

Affinity-purified *Dermatophagoides farinae* antigen induces CD23 on T and B lymphocytes and monocytes specifically in patients with atopic dermatitis

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Received 4 November 1994; revision received 2 March 1995; accepted 28 April 1995

Abstract

The purpose of this work is to analyze CD23 expression on subpopulations of peripheral blood mononuclear cells (PBMC) from the patients with atopic dermatitis (AD) after stimulation with affinity-purified *Dermatophagoides farinae* antigen (Af-DF). PBMC from mite-allergic AD patients stimulated with Af-DF induced striking expression of CD23 after the culture. The frequencies of CD23-positive (CD23⁺) PBMC in the culture were significantly higher than those without stimulation. Most of the CD23⁺ cells belonged to B lymphocytes and monocytes subsets. CD23 was induced in less than 1% of CD3⁺, CD4⁺ and CD8⁺ lymphocytes by Af-DF. However, significant differences were observed compared to the spontaneous expression, and dose-dependencies were confirmed. No significant increase in the frequencies of CD23⁺ PBMC was found in non-mite-allergic AD patients and non-atopic donors after stimulation with Af-DF. The kinetics of CD23 expression by Af-DF showed similar patterns to that by IL-4 stimulation. Our results demonstrate that Af-DF can induce CD23 expression on PBMC in mite-allergic AD patients. Based on the high titer of mite-specific IgE antibody in blood of AD, mite antigen may, at least in part, contribute to high CD23 expression in AD.

Keywords: Affinity-purified *Dermatophagoides farinae* antigen; CD23; Atopic dermatitis; Peripheral blood mononuclear cell; Interleukin-4

1. Introduction

CD23, known as a low affinity receptor for IgE, belongs to a novel super family of type-II integral

membrane glycoprotein [1]. CD23 is expressed on a variety of hematopoietic cells and is regulated by several cytokines [1]. In some pathological conditions such as Epstein-Barr virus (EBV) in-

Abbreviations: AD, atopic dermatitis; DF *Dermatophagoides farinae*; Af-DF, affinity-purified DF antigen; PBMC, peripheral blood mononuclear cells; rIL-4, recombinant interleukin-4.

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fection, the CD23 gene is abnormally upregulated and results in CD23 overexpression [2]. CD23 plays a role in the spontaneous IgE synthesis by B cells. CD23 is also involved in the regulation of growth and differentiation of B cells [3] and antigen presentation [4].

Atopic dermatitis (AD) is a chronic and eczematous disorder accompanied by severe pruritus. IgE hyperproduction is considered to be involved in the etiology of atopic dermatitis. Elevation of serum IgE levels [5], positive immediate skin tests and high titers of IgE antibody to a variety of antigens [6] and CD23 overexpression on lymphocytes [7] were reported in AD.

Based on the results of high IgE titer against mite antigen and high positive rates of patch test with mite antigen [8], mite antigen is a major allergen in AD. The purpose of this study is to test whether mite antigen induces CD23 expression on PBMC or not, and to assess the CD23 expression on all subpopulations of PBMC in AD.

2. Materials and methods

2.1. Subjects

Ten AD patients allergic to mite antigen, confirmed both by mite patch test described before [8] and IgE-radioallergosorbent test (RAST) for DF, were selected for this study. Six non-mite-allergic AD patients and five healthy non-atopic donors were selected as controls. AD was diagnosed according to the criteria defined by Hanifin and Rajka [9]. The AD group consisted of seven females and nine males (average: 23 years old, range 10–42 years old). The healthy control group consisted of five males (average: 33 years old, range 27–41 years old). None of the patients received systemic corticosteroid therapy. Most of patients had been treated with oral antihistamine and topical steroids.

2.2. Preparation of peripheral blood mononuclear cells (PBMC)

PBMC were isolated from heparinized venous blood by means of Ficoll-Paque gradient density centrifugation. Concentration of PBMC were adjusted at 1×10^6 cells/ml with RPMI 1640 containing 10% heat-inactivated fetal bovine

serum (FBS), sodium bicarbonate (2 g/l), streptomycin (100 μ g/ml) and penicillin (100 u/ml) (Gibco).

2.3. Reagents

Extract from whole culture of the house dust mite *Dermatophagoides farinae* (DF-WCE) and affinity purified rabbit antibodies to DF-WCE were processed as described in previous papers [8,10]. To obtain further purified antigens, affinity purified rabbit antibodies to DF-WCE were coupled to a CNBr-activated Sepharose 4B column. A crude extract was applied to the column in a 0.02 M phosphate buffer, pH 7.4, containing 0.15 M NaCl (PBS) and the bound antigens were eluted with 0.2 M glycine-HCl buffer, pH 2.5, immediately neutralized with 2 M Tris base, dialysed against PBS, freeze-dried and stored at -20°C until use.

