

Short communication

CD86 (B7-2) antigen on B cells from atopic patients shows selective, antigen-specific upregulation

Nakada M, Nishizaki K, Yoshino T, Okano M, Masuda Y, Ohta N, Akagi T. CD86 (B7-2) antigen on B cells from atopic patients shows selective, antigen-specific upregulation.

Allergy 1998; 53: 527-531. © Munksgaard 1998.

To determine whether B7 signals are associated with atopic responses in man, we assayed CD80 and CD86 expression on B cells and monocytes from atopic patients and controls. Peripheral blood mononuclear cells from 10 patients with perennial allergic rhinitis and from 10 normal subjects were cultured in the presence or absence of house-dust-mite antigen, and B cells and monocytes were assayed for expression of CD80 and CD86 by flow cytometry. CD86 on B cells was significantly and selectively upregulated in all atopic subjects, but not in normal subjects, whereas CD80 expression was not altered in B cells from the atopic subjects or controls. In contrast, both CD80 and CD86 were upregulated in monocytes from the atopic subjects as well as the controls. However, CD86 upregulation was significantly higher in the atopic subjects than in controls. Our results seem to suggest that selective upregulation of CD86 on B cells by a challenging antigen may play a critical role in the development of Th2 cells in patients with atopic disease.

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Key words: antigen-presenting cell; atopic
disease; CD80; CD86; costimulatory molecule;
Th2 cell.

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Accepted for publication 16 January 1998

Atopic disease of the immediate type of hypersensitivity is triggered by the production of allergen-specific IgE antibodies, which, in turn, is controlled by allergen-specific CD4⁺ Th cells (1). The latter have been classified into two distinct subsets by their different patterns of cytokine production and effector functions (2). Th1 cells mainly produce interferon-gamma (IFN- γ), interleukin-2 (IL-2), and tumor necrosis factor-beta (TNF- β), and function as effectors of cell-mediated immunity, whereas Th2 cells produce IL-4, IL-5, IL-10, and IL-13 and are mainly responsible for humoral immunity (3). IL-4 secreted by Th2 cells stimulates the production of allergen-specific IgE antibodies; conversely, IFN- γ secreted by Th1 cells inhibits IgE production (4). An imbalance in the production of these cytokines has been shown to be closely associated with atopic disease (5), and allergen-specific Th2 clones are dominantly established from the peripheral blood of patients with atopic disease (6-8). Although the differentiation of Th1/Th2 cells has been reported to be related to the nature and dose of antigen (9), the cytokine microenvironment (10), and the type

of antigen-presenting cell (APC) (11), the mechanism of this differentiation is not fully understood.

Antigen-specific T-cell responses require two distinct signals from APC (12). The first is provided by the interaction of the antigen/MHC complex with the T-cell receptor, and the second is provided by costimulatory molecules expressed on APC. Among these molecules, the most important are CD80 (B7-1) and CD86 (B7-2), members of the B7 family that bind to the counterreceptor CD28 on T cells (13-15). It has been reported that B7-associated signals regulate the strength of T-cell responses as well as the differentiation of T cells into Th1 or Th2 cells. Although CD80 and CD86 are preferentially associated in mice with the induction of Th1 and Th2 cells, respectively (16-19), the expression of these molecules on APC in atopic subjects has not yet been examined.

To determine whether B7 signals are associated with human atopic disease, we assayed the expression of CD80 and CD86 on B cells and monocytes from patients with perennial allergic rhinitis and from nonallergic subjects.

Material and methods

Subjects

Ten Japanese patients (15–31 years old, seven males and three females) with perennial allergic rhinitis and 10 healthy Japanese volunteers (24–40 years old, eight men and two women) with no history of allergic diseases were studied. Informed consent for participation in the study was obtained from each subject. All of the allergic patients exhibited specific IgE antibodies against *Dermaphagoides pteronyssinus* when assayed by the radioallergosorbent test. None of the patients received immunotherapy or used immunosuppressive drugs during this study.

Antigen

Crude extracts of *D. pteronyssinus* (Dp-c), a species of house-dust mite that causes perennial allergic rhinitis (20), were used as a stimulating antigen; the optimal Dp-c concentration for T-cell proliferation has been shown to be 10 µg/ml (7).

