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Mite-antigen-stimulated cytokine production by peripheral blood mononuclear cells of atopic dermatitis patients with positive mite patch tests

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To investigate possible involvement of Th1-type immunoreaction in the development of skin lesions of atopic dermatitis (AD), we measured mite-antigen-stimulated production of IL-2, IL-4 and IFN- γ , using peripheral blood mononuclear cells (PBMC) obtained from 23 patients with AD who developed positive patch test reactions to house dust mite antigens extracted from *Dermatophagoides pteronyssinus* (Dp). Incubation of these PBMC with the Dp antigen for 72 h produced marked secretion of IL-2 and IFN- γ , but not IL-4, indicating that Dp-specific T cells were found in these patients' circulating peripheral blood and were capable of producing IL-2 and IFN- γ , known as major mediators of the delayed-type allergic reaction. Our study suggests that Th1-type cells may be involved in the development of skin lesions of AD patients with positive patch test reactions to house dust mite antigen.

Key words: cytokines; atopic dermatitis; house dust mite antigen; positive patch test.

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Certain immunologic mechanisms contribute to the development of skin lesions in atopic dermatitis (AD). Elevated serum levels of IgE for environmental and/or food antigens have been found in most patients with AD, suggesting the involvement of immediate hypersensitivity in its development (1). We have shown, however, that nearly 40% of AD patients show positive patch test (PT) reactions to house dust mite antigens (2). Furthermore, reduction of environmental mites improved the skin lesions of patients with positive PT reactions (3). These observations suggested that a delayed-type hypersensitivity (DTH) reaction to house dust mite antigen might be involved in the development of skin lesions in a certain proportion of patients with AD.

Over the past few years, it has become evident that IgE production is mediated largely by 2 cytokines: interleukin-4 (IL-4), a Th2 cell-derived cytokine, and interferon- γ (IFN- γ), a Th1 cell-derived cytokine; IL-4 is a necessary stimulus for IgE production and IFN- γ blocks the stimulatory effect of the IL-4 (4-6). Together with IL-2, another Th1 cell-derived cytokine, IFN- γ , is believed to play an important role in eliciting the DTH reaction (7-9).

Therefore, an analysis of these cytokines could be helpful for the better understanding of the development of skin lesions in patients with AD.

We previously demonstrated, by analysing a few cases of AD patients with positive PT reactions to house dust mite antigens, that peripheral blood mononuclear cells (PBMC) of these patients secreted IL-2 and IFN- γ , but not IL-4, after stimulation with house dust mite antigens (10, 11). On the contrary, these are some controversial reports that T lymphocyte clones generated from AD patients with house dust mite allergy exhibited a higher frequency of IL-4-producing T cells (12-14).

In this study, we investigated antigen-stimulated production of IL-2, IL-4 and IFN- γ by PBMC obtained from 23 AD patients who developed positive PT reactions to Dp antigen.

Patients and Methods

Patients

23 patients (14 male and 9 female; median age 24.8; range 6-58 years) with moderate to severe AD were included in the study. These patients developed positive PT reactions to Dp antigen, according to

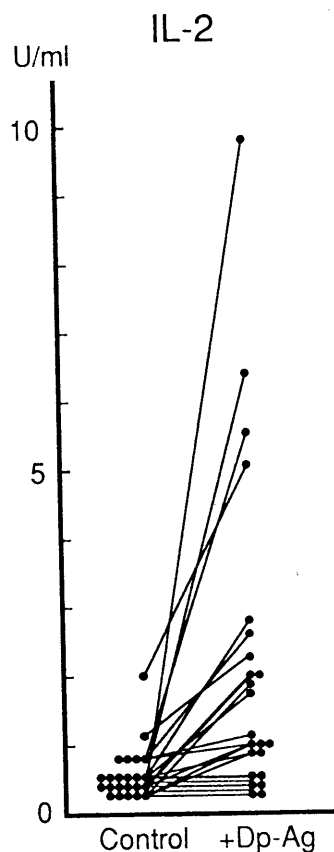


Fig. 1. The activity of IL-2 in the culture supernatant incubated with Dp antigen was considerably higher than that in the control supernatant. The difference between 2 groups was statistically significant ($p < 0.01$).

the criteria of the *International Contact Dermatitis Research Group* (ICDRG). With regard to specific IgE for Dp antigen, 5 patients had low levels of specific IgE (Dp-RAST score 0, 1, 2) and 18 had high levels of such IgE (Dp-RAST score 3, 4) (2).

