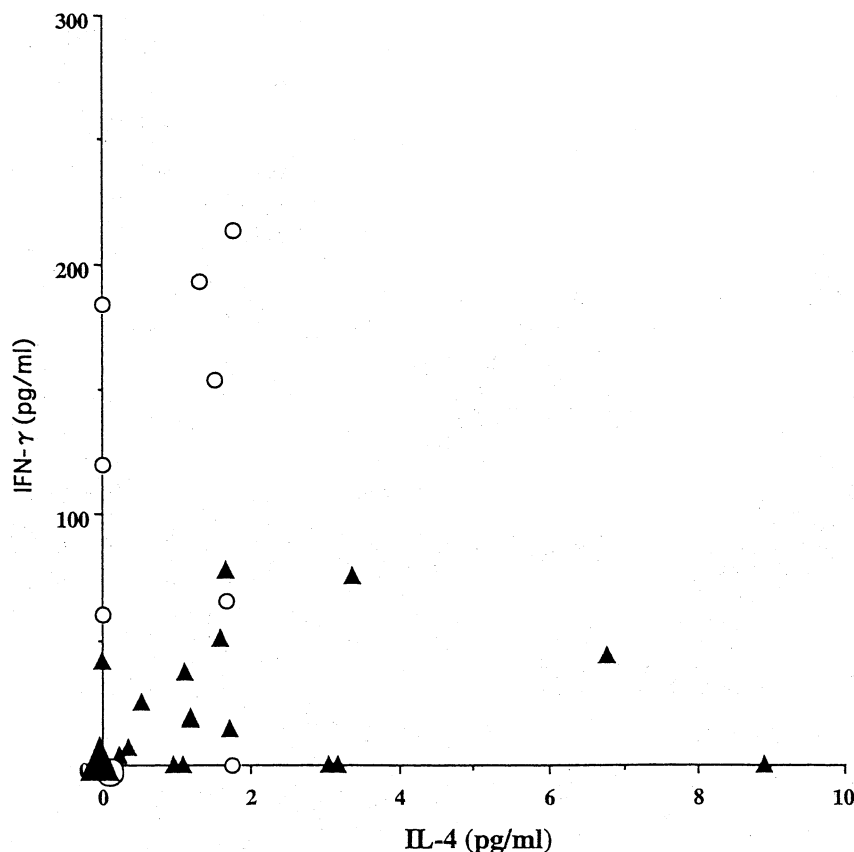


Fig. 5. (A) Comparison of IL-4 and IFN- γ production by PBMC in response to *D. farinae* between patients with AD ($r = 0.252$, $P = 0.197$) and HC ($r = 0.436$, $P = 0.139$). ▲, AD ($n = 29$) ▲ = ▲ $\times$ 13; ○, HC ($n = 13$) ○ = ○ $\times$ 5. (B). Relationship between IL-4 and IFN- γ production by PBMC in response to *Alternaria* between patients with AD ($r = -0.038$, $P = 0.851$) and HC ($r = 0.425$, $P = 0.151$). ▲, AD ($n = 29$) ▲ = ▲ $\times$ 18; ○, HC ($n = 13$) ○ = ○ $\times$ 5.

The aggravating factors for AD are quite heterogeneous among different patients [4,5,17–23]. Although some patients may show high titers of IgE RAST, while others show no abnormal change in IgE RAST, Tanaka et al. [4] reported that a high RAST score with decreased contact sensitivity to *C. albicans* allergen, to which most people have frequently been exposed since infancy, is specific to severe adult AD patients in contrast to the fact that normal healthy individuals or patients with atopic respiratory diseases only show a contact hypersensitivity reaction without any increased IgE RAST [24]. In our present study, we also found that the PBMC produced more IL-2 and IFN- γ with *C. albicans* allergen than with *D. farinae* allergen, which

might suggest a relatively stronger Th1 related response to *C. albicans* than to *D. farinae* even in AD patients. Our present finding that IL-2 and IFN- γ production to *C. albicans* was not specifically reduced in our AD patients probably reflects the fact that we performed the measurements in a rather limited number of patients.

In this study, although we used a simple prick test as a more practical and safer method than the intradermal skin test [28], we were able to reproducibly detect both cutaneous IMR and LPR for at least 24 h. In a few patients, the erythematous reaction tended to persist even for 48 h after pricking, although the reactions at 48 h did not correlate with the atopic patch test (data not shown). We found that 13 out of 23 AD patients