Recombinant IL-4 was purchased from Genzyme Corporation Boston, MA, USA. Phycoerythrin (PE)-conjugated antibodies to CD3, CD4, CD8, CD19 (DAKO, Glostrup, Denmark), CD14 (Immunotech Luminy, France), and FITC conjugated anti-CD23 monoclonal antibody (Immunotech) were used in this experiment. Propidium Iodide was obtained from Molecular Probes, Inc. OR, USA.

2.4. Culture conditions

For kinetic studies, PBMC suspensions from four mite-allergic AD patients and three non-atopic donors were cultured in stimulation with either Af-DF (21 μ g/ml), or recombinant interleukin-4 (rIL-4, 100 u/ml), or without stimulation at 37°C for 7 days in 96-well flat-bottom plastic plates (Corning). Cultures for analysis of CD23⁺ PBMC and of CD23⁺ B cells were performed in five and three replicate, respectively. For dose-response experiments, PBMC obtained from five mite-allergic AD patients and five non-atopic donors were cultured with graded concentrations of Af-DF (0–42 μ g/ml) or rIL-4 (0–150 u/ml) for 3 days. To confirm the specific effect of Af-DF on mite-allergic AD patients, PBMC from mite-allergic AD patients, non-mite-allergic AD patients and non-atopic donors were cultured with or without Af-DF (21 μ g/ml) for 3 days and were

analyzed CD23 induction in each subpopulation of PBMC. Monocyte subpopulation was analyzed on 2 days of culture.

2.5. Double-immunofluorescence staining

After culture, double-immunofluorescence staining was performed to identify the CD23⁺ subpopulations. PBMC (1×10^5) was incubated with FITC-conjugated antibodies against CD23 and PE-conjugated antibodies against either CD3, CD4, CD8, CD14 or CD19 successively. The corresponding isotype-matched antibody (Immunotech) were used as controls.

2.6. Flow cytometric analysis

Twenty thousand living cells were analyzed to evaluate the percentage of positive cells with a FACScan flow cytometer (Becton Dickinson, Mountain View, CA). Dead cells were excluded by selectively gating on forward scatter and FL-3 parameter from analysis using a red fluorescent dye (Propidium Iodide) that is bound to nucleic acid of dead cells.

Using isotype-matched negative control antibodies to determine regions of specific staining, in which the cells were labeled by anti-CD23 mAb or other mAbs but not labeled by negative control antibodies.

Counts of CD23⁺ PBMC, T cells, B cells and monocytes were expressed as percentages of the corresponding cells positively stained with anti-CD23.

2.7. Statistical analysis

The statistical significance was evaluated using a Wilcoxon signed-rank test (paired data). In kinetic and dose-response experiments, only data which were reproducible in all independent experiments are shown. Due to the inter-individual variances, the results of a representative experiment are shown. The standard deviations given in the kinetic figures are the deviations calculated from five and three replicate cultures out of a single experiment.

3. Results

3.1. Kinetic study of the expression of CD23 on the cultured PBMC

CD23 expression on PBMC from mite-allergic

AD patients stimulated with Af-DF antigen reached a striking increase after culture. The kinetic pattern showed the same manner as that by rIL-4 stimulation. Without stimulation, CD23 expression decreased with time (Fig. 1a). In non-atopic healthy donors, rIL-4, but not Af-DF induced CD23 expression on PBMC (Fig. 1b).

Addition of Af-DF or rIL-4 in the cell cultures had an effect on the increase of CD23 on B cells (CD19⁺) from mite-allergic AD patients (Fig. 1c). This phenomenon was not observed in Af-DF-stimulated PBMC from normal donors (Fig. 1d).