Separation of peripheral blood mononuclear cells (PBMC) and cell culture

Heparinized blood samples were obtained from the patients and controls. PBMC were separated by Ficoll-Paque density gradient centrifugation (Pharmacia Biotech, Uppsala, Sweden). The cells were washed in RPMI 1640 (Nikken Co, Kyoto, Japan) supplemented with 2% heat-inactivated FCS (fetal calf serum) and then resuspended in RPMI 1640, supplemented with 10% heat-inactivated FCS, 100 µg/ml streptomycin, 100 U/ml penicillin, and 20 mM L-glutamine (Gibco, Grand Island, NY, USA), at a concentration of 1.5×10^6 /ml. Cells were cultured in 48-well, flat-bottomed plates (Corning Co, Corning, NY, USA) for 12 h at 37°C in 5% CO₂/95% air in the presence or absence of Dp-c (10 µg/ml). In experiments assaying the kinetics of CD80 and CD86 expression, the PBMC of a patient with allergic rhinitis were cultured for 6, 12, and 24 h.

Flow cytometric analysis

Cultured cells were harvested and incubated on ice for 30 min with FITC-labeled anti-CD19 monoclonal antibodies (mAb) (Dako Japan, Kyoto, Japan), anti-CD14 mAb (Dako Japan, Kyoto, Japan), biotin-labeled anti-CD80 mAb (Pharmingen, San Diego, CA, USA), or biotin-labeled anti-B7-2 mAb (Pharmingen, San Diego, CA, USA). After washing with phosphate-buffered saline (PBS) supplemented with 0.4% horse serum, 7 mM NaN₃, and 1% HEPES,

the cells were stained with phycoerythrin-labeled streptavidin (Ancell Co, Bayport, MN, USA) for 30 min on ice. The cells were again washed, fixed with 2% paraformaldehyde, and analyzed with FACScalibur (Becton Dickinson, Mountain View, CA, USA). B cells were determined by gating CD19+ cells, whereas monocytes were gated on CD14+ cells and/or FSC/SCC dot plots. For comparison of antigen-specific expression between atopic and nonatopic subjects, percent modulation was calculated by the following formula:

$$\text{percent modulation} = (a - b) / b \times 100 (\%)$$

where *a* is the mean fluorescence intensity (MFI) of APC cultured with antigen and *b* is the MFI of APC cultured with medium alone (21).

Statistical analysis

Data are reported as percent modulation. Statistical comparisons were performed with Student's *t*-test. A level of $P < 0.05$ was accepted as statistically significant.

Results

Expression of CD80 and CD86 on B cells

We assayed the expression of CD80 and CD86 on B cells and monocytes by cytofluorography (Fig. 1) in primary cultures of PBMC in the presence or absence of antigen. We observed a significantly increased expression of CD86 on B cells of all atopic subjects, but not on B cells of nonatopic subjects (Fig. 2b). In contrast, we observed no significant alterations in CD80 expression in atopic or nonatopic subjects (Fig. 2a).

Expression of CD80 and CD86 on monocytes

In monocytes, expression of CD80 and CD86 was increased in cells from both atopic and nonatopic subjects cultured in the presence of antigen (Fig. 1). Percent modulation of CD86 in atopic subjects was significantly higher ($P < 0.01$) than in nonatopic subjects (Fig. 2d). The difference in CD80 expression was not statistically significant (Fig. 2c).

Kinetics of CD80 and CD86 expression on B cells and monocytes

When we assayed the kinetics of CD80 and CD86 expression on B cells, we observed that CD86 was rapidly induced at 6 h and peaked at 12 h. In contrast, we observed no significant changes in CD80 expression on B cells (Fig. 3). In monocytes, the expression of both CD80 and CD86 increased

