

Upregulation of B7.2, but not B7.1, on B cells from patients with allergic asthma

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Background: Allergic asthma is associated with T_{H2}-like cell responses and increased IgE production. Recent studies in mice have suggested that the costimulatory molecule B7.2 (CD86) may influence the development of T_{H2} cells.

Objective: We sought to determine the potential role of B7.2 in patients with asthma.

Methods: We performed an analysis of B cells from patients with allergic asthma and healthy control subjects for expression of B7.1 and B7.2 on B cells using five-parameter flow cytometry.

Results: We report that atopic patients with asthma who are exposed to allergens have significantly ($p < 0.005$) higher levels of B7.2 expression on B cells than atopic asthmatic subjects not exposed to allergen *in vivo* or nonatopic control subjects. In contrast, there were no differences in B7.1 (CD80) expression among the three study subject groups. When peripheral blood mononuclear cells from asthmatic patients or normal control subjects were stimulated with IL-4 or IL-13, the expression of B7.2, but not B7.1, was significantly increased ($p < 0.005$) on B cells. Interferon- γ or IL-12 did not affect the expression of either molecule. The functional significance of B7.2 induction by IL-4 in allergic disease was suggested by the increased expression of this molecule on CD23⁺, but not CD23⁻, B cells.

Conclusion: These results indicate that the same B cell involved in allergen presentation also expresses the costimulatory molecule B7.2 and support the hypothesis that this molecule is an important costimulatory molecule in allergic responses, the expression of which can be modulated by T_{H2}-like cytokines. (*J Allergy Clin Immunol* 1998;101:96-102.)

Key words: B cells, asthma, costimulatory molecules, interleukin

The B7 antigen is an accessory molecule that plays an important role in T-cell/B-cell interactions.^{1,2} B7 is present mainly on antigen-presenting cells and is the ligand for CD28/CTLA4 expressed on both resting and activated T cells, respectively.³ Interaction between B7 and CD28/CTLA4 allows T cells to respond to an

Abbreviations used

FITC:	Fluorescein isothiocyanate
IFN- γ :	Interferon- γ
PBMC:	Peripheral blood mononuclear cells
PE:	Phycoerythrin
PerCP:	Peridinin chlorophyll- α protein

activated B cell by dividing and producing cytokines.⁴ The B7 antigen is present in at least two forms, B7.1 (CD80) and B7.2 (CD86). A third form of B7 (B7-3) has recently been described, but its function has not yet been defined.³ Both B7.1 and B7.2 bind to CD28 and CTLA4 but use different binding determinants and have different binding kinetics,⁵ suggesting functional differences between these molecules. Work done in mice suggests that B7.1 and B7.2 influence the type of T-helper cell activation differently,⁴ with B7.2 involved in the development of T_{H2} cells. However, the relevance of B7.1 and B7.2 in T_{H2}-like responses is still unknown in human beings.

Asthma is a prototypic allergic disease associated with expansion of T_{H2} cell-like responses and elevated IgE responses.^{6,7} This predominance of IL-4- and IL-13-secreting T_{H2}-like cells in allergic asthma makes it an ideal model of human disease to determine whether there is an imbalance in the expression of the B7 family of molecules in human T_{H2}-mediated responses. In this study we therefore analyzed the expression of B7.1 and B7.2 on the surface of freshly isolated B cells from atopic asthmatic subjects undergoing exposure to allergen *in vivo*, as compared with atopic asthmatic subjects not exposed to allergen or nonatopic controls. We also investigated the effect of T_{H1}-like versus T_{H2}-like cytokines on the expression of B7 molecules.

METHODS

Reagents

Anti-B7.1 and anti-B7.2 antibodies (IgG1) were purchased from Ancell (Bayport, Minn.). Recombinant human IL-4 (rIL-4) was obtained from Schering Research Institute (Bloomfield, N.J.). Human rIL-13 was obtained from Peprotech (Rocky Hill, N.J.), recombinant interferon- γ (rIFN- γ) from Genentech Inc. (San Francisco, Calif.), and rIL-12 from R&D Systems (Minneapolis, Minn.). The following monoclonal antibodies were obtained from Becton Dickinson Immunocytometry Systems (San Jose, Calif.): anti-CD19 (fluorescein isothiocyanate [FITC], Peridinin chlorophyll- α protein [PerCP]), anti-CD80 or B7.1 (phycoerythrin [PE]), G1CL (mouse IgG1

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control; FITC, PE, PerCP), and G2GL (mouse IgG2 control; FITC, PE, PerCP). The anti-CD86 or B7.2 PE-conjugated monoclonal antibody was obtained from Ancell (Bayport, Minn.). The anti-CD23 FITC-conjugated monoclonal antibody was obtained from Pharmingen (San Diego, Calif.).