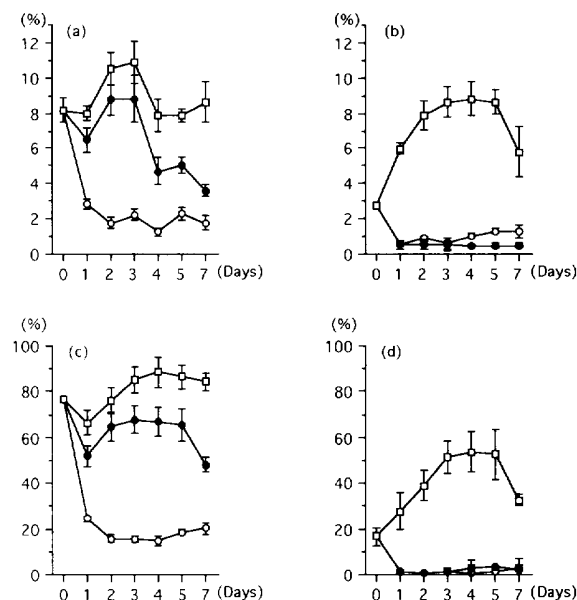


Fig. 1. Kinetics of spontaneous (○), Af-DF (●)- and rIL-4 (□)-stimulated CD23 expression on PBMC from a mite-allergic AD patient (a), from a normal donor (b), and on B cells from a mite-allergic AD patient (c), from a normal donor (d). Means $\pm$ S.D. calculated from five (a,b) and three (c,d) replicate cultures of one representative experiment out of four (a,c) or three (b,d), respectively, is shown. In 3 days, CD23 was decreased without stimulation. Addition of Af-DF (21 μ g/ml) or rIL-4 (100 u/ml) increased CD23 expression on PBMC (a) and on B cells (c) in mite-allergic AD patients. CD23 expression was induced by rIL-4, whereas it could not be induced by Af-DF in normal donors either on PBMC (b) or on B cells (d).

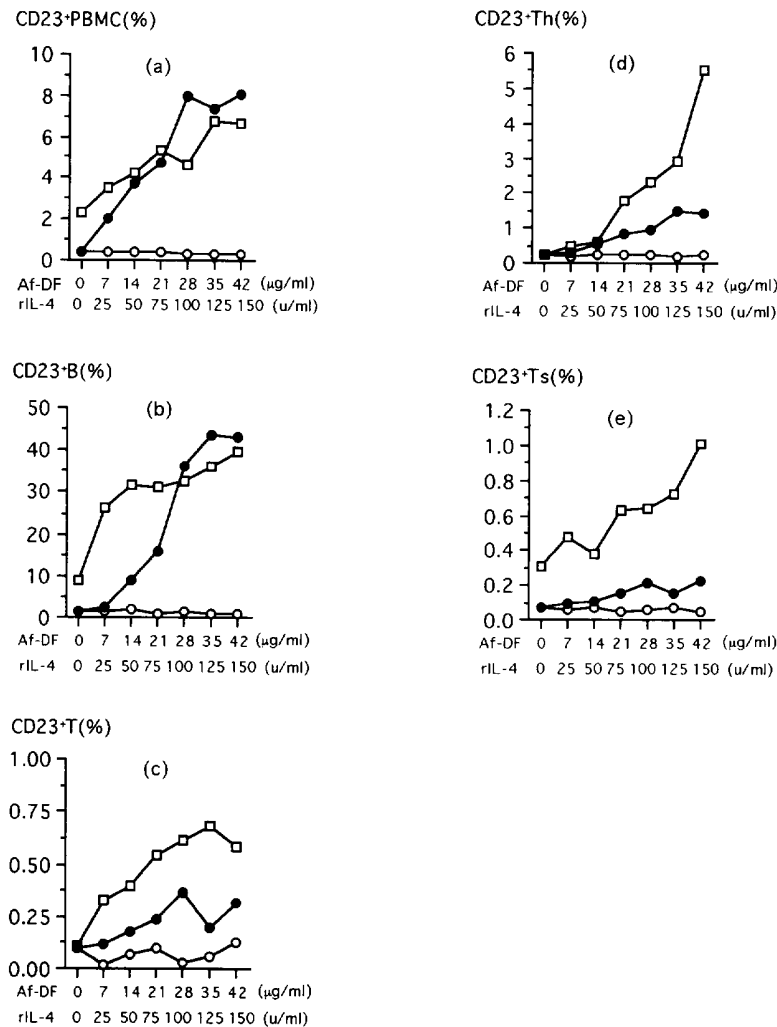


Fig. 2. Dose-response of CD23 expression to Af-DF and IL-4 on PBMC. The results of a typical experiment out of five mite-allergic AD patients or five non-atopic donors are shown. PBMC were cultured with graded concentrations of Af-DF or rIL-4 at 1×10^6 cells/ml for 3 days. Percentages of CD23+ PBMC (a), CD23+ B cells (b) CD23+ T cells (c), CD23+ helper T cells (Th) (d) and CD23+ suppressor T cells (Ts) (e) obtained from mite-allergic AD patients ($\square$) non-atopic donors ($\circ$) after culture with Af-DF antigen were shown. Af-DF dependent enhancement of the CD23 expression in mite-allergic AD patients was observed in all subpopulations. CD23 was induced in all subpopulations from non-atopic donors by addition of rIL-4 ($\bullet$) in the same manner.

