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The Effect of Dermatophagoides Pteronyssinus Allergens on Proliferation and CD23 Antigen Expression of Peripheral Blood Lymphocytes from Atopic Patients

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The low affinity IgE receptor (FcεRII/CD23) has been proposed to be involved in the regulation of IgE synthesis.

The present study was undertaken to investigate the responses to in vitro stimulation by allergen (Dermatophagoides pteronyssinus; D.p) and/or interleukin-4 (IL-4) of peripheral blood lymphocytes (PBLs) isolated from atopic and non-atopic subjects. IL-4 induced up to 5 fold increase in CD23 expression on PBLs from both atopic patients and normal controls, whereas the D.p extract increased CD23 expression on cells from 7 of 8 atopic donors and from 2 of 8 normal controls. The combination of IL-4 and allergen had an additive effect of CD23 expression. PBLs from 6 of 8 atopic patients but 1 of 8 normal controls showed significant proliferative responses to D.p extract whereas IL-4 did not induce any cell proliferation. The dose of D.p extract required for the maximal CD23 expression was 20 fold higher than that for cell proliferation.

These results imply that allergen stimulation, presumably through proliferating allergen specific T cells which secrete IL-4, activates B cells from most atopic donors and a few non-atopic donors resulting in increased CD23 expression. This allergen-mediated CD23 expression may play an important role in specific IgE production.

Key Words: *FcεRII/CD23, IL-4, Dermatophagoides spp., Atopy*

INTRODUCTION

The interaction between environmental allergens and the immune system is critical in the development of specific allergy in humans. Lymphocyte responses to allergen challenge in vitro, assessed by blast transformation[1], [^{3}H] thymidine incorperation[2] or lymphokine production[3], have been reported by many investigators. This interaction is presumably initiated by uptake and presentation of allergen by MHC class II positive accessory cells to allergen specific helper T cells[4,5]. Activated T cells then induce B cells to produce allergen specific IgE antibody. Recent studies suggested that IgE is regulated in part by IL-4[6,7], and the major interest has been addressed to the role of the low affinity recptor for IgE (FcεRII), which has been shown to be identical to a B cell differentiation antigen, Blast-2, or CD23[8,9]. The CD23 antigen and its proteolytic cleavage product, the IgE binding factor (IgE-BF, sCD23), may function as regulatory molecules in IgE synthesis[10]. The existence of CD23 has been shown on B cells[11], T cells[12], monocytes[13], eosinophils[14] and epidermal Langerhan's cells[15] etc.. Although CD23 is expressd only weakly in a

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minority of resting B cells from peripheral blood or tonsil, its expression is dramatically upregulated following activation by stimuli such as Epstein-Barr virus (EBV)[16], phorbol esters[17] and interleukin-4 (IL-4)[18]. The basic differences between atopic and non-atopic individuals in the regulation of IgE synthesis, however, has not been resolved. The investigation in such subject has been hindered by the absence of suitable allergen dependent in vitro system for IgE production.

This study was performed to assess the in vitro response of peripheral blood lymphocytes (PBLs) to the house dust mite, Dermatophagoides pteronyssinus (D.p) antigen, which is one of the most common aeroallergens in Korea. For this purpose, we analysed the expression of CD23 in PBLs using flow cytometry and lymphocyte proliferative response by [^{3}H] thymidine incorporation assay. We compared these responses of PBLs isolated from atopic patients and those of cells from non-atopic controls.

MATERIALS AND METHODS

1. Cells

Peripheral blood mononuclear cells (PBMCs) were separated from heparinized blood by centrifugation on Hystopaque (Sigma, U.S.A.) gradient according to the method of Boyum[19]. Eight patients suffering from house dust mite allergy, not treated with systemic or topical steroids, were investigated. The mite-sensitivity was evaluated by skin prick tests with D.p extract (Bencard, U.K.). In all patients, the serum IgE levles were above 400 I. U./ml.