Subjects and cell cultures

We investigated 16 patients with allergic asthma, including 10 patients who were allergic to grass pollen, ragweed, or both. We also investigated six patients who were allergic to cats, as demonstrated by positive skin prick test responses to the involved allergen. During the study, nine patients were exposed to the allergen to which they were allergic. In the patients allergic to pollen, blood was drawn during pollen season. On the basis of daily pollen counts, the peak of grass-pollen season in Denver, Colorado was between mid-May and early June; and the peak of ragweed season was during late August and early September. Patients allergic to cats were considered exposed if they were living with a cat. These nine asthmatic subjects were on as-needed inhaled β -agonist therapy at the time of study. None had previously received oral glucocorticoid therapy. Seven allergic asthmatic subjects had their blood drawn at a time when there was no in vivo exposure to pollen (i.e., during the winter or when exposed to cats).

Seven nonatopic healthy subjects who had negative skin test responses to a panel of common allergens were also studied. Informed consent was obtained from all subjects before entering the study.

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood by density gradient centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden). In selected experiments PBMCs were cultured in the presence and absence of either rIL-4 (400 U/ml), rIL-13 (100 ng/ml), rIFN- γ (100 ng/ml), or rIL-12 (5 ng/ml) for 48 hours at 2×10^6 cells/ml in RPMI 1640 (Bio-Whitaker, Walkersville, Md.) supplemented with 10% heat-inactivated fetal calf serum (Gibco, Grand Island, N.Y.), 20 mmol/L N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid buffer, antibiotics, and 2 mmol/L L-glutamine (Gibco).

Cell staining and flow cytometric analysis

Five-parameter analysis was performed by using a FACScalibur flow cytometer (Becton Dickinson) with FITC, PE, and PerCP as the three fluorescent parameters. Immunofluorescence staining for this multiparameter analysis was performed as previously described.⁸ Methods of cytometer set up and data acquisition were as described previously.⁸ List-mode multiparameter data files (each file with forward scatter, side scatter, and three fluorescent parameters) were analyzed by use of the CellQuest Macintosh program (Becton Dickinson). Analyses were performed using a light-scatter gate including only viable lymphocytes and a gate based on expression of CD19+ B cells. Negative control reagents were used to verify the staining specificity of experimental antibodies.

Statistical analysis

Data are expressed as individual values or the mean $\pm$ SEM for each subject group. Statistical comparisons were made with an unpaired two-tailed Student's *t* test to compare different groups of study subjects and a paired Student's *t* test to compare PBMCs from the same donors cultured under different conditions. Differences between groups were considered significant at *p* values less than 0.05.