3.2. Dose response of CD23 expression to Af-DF

The treatment with Af-DF for 3 days markedly enhanced the expression of CD23 on PBMC in a dose-dependent manner (Fig. 2a) in AD. The most striking difference in CD23 expression by Af-DF was seen between T and B cells. CD23 expression on B cells was markedly enhanced, whereas induction of CD23 on T cells was far

lower than that of B cells. Only low frequency and weak expression on T cells were found, but a dose dependency was observed. The same phenomenon was also seen in stimulation with rIL-4 (Fig. 2b,c). Af-DF enhanced expression of CD23 on B cells in the same manner as rIL-4. It may imply that IL-4 is involved in the mechanism of CD23 induction by Af-DF. Among T cells, better

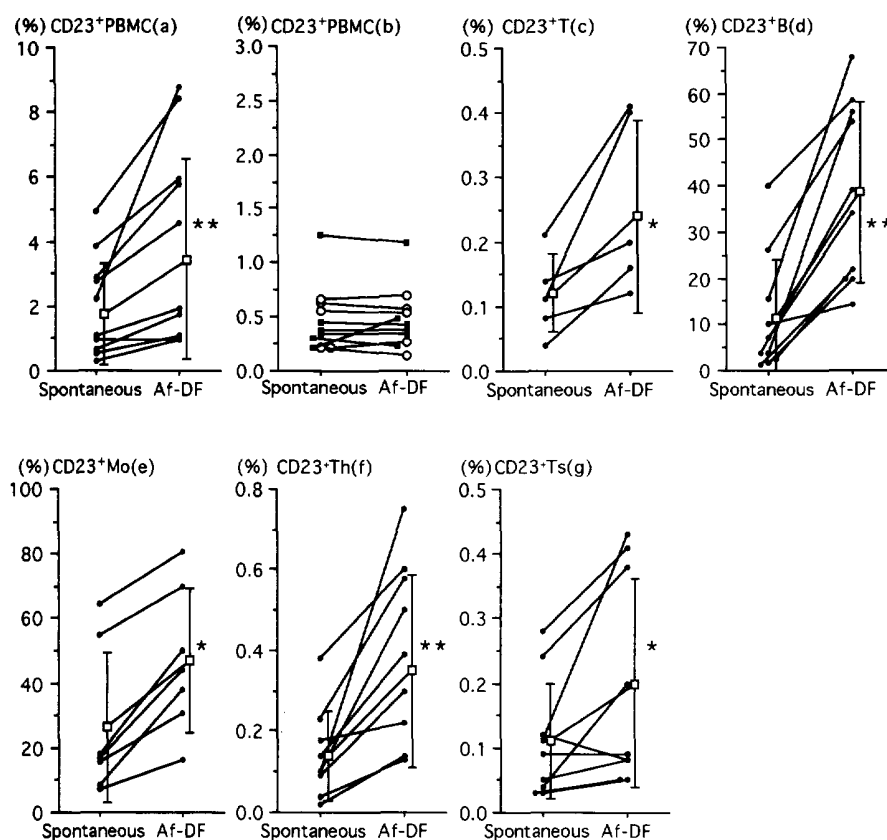


Fig. 3. Comparison of CD23 expression on PBMC stimulated with and without Af-DF. After 3 days incubation in the presence of Af-DF stimulation (21 μ g/ml), the percentages of CD23⁺ PBMC were higher than those without stimulation (spontaneous) in mite-allergic AD patients ($n = 10$) (a). In contrast, the percentages of CD23⁺ PBMC showed no changes even though stimulated by Af-DF either in non-atopic donors ($n = 5$) ($\circ$) and in non-mite-allergic AD patients ($n = 6$) ($\blacksquare$) (b). In T (c) ($n = 5$), B (d) ($n = 10$), Th (f) ($n = 9$), Ts (g) ($n = 9$) lymphocytes and monocytes (e) ($n = 7$), increase of CD23 expression was seen after stimulation with Af-DF in mite-allergic AD patients for 3 days (lymphocytes) or 2 days (monocytes) culture; ($\square$, mean $\pm$ SD; * $P < 0.05$; ** $P < 0.01$).

responses were observed in CD4⁺ cells than in CD8⁺ cells (Fig. 2d,e).