Eight healthy laboratory workers who had no history of atopic disease and were negative for allergen skin tests served as normal controls.

2. Culture Conditions

RPMI-1640 culture medium (Gibco, U.S.A.) was supplemented with 10% fetal calf serum (Gibco), 2 mM glutamine, 10 mM HEPES, 100 μg/ml of streptomycin and 100 U/ml of penicillin. The cells were cultured at a density of 1×10^6 cells/ml, and generally 2 ml cultures were used for surface marker analysis. Cultures were maintained at 37°C in 5% CO_2 atmosphere for three to seven days.

3. Reagents

Recombinant human IL-4 was kindly supplied by Dr. Hiroku Tsutsui (Osaka City University Japan). Dialyzed extract of D.p additives was kindly provided by Bencard Co. (U.K). The amount of protein in the extract solution was measured using Bio-rad protein assay reagents (U.S.A.). Phycoerythrin (PE)-conjugated monoclonal anti-CD23 antiboidy and Fluorescein isocyanate (FITC)-conjugated monoclonal anti-CD19 antibody were obtained from Becton Dickinson Co. (U.S.A.).

4. Detection of CD23 Antigen

Cultured cells were harvested and washed in phosphate buffer solution. Aliquots of 100μl of cell suspension (1×10^6 cells/ml) were incubated for 45 min at 4°C with 10μl of PE-anti-CD23 and FITC-anti-CD19 antibody. Murine FITC-IgG1 and PE-IgG2 were used to evaluate nonspecific bindings. A two color analysis was performed on FACscan (Becton-Dickinson) equipped with argon laser tuned to 488 nm and the data was analyzed by Consort 30 and Lysis software. After gating live cells on the basis of forward and right angle light scatter, 3×10^4 lymphocytes were evaluated in each determination. Fluorescence-positive cells were quantified as percentage of lymphocytes in the range of fluorescence intensity channels above a predefined threshold channel. We further analyzed the surface antigen expression in relation to the cell size.

5. Proliferation Assay

PBMCs were cultured in triplicate in 96-U bottom microwell plates (1×10^5 cells/well), either alone or with IL-4, phytohemagglutinin (PHA) and/or D,.p extract. After incubating cells for 4 days, individual wells were pulsed with 1 μCi of ^{3}H-thymidine (^{3}H-TdR, Amersham, U.K.) 24 hours before harvesting and gamma counting. The standard deviation of triplicated values was below 10%. The response was considered positive when the stimulation index (S.I.: the ratio between the mean of the treated to the untreated) was above 2.5

RESULTS

1. The Effect of IL-4 and D.p Extract on CD23 Expression

In control cultures, the mean percentage of CD23 positive B cells from atopic patients (10.7$\pm$6.5% per B cells) was similar to that in non-atopic controls (11.6$\pm$6.5%). The CD23 expression was increased by IL-4 and by D.p extract in dose-dependent manners. The CD23 expression was induced maximally at 0.2 to 2 ng/ml of IL-4 con-

centration and at 150 ng/ml of D.p extract (Fig. 1). The highest increase was obtained at 48 to 72 hours of culture. The responses to IL-4 stimulation in atopic subjects did not differ from those in non-atopic controls (42.0±19.1% v.s. 38.0±17.8%).

However, the D.p extract-stimulated cultures showed significantly higher percentages of CD23 positive cells in atopic subjects (36.0±14.0%) as compared to those in controls (16.4±8.0%). The addition of the D.p extract in conjunction with IL-4, further increased the percentage of CD23 positive B cells. The results of all experiment on the CD23 expression are presented in Fig. 2. When the individual values were compared between control cultures and D.p cultures, 7 of 8 atopic patients showed more than 2 fold increase in the numbers of CD23 positive B cells, whereas it was observed in only 2 of 8 non-atopic subjects (data not shown).