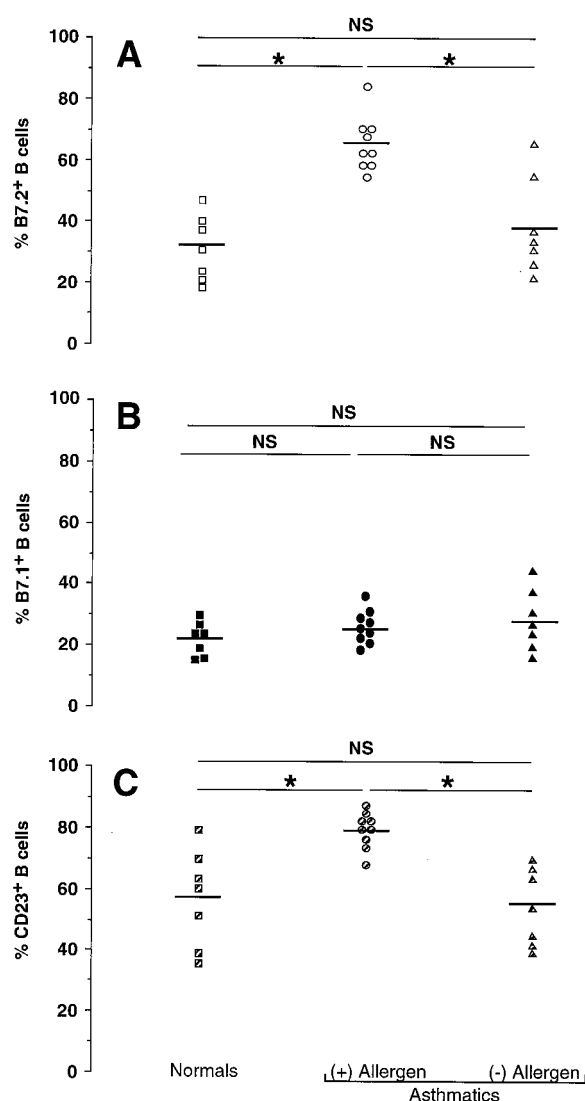


FIG. 1. B7.2 and CD23, but not B7.1, expression on B cells is increased in asthmatic patients exposed to allergen. Freshly isolated PBMCs from nonatopic control subjects (*squares*), asthmatic patients exposed to allergen (*circles*), and asthmatic patients not exposed to allergen (*triangles*) were stained with anti-CD19 FITC-conjugated antibodies and either anti-B7.2 PE-conjugated (**A**), anti-B7.1 PE-conjugated (**B**), or anti-CD23 PE-conjugated antibodies (**C**). They were analyzed by flow cytometry. Results for individual values are shown (nonatopic control subjects vs asthmatic subjects exposed or not exposed to allergen). NS, Not significant. **p* < 0.005.

RESULTS

Increased expression of B7.2 on B cells of atopic patients with asthma

Freshly isolated PBMCs from patients with atopic asthma and normal control subjects were analyzed by flow cytometry for the expression of B7.1, B7.2, and CD23 on B cells. As shown in Fig. 1, *A*, B7.2 expression was significantly higher on B cells from asthmatic patients exposed to allergen ($63.3\% \pm 2.9\%$) compared with nonatopic control subjects ($34.1\% \pm 4.1\%$, *p* <

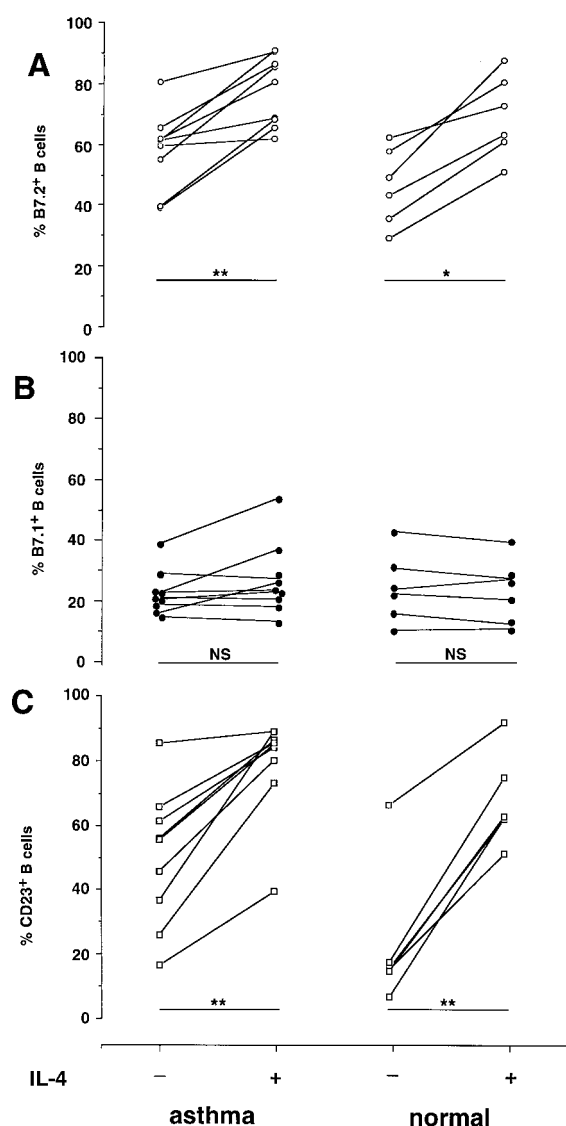


FIG. 2. B7.2 and CD23, but not B7.1, expression on B cells is increased by IL-4. PBMCs from nine asthmatic patients and seven nonatopic control subjects were cultured for 2 days in the presence or absence of rIL-4. At the end of the culture period, PBMCs were stained with anti-CD19 FITC-conjugated antibodies and either anti-B7.2 PE-conjugated (A), anti-B7.1 PE-conjugated (B), or anti-CD23 PE-conjugated antibodies (C). They were then analyzed by flow cytometry. Results are expressed as individual values (medium alone vs IL-4). NS, Not significant. * $p < 0.005$. ** $p < 0.001$.