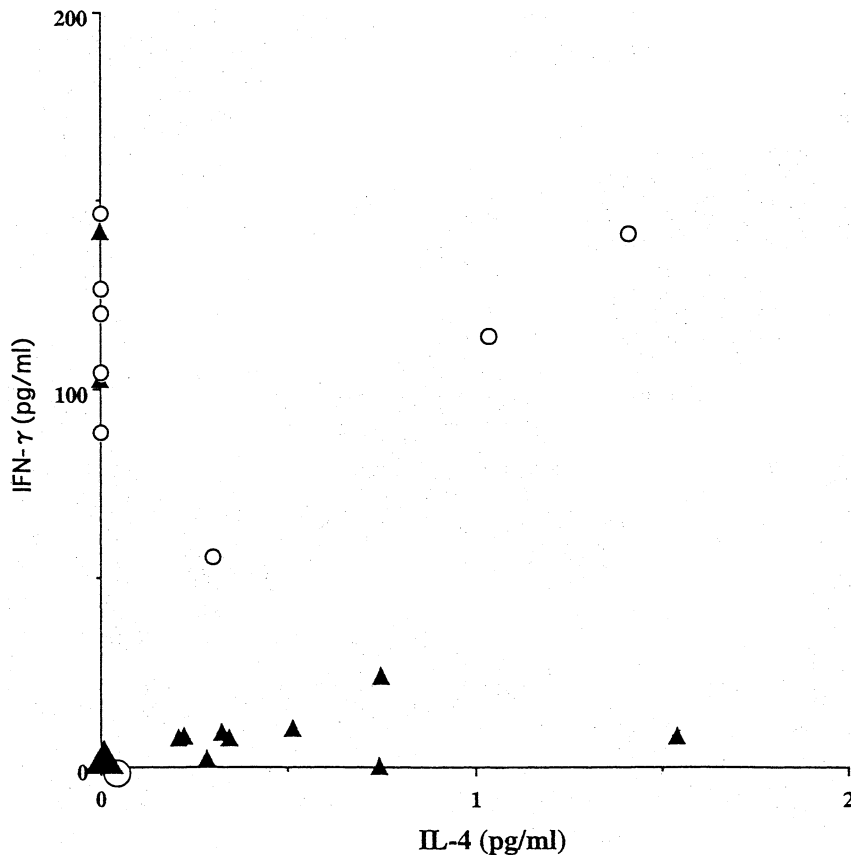


Fig. 5. (Continued)

Table 3

Results of cutaneous immediate and LPRs at prick test sites in 23 AD patients with high IgE antibody against *D. farinae*

Type of responses (time after prick)	<i>D. farinae</i> (%)	<i>Alternaria</i> (%)	<i>C. albicans</i> (%)
Immediate (15 min)	23/23 (100)	0/23 (0)	1/23 (4)
Late phase (8 h)	13/23 (57)	0/23 (0)	0/23 (0)
Delayed (48 h)	3/23 (13)	0/23 (0)	0/23 (0)

Table 4

Results of cutaneous immediate, late phase and delayed reactions at prick test sites in 10 HCs

Type of responses (time after prick)	<i>D. farinae</i> (%)	<i>Alternaria</i> (%)	<i>C. albicans</i> (%)
Immediate (15 min)	4/10 (40)	0/10 (0)	2/10 (20)
Late phase (8 h)	1/10 (10)	0/10 (0)	0/10 (0)
Delayed (48 h)	1/10 (10)	0/10 (0)	1/10 (10)

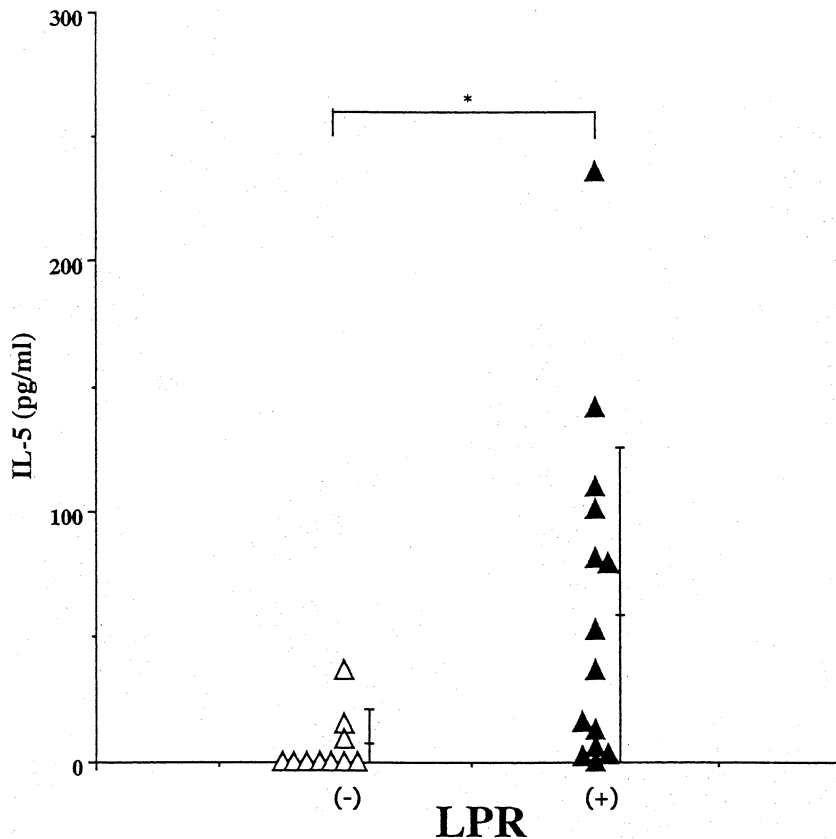


Fig. 6. (A) IL-5 production is higher in LPR-positive AD patients than in LPR-negative patients. ▲, LPR-positive AD patients ($n = 13$); △, LPR-negative AD patients ($n = 10$). * $P < 0.05$. (B) Positive correlation between IL-5 production and the mean diameter of LPR in AD patients ($r = 0.433$, $P < 0.05$) ▲, AD ($n = 23$) ▲ = ▲ $\times 4$.