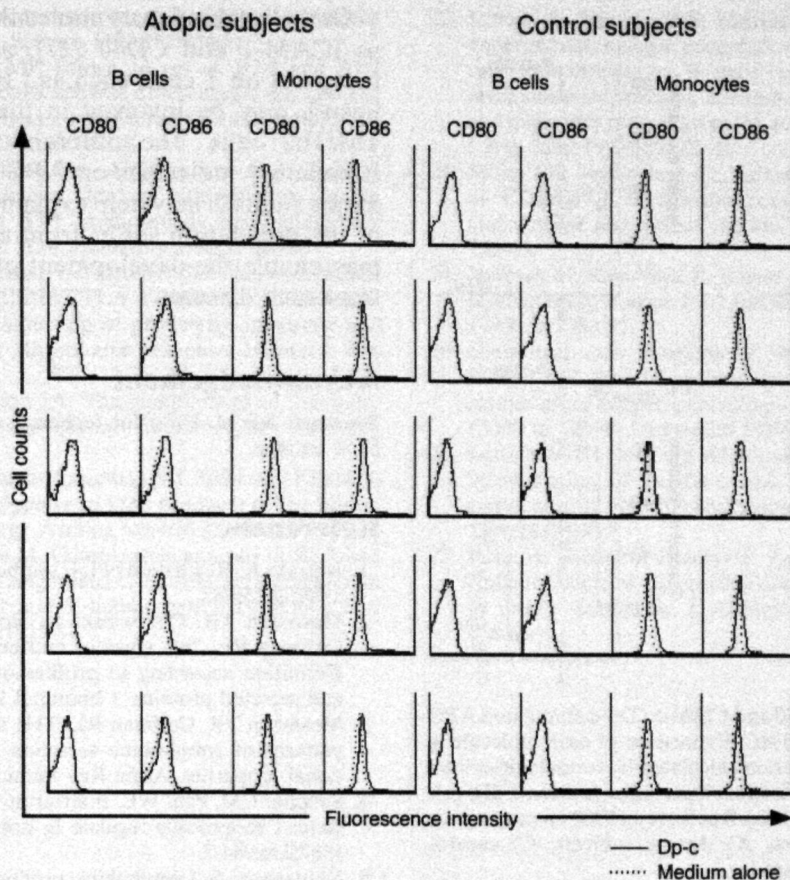


Fig. 1. Flow cytometric analysis of expression of CD80 and CD86 on APC. PBMC from atopic patients and controls were cultured in presence (solid line) or absence (dotted line) of Dp-c (10 µg/ml) for 12 h. Histogram plots from five unselected subjects are shown. x-Axis (log scale) shows fluorescence intensity and y-axis shows cell counts.

significantly 6 h after exposure to antigen, while CD86 showed more intense induction, peaking at 6 h and then gradually declining (Fig. 3).

Discussion

We have shown a selective upregulation of CD86 expression on antigen-stimulated B cells in primary culture of PBMC from atopic, but not from non-atopic, subjects, as well as an enhanced expression of CD86 on monocytes from both atopic and nonatopic subjects. These findings suggest that CD86 on B cells play an important and selective role in antigen presentation, leading to the development of Th2 cells in atopic subjects. The expression of CD86 on B cells may be regulated by direct and/or indirect (e.g., via allergen-specific T cells) interaction with allergens. In either case, the upregulated CD86 on B cells should interact strongly with CD28 on T cells, and this paracrine-like stimulation may induce a strong and prolonged allergen-specific hypersensitive state. Although the number of allergen-specific B cells in PBMC is not known, our findings suggest that allergen stimula-

tion in atopic subjects may enhance CD86 expression on B cells to a greater extent than in nonatopic subjects, whether or not the subjects had previously been exposed to antigen.

It has lately been suggested that CD86 induces the differentiation of Th2 cells, whereas CD80 induces Th1 differentiation (16–19). For example, in experimental allergic encephalomyelitis, costimulation by CD86 was found to generate Th2 responses, whereas costimulation by CD80 produced Th1 responses (16). Freeman et al. reported similar evidence in human CD4⁺ CD45RA⁺ T cells stimulated by CD80 or CD86 transfectants (17). It has also been reported that Th2 differentiation is induced by antigen presentation by B cells (22), while Th1 differentiation is associated with the expression of CD80 on macrophages (23). Our finding that CD86 on B cells was selectively upregulated by antigen stimulation in atopic subjects may support this hypothesis, although some reports contradict it (24, 25).