0.005). However, the percentage of B7.2 expression on B cells ($37.2\% \pm 7.5\%$) from asthmatic patients without symptoms not exposed to allergen at the time that blood was drawn was similar to that of normal control subjects, but significantly less ($p < 0.005$) than that of asthmatic patients exposed to allergen (Fig. 1, A). B7.1 expression on B cells (Fig. 1, B) was not significantly different between allergic patients and nonatopic control subjects. As a control, we measured B-cell expression of CD23, the low affinity Fc receptor for IgE, which is known to be increased in allergic diseases.⁹ CD23 expression on B

cells (Fig. 1, C) was significantly increased in asthmatic patients exposed to allergen ($78.5\% \pm 2.0\%$) compared with nonatopic control subjects ($56.1\% \pm 6.2\%$, $p < 0.01$) or asthmatic patients not exposed to allergen ($58.9\% \pm 5.1\%$).

Increased expression of B7.2 on B cells cultured with IL-4 or IL-13

The observation that increased B7.2 expression on B cells was found only in allergic patients exposed to allergen at the time that blood was drawn suggested that the level of B7.2 expression was not genetically determined but rather the result of in vivo allergen-driven T_{H2} -like cell activation. To test this hypothesis, we cultured PBMCs from patients with allergic asthma and from nonatopic control subjects in the presence of various T_{H1} -like and T_{H2} -like cytokines for 2 days. This time point was chosen on the basis of preliminary experiments that showed that maximal induction of B7.2 is achieved after 24 hours and that maximal induction of B7.1 is achieved after 48 hours.

As shown in Fig. 2, IL-4 significantly increased the percentage of B cells positive for B7.2 in both groups ($p < 0.005$). However, IL-4 did not influence the expression of B7.1 on B cells. As expected, IL-4 increased the expression of CD23 on B cells in both groups. In contrast to CD23, in which both the percentage of positive cells and the fluorescence intensity were increased by IL-4, only the percentage of B7.2⁺ cells was increased after culture with IL-4 (Fig. 3).

We considered the possibility that our inability to detect changes in B7.1 after IL-4 stimulation was due to a defective reagent. However, this is highly unlikely for two reasons. First, the commercially available anti-B7.1 we used stained 20% to 40% of B cells at baseline in both asthmatic and normal subjects (Figs. 1 and 2). Second, addition of 10 mg/ml phytohemagglutinin to unfractionated PBMCs led to a significant rise in B7.1 expression on B cells ($49.5\% \pm 6.3\%$; $p < 0.05$) when compared with medium alone ($23.7\% \pm 2.0\%$).

We also analyzed the effects of three other cytokines on B7 expression on B cells. The cytokine concentrations were determined by a dose-response curve in preliminary experiments. As shown in Table I, IL-13 had the same effect as IL-4 on B7.2 and CD23 expression on B cells from both asthmatic patients and normal control subjects. However, IFN- γ and IL-12 did not have any effect on the baseline expression of B7.2 or B7.1, although IL-12 and IFN- γ significantly decreased the expression of CD23 on B cells. Furthermore, neither IL-12 nor IFN- γ inhibited IL-4-induced B7.2 expression (Table II). Thus B7.2, which had a higher expression in asthmatic subjects exposed to allergen, was increased on B cells by T_{H2} -like, but not T_{H1} -like, cytokines.

IL-4 stimulates double-positive expression (B7.2 and CD23) on B cells

Because CD23 is involved in IgE-dependent antigen presentation¹⁰ and the upregulation of IgE synthesis,¹¹