(57%) with high serum IgE to *D. farinae* as well as definite IMR to *D. farinae* demonstrated positive LPR to *D. farinae*. About one half of the HC subjects, despite their low RAST scores, displayed IMR to *D. farinae*, but only one of them revealed LPR. None of the AD patients or HCs revealed LPR to *Alternaria* or *C. albicans*, suggesting that the LPR to *D. farinae* phenomenon was allergen-specific.

Among the various cytokines we measured for their *in vitro* production (IL-2, IFN- γ , IL-4, IL-5) by PBMC, we found a significantly higher production of IL-5 in the LPR-positive AD patients when compared to that in the LPR-negative AD patients. Despite the Th2-dominant activation to generate IL-5 under the allergen stimulation, there was no significant difference in IL-4 production. With regard to the infiltrating

cells in LPR, eosinophils have been shown to be closely related to the formation of LPR in humans [12,13] and in our recent experimental animal models [29,30]. Even though not many eosinophils could be seen in the ordinary hematoxylin and eosin sections, immunohistochemical study using antibodies against major basic protein and eosinophil cationic protein suggested the importance of eosinophilic infiltration in the LPR as well as the early destruction of eosinophils in the skin [12].

The enormous ingress of mite antigens allowed by the impaired barrier in AD skin would be expected to generate prodigious IgE and Th2 responses, but it does not necessarily indicate that the AD lesion is a simple result of the LPR, which has little resemblance to the AD lesional skin histologically and certainly does not pro-

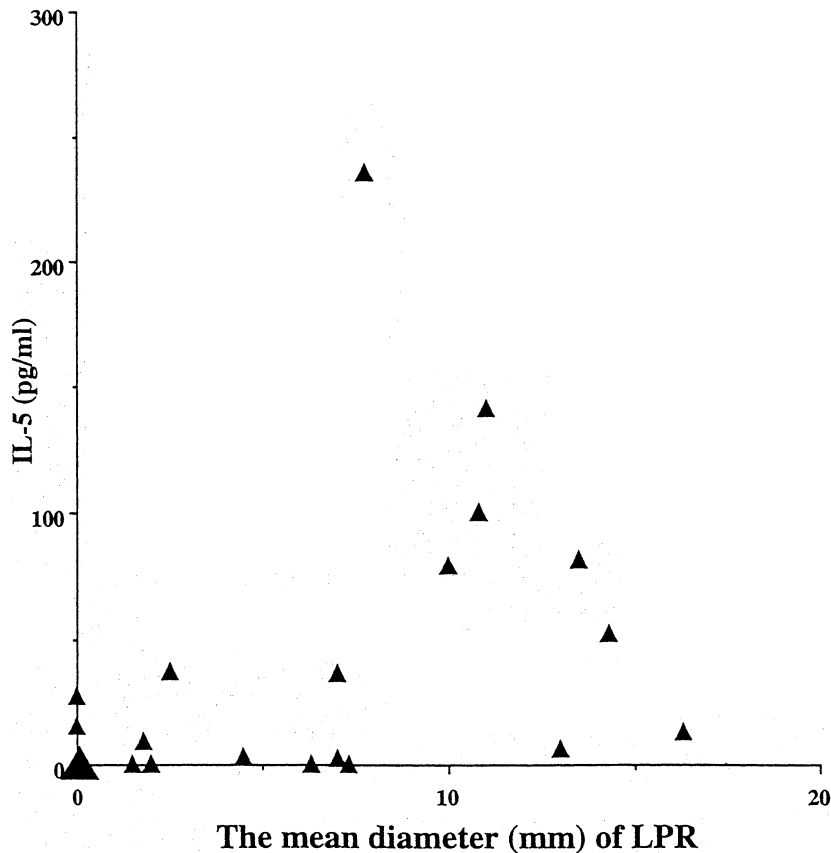


Fig. 6. (Continued)

duce a chronic, eczematous spongiotic lesion [12].

Based on the results of our present study, we should at first clarify the triggering or aggravating factor(s) in each patient and subsequently examine which types of allergic reactions are dominant in them, IMR, LPR or DTH, to carry out proper treatment. Continuous observation at the prick test sites for IMR and LPR can be a practical way to classify AD patients into different subgroups, and analysis of these results will enable us to employ a more appropriate therapeutic modality for each patient.

Acknowledgements

This study was supported by grants from the

Ministry of Education, Science and Culture, Japan (Nos. 07807079, 0845234, 09670865) and Special Coordination Funds for Promoting Science and Technology from the Science and Technology Agency, Japan. We thank Ms. Y. Yoshino for skillful technical assistance.

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