When we assayed the kinetics of CD80 and CD86 expression on B cells and monocytes from an atopic subject, we found that CD86 expression

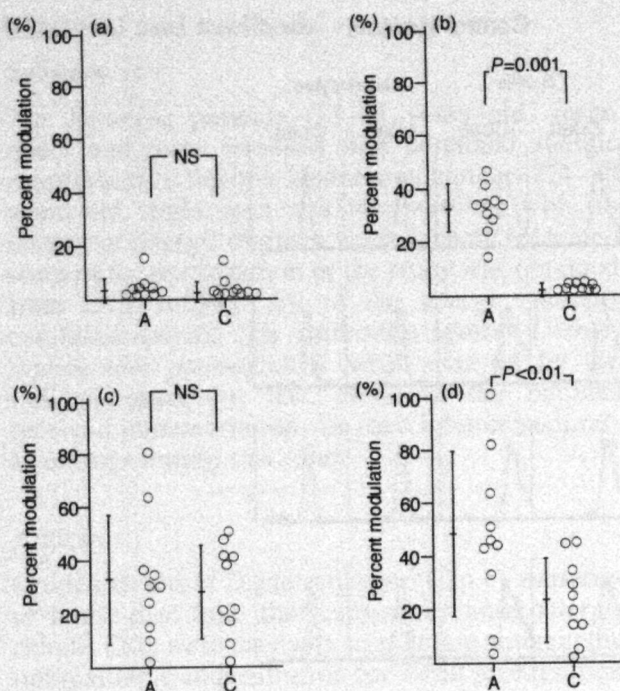


Fig. 2. Expression of CD80 and CD86 on Dp-c-stimulated APC in primary cultures of PBMC. Expression of each molecule is shown by percent modulation calculated by formula described in Material and methods section. Bars indicate mean $\pm$ SD. (a): CD80 on B cells; (b): CD86 on B cells; (c): CD80 on monocytes; (d): CD86 on monocytes. A) Atopic subjects; C) control subjects. NS: not significant.

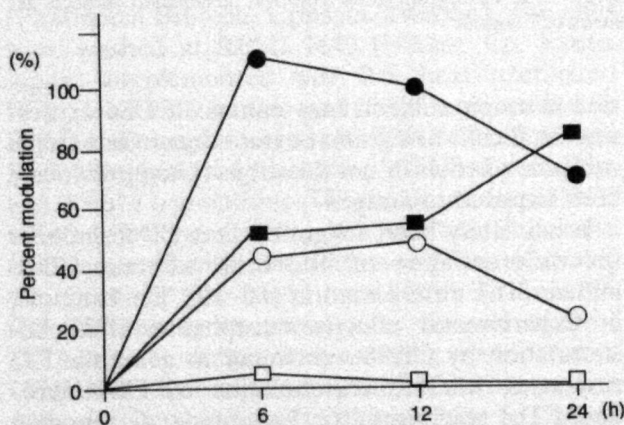


Fig. 3. Kinetics of CD80 and CD86 expression on APC. PBMC were cultured in presence or absence of Dp-c (10 μ g/ml) for 6, 12, and 24 h, and expression of CD80 and CD86 was determined by percent modulation. $\circ$: CD86 on B cells; $\bullet$: CD86 on monocytes; $\square$: CD80 on B cells; $\blacksquare$: CD80 on monocytes.

was enhanced on B cells at 6 h and peaked at 12 h, in agreement with the results of Lenschow et al. (26). However, the enhanced expression of CD86 on monocytes peaked at 6 h, suggesting that, in the early stages of immune reactions, antigen presentation by monocytes may be more important than that by B cells.

Other costimulatory molecules on B cells, such as ICAM-1 and CD40 (27), as well as counter-receptors on T cells, such as LFA-1 and the CD40 ligand, may be involved in the differentiation of Th1/Th2 cells. The different expression of costimulatory molecules on APC in atopic and non-atopic subjects may help explain why only a portion of the population suffer from atopic diseases, and may enable the development of novel methods to treat such diseases.

Acknowledgement

We thank Ms M. Hato for technical assistance in preparing Dp-c antigen.

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