We also analysed the effects of D.p extract on the cell size, as presented in Fig. 3. Stimulation with D.p extract increased the population of large cells in atopic donors. Of these large activated cells, 40% were B cells among which 50% expressed CD23.

2. The Effect of IL-4 and D.p Extract on Cell Proliferation

To investigate whether stimulation with the D.p or IL-4 could induce cell proliferation, [^{3}H] thymidine incorporation studies were undertaken. IL-4 stimulation did not induce significant proliferation of cells from eitheir atopic patients or normal control. However D.p extract induced significant proliferation (S.I.>2.5) in 6 of 8 atopic patients and 1 of 8 normal controls (Fig. 4). The response was dose-dependent and the maximal proliferation was observed on the 4th day of culture with 7.5 ng/ml

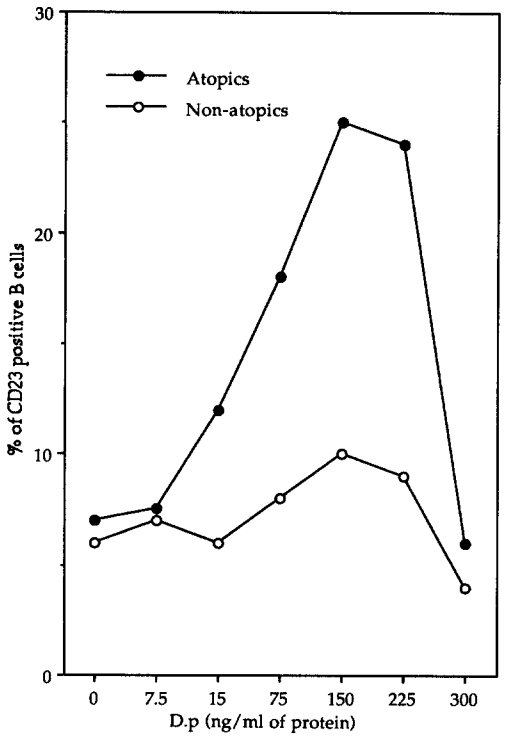

Fig. 1. *The dose effect of the D.p extract on CD23 expression of B cells on the 3rd day of culture.*

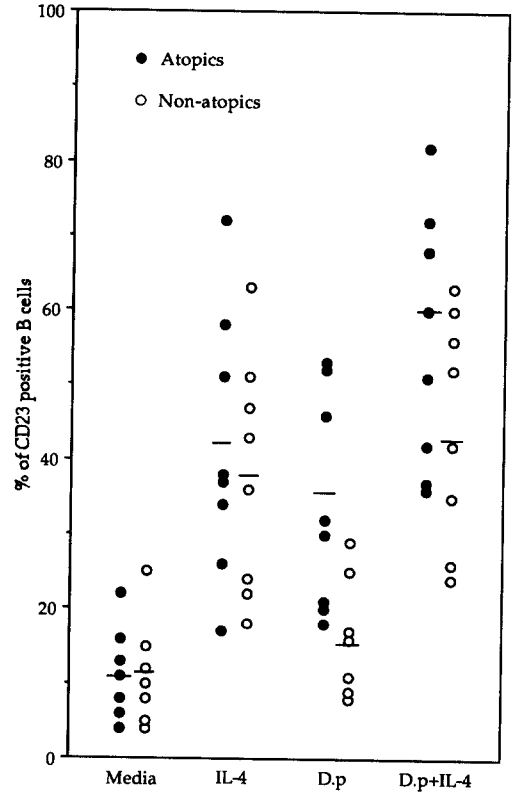

Fig. 2. *The CD23 expression on B cells from atopic and non-atopic subjects on the 3rd day of culture with IL-4 (0.2 mg/ml), D.p extract (150 ng/ml) and IL-4 plus D.p extract*