3.3. Comparison of CD23 expression on PBMC stimulated with and without Af-DF

The frequencies of CD23⁺ PBMC after stimulation with Af-DF were significantly higher than the cells without stimulation on 3 days of culture. Furthermore, the whole PBMC subsets showed an increased expression of CD23. Although less than 1% of CD3⁺, CD4⁺ and CD8⁺ lymphocytes were induced to express CD23, a statistical significance was observed compared with control. No

significant increase in frequency of CD23⁺ PBMC was found in non-mite-allergic AD patients and non-atopic donors after Af-DF stimulation (Fig. 3).

The increase in frequency of CD23⁺ monocytes by stimulation with Af-DF was also found in mite-allergic AD patients 2 days after the culture (Fig. 3e).

4. Discussion

In this study, it was demonstrated that Af-DF had a stimulatory effect on CD23 expression by

whole subpopulations of PBMC. CD23 induction was obtained mainly on B cells and monocytes subsets, whereas Af-DF induced a small percentage of CD23⁺ T cells. Our data support the previous findings [11] that most of cells expressing CD23 were B cells and monocytes but not T cells, after stimulation with IL-4. The effect of Af-DF on CD23 expression was similar to that of IL-4. It suggests that the induction of CD23 expression by Af-DF may be associated with IL-4. Rawle et al. [12] have reported that helper T lymphocytes predominantly proliferated due to stimulation with mite allergen in mite-allergic AD patients. In addition, T cell clones derived from atopic patients with high specific IgE allergic to DF produced significantly more IL-4 and less IFN- γ [13]. Other investigators showed that the supernatants of mononuclear cells from mite-allergic patients cultured with DF antigen, as well as IL-4, induced CD23 expression on U937 cells, while anti-IL-4 antibody inhibited the induction of CD23 expression on U937 cells by the supernatants [14]. It can be postulated that Af-DF induces the antigen-specific T cells to secrete IL-4, which may induce synthesis of CD23 on lymphocytes and monocytes.

CD23 is known to have various functions in B cells. CD23 constitutively expressed on normal B cells may be involved in the regulation of growth and differentiation of B cells [15]. It is not only an IgE receptor but also a membrane-bound precursor of soluble CD23 (sCD23) that still binds IgE. sCD23 released by CD23-bearing B cells are capable of increasing the spontaneous IgE synthesis by preactivated B cells in allergic donors [16], whereas it has been reported by several authors that monoclonal antibody against CD23 inhibited IgE production [17]. These results suggested that CD23 may play a role in IgE regulation. Recently, IgE-dependent antigen presentation by EBV-transformed B cells was demonstrated [18]. The CD23-bearing B cells pretreated with IgE efficiently captured antigen and showed enhanced antigen presentation, as compared with non- or IgG-treated B cells [19]. Recent studies further suggested that cross-linking of surface CD23 on such non-antigen-specific antigen-presenting B cells prevents their differentiation into Ig-secreting cells [20].

In contrast, the role of CD23 on T cells has been not yet well known; possibly because of the small percentage of CD23⁺ T cells. Our results also demonstrated that CD23 was induced by stimulation with Af-DF only in a small number of T cells (<1%). It was previously reported that T cells stimulated with phytohemagglutinin (PHA) expressed a high level of CD23 (>10%) on their surface, whereas IL-4 induced a small amount of CD23 on T cells (<4%) [21]. It may suggest that the mechanism of the PHA-induced CD23 expression on T cells is different from that of IL-4.

Our results confirmed the observation reported by Vercelli et al. [22] that IL-4 can strongly induce the expression of CD23 on monocytes (data not shown). In addition, we confirmed that Af-DF can also induce CD23 expression on monocytes. As described above, CD23 upregulation on monocytes induced by Af-DF may be associated with IL-4 released from Af-DF-specific helper T cells. It was reported that CD23 accounted for IgE-dependent release of inflammatory mediators from monocytes [23].

In conclusion, CD23 was upregulated on whole lymphocyte subpopulations and monocytes by Af-DF. The majority of CD23⁺ cells were B cells and monocytes; a small percentage of T cells expressed CD23. The effect of Af-DF on CD23 induction was specific, because Af-DF cannot lead to CD23 upregulation in non-mite-allergic AD patients as well as in normal donors. In addition, this effect was dose-dependent. It may be considered that exposure to mite antigen accounts for the elevated levels of CD23 in patients with AD. Considering that CD23 is closely associated with IgE production and IgE-dependent reactions, mite antigen not only directly, but also through CD23 indirectly, may be involved in pathogenesis of AD.

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