Isolation of PBMC and cell culture

PBMC were isolated from the patients' peripheral blood by densimetric centrifugation and washed with RPMI medium 1640. The lymphocyte concentration was adjusted to a volume of 2×10^6 by the addition of 100 U/ml penicillin, 100 μ g/ml streptomycin, and 10% fetal calf serum (FCS) to the RPMI 1640 culture medium. 0.5 ml of Dp antigen (Shionogi Pharmaceutical Co. Osaka, Japan), in a concentration of 80 μ g/ml, was added to 1.5 ml of the PBMC suspension, and the mixture was incubated in 24-well plate for 72 h at 37°C in a humidified atmosphere containing 5% CO₂. Culture period was 72 h, since we have shown this period to be sufficient to measure the levels of cytokines on PBMC (10). Following incubation,

the cell-free supernatant was collected and stored frozen at -70°C .

A control supernatant was obtained by adding 0.5 ml of culture medium, instead of the Dp antigen, to the cellular suspension. The cellular mixture was incubated and the supernatant was collected in the manner described above.

Assays

Interleukin-2 (IL-2) activity in the culture supernatant was measured by a radioimmunoassay (RIA) test kit (Medgenix Diagnostics S.A., Brussels, Belgium) with a sensitivity of 0.8 U/ml. The IL-4 content was estimated using a specific solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) with a sensitivity of 31 pg/ml (R&D systems, Minneapolis, USA). IFN- γ activity in the culture supernatant was measured by a RIA test kit (Centocor, Malverne, PA, USA) with a sensitivity of 0.1 U/ml.

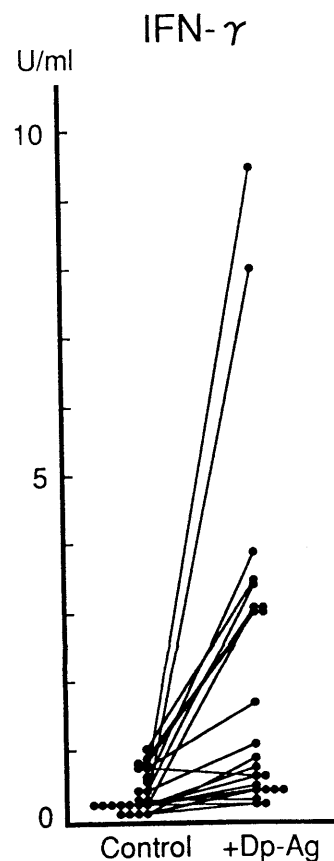


Fig. 2. The activity of IFN- γ in the culture supernatant incubated with Dp antigen was considerably higher than that in the control supernatant. The difference between 2 groups was statistically significant ($p < 0.01$).

Statistics

Statistical analysis was performed by using the paired *t*-test, and a *p*-value of <0.01 was taken as the level of significance.

Results

The activities of IL-2 (Fig. 1) and IFN- γ (Fig. 2) in the culture supernatant incubated with Dp antigen were considerably higher than that in the control supernatant ($p < 0.01$). In contrast, no secretion of IL-4 (data not shown) was found in either the Dp antigen stimulated supernatant or the control (less than 31 pg/ml).

Discussion

In this study, we have shown that AD patients with positive PT reactions to house dust mite antigens are equipped with circulating Dp-specific T lymphocytes, which are capable of producing IL-2 and IFN- γ when stimulated by the same mite antigens. With this secretion pattern, Dp-specific T cells obtained from AD patients resembled Th1 cells. It is known that IL-2 and IFN- γ , produced by Th1 cells, play an important role in the development of the DTH reaction in the skin (7-9). Our data appears to be in line with findings that nickel-specific T lymphocytes derived from patients with nickel contact dermatitis produced IL-2 and IFN- γ (15).

These results may conflict with reports that T-lymphocyte clones, generated from AD patients with house dust mite allergy, frequently exhibit Th2-like cell behavior (12-14), yet some Dp-specific T-lymphocyte clones produced detectable amounts of IFN- γ (13). The difference may be attributable to the cloning procedure of T cells. It is difficult to ascertain whether cloned T cells quantitatively represent those T cells that are present in the blood and skin lesions.

Our present study may serve as support for the hypothesis that the DTH reaction to Dp-antigen plays a role in the development of the skin lesions of a certain proportion (nearly 40%) of patients with AD (2).

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