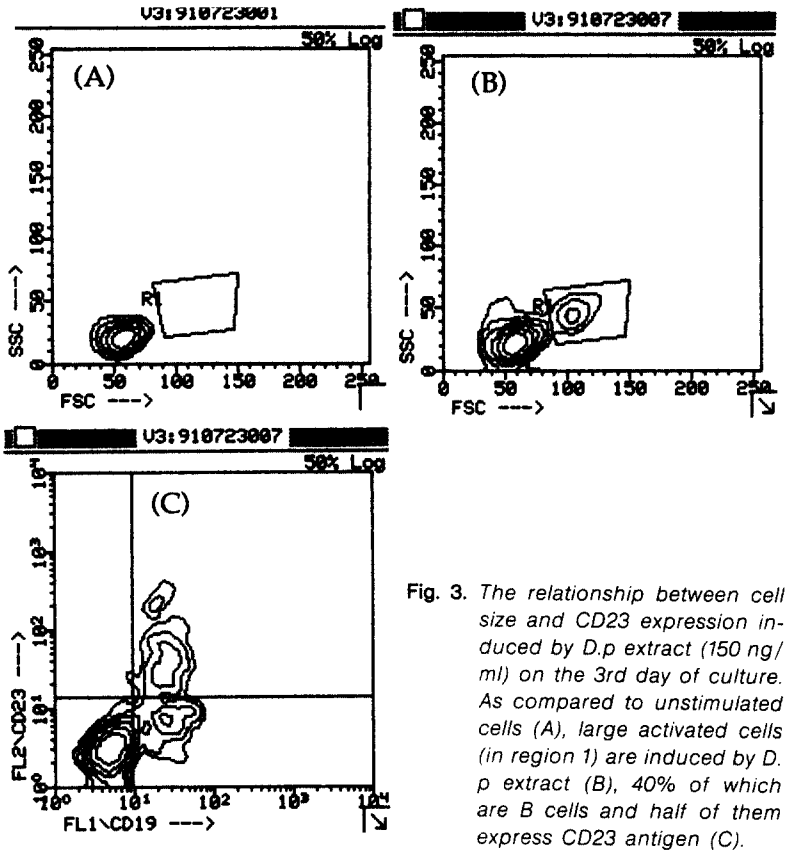

Fig. 3. *The relationship between cell size and CD23 expression induced by D.p extract (150 ng/ml) on the 3rd day of culture. As compared to unstimulated cells (A), large activated cells (in region 1) are induced by D. p extract (B), 40% of which are B cells and half of them express CD23 antigen (C).*

protein concentration of the extract (Fig. 5), which was about 20 fold lower than that for CD23 induction.

DISCUSSION

IgE is responsible for allergic sensitivity in atopic individuals. Earlier studies suggested that IgE production could be regulated not only by antigen specific T cells, but also through isotype-specific factors (reviewed in 20, 21). Now it is evident that IgE synthesis is regulated in part by IL-4, which is secreted by a particular subset of helper T cells, and it has been described that CD23 molecules might be related with IL-4 induced IgE production[22~24]. However, basic differences between atopic and non-atopic individuals in the regulation of IgE synthesis have not been resolved.

In this experiment, the CD23 expression in unstimulated or IL-4 stimulated cells from atopic patients were not different from that of cells from normal controls. However, D.p extract showed different responses. Whereas it increased CD23 expression of PBLs from 7 of 8 atopic patients, D. p extract increased CD23 on cells from 2 of 8 skin test-negative non-atopic subjects. The cells from one of these non-atopic two subjects also showed significant [^{3}H] thymidine incorhoration upon the antigen challenge. This subject was a technician at allergy clinic who had a lot of chances of exposure to common environmental allergens used in skin tests. Again, we noticed that D.p extract increased the size of cells from some non-atopic controls (data not shown), which would mean the activation of cells. Several controversial observations have been reported in this regards. While Buckley et al[25]. showed proliferative response of PBLs to a ragweed allergen in both atopic and non-atopic patients, Rawle et al[26]. reported that the D.p specific proliferative response was observed almonst

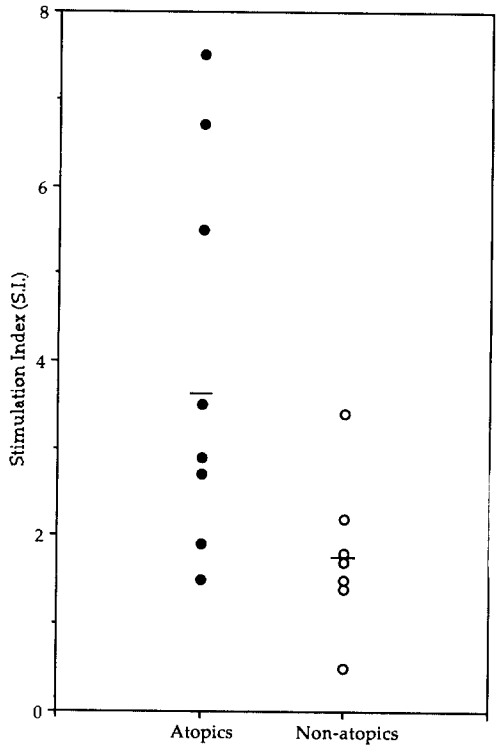

Fig. 4. *The proliferative response of cells by D.p extract (7.5 ng/ml) on the 4th day of culture.*

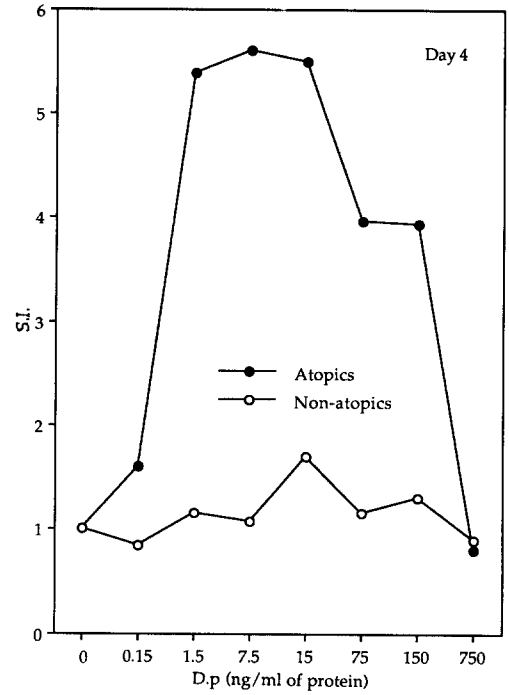

Fig. 5. *The dose effect of the D.p extract on cell proliferation on the 4th day of culture.*

exclusively in cells of atopic patients. More recently, O'Hehir et al.[27], showed that D.p extract induced only clonal expansion of reactive T cells without IgE production in non-atopic donors. The reason why D.p allergens cause proliferative response of PBLs without specific IgE production from some non-atopic subjects might be explained by the possible absence of D.p-reactive B cells or the presence of suppressor T cell activities.

The significant discrepancy between the dose of D.p extract required for CD23 expression and that for cell proliferation might have a sense if CD23 antigen or its cleavage products are involved in specific IgE production.

The allergen-induced CD23 expression may be dependent on IL-4 secreted by helper T cell clones stimulated in atopic donors[23]. Parronchi et al[28]. recently described that many allergen-specific T cell clones secreted IL-4, which suggested that clonal expansion of the IL-4 producing T cells is important for IgE production. There might be a possibility that D.p extract could act as a polyclonal activator like PHA[29] or a direct B cell stimulator. However, these are unlikely because the proliferated cellular response by D.p was delayed and sustained for a substanital period as compared to that by PHA (unpublished observation).

The results from our experiments suggest that allergen stimulation, presumably through proliferating allergen-specific T cells whcih secrete IL-4, activate B cells from atopic donors resulting in increased CD23 expression. This allergen-mediated CD23 expression may play an important role in IgE production.

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