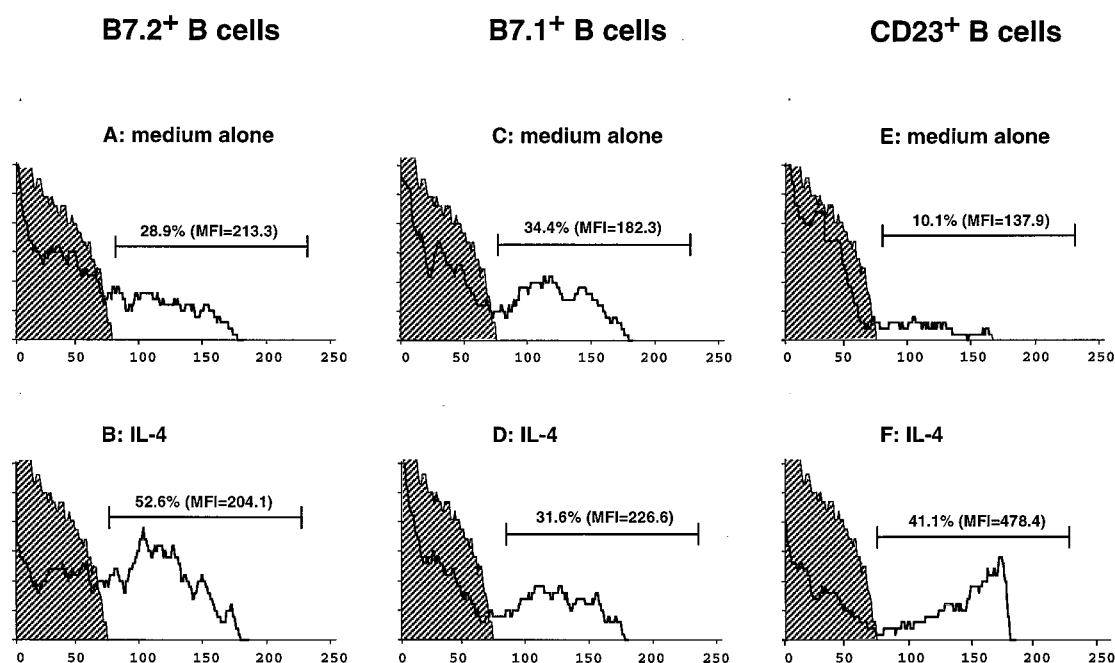


FIG. 3. Percentage of B7.2- and CD23-expressing B cells and the MFI of CD23 surface molecules are increased after culture with IL-4. Cultures were done as in Fig. 3. Representative histogram plots show isotype-control (hatched area) B7.2 (A and B), B7.1 (C and D), and CD23 (E and F) expression on gated B cells (solid line) in medium alone (A, C, and E) and in presence of IL-4 (B, D, and F). Percentage of positive B cells and MFI (horizontal axis) of positive cells are indicated in each panel. Similar histograms were found in all other experiments. MFI, Mean fluorescence intensity.

TABLE I. B7 and CD23 expression on B cells cultured with cytokines from patients with asthma

Cell surface marker	Positive B cells after incubation with (%)				
	Medium	IL-4	IL-13	IFN- γ	IL-12
B7.1	20.4 $\pm$ 3.4	24.2 $\pm$ 5.0	24.8 $\pm$ 4.4	17.9 $\pm$ 3.0	24.0 $\pm$ 2.5
B7.2	56.5 $\pm$ 5.5	78.2 $\pm$ 3.7†	76.0 $\pm$ 3.6†	61.5 $\pm$ 4.5	54.0 $\pm$ 4.8
CD23	46.9 $\pm$ 11.3	81.7 $\pm$ 2.6*	74.8 $\pm$ 4.2*	34.7 $\pm$ 8.1*	39.4 $\pm$ 10.3*

Results are expressed as mean $\pm$ SEM of six experiments (medium alone vs cytokine stimulation).

* $p < 0.01$.

† $p < 0.005$.

we determined whether B7.2 and CD23 are expressed on the same cell and what the effect of IL-4 might be on the coexpression of these molecules. Therefore we measured the expression of B7.2⁺ CD23⁺ B cells in PBMCs from three normal donors incubated in medium alone versus IL-4 and compared it with B7.2⁺ CD23⁻ B cells. As shown in Table III, IL-4 significantly increased the expression of B7.2⁺ CD23⁺ B cells (35.3% $\pm$ 7.5%) compared with medium alone (3.7% $\pm$ 0.3%, $p < 0.05$). In contrast, IL-4 decreased the expression of B7.2⁺ CD23⁻ B cells (medium alone: 22.7% $\pm$ 2.3%; IL-4: 16.3% $\pm$ 1.2%, $p < 0.05$). B7.2 fluorescence profiles of the CD19⁺ CD23⁺ and CD19⁺ CD23⁻ cells before and after IL-4 treatment is shown in Fig. 4. These results indicate that IL-4 enhances the expression of B7.2⁺ CD23⁺ B cells.

DISCUSSION

Costimulation through the B7/CD28 pathway plays a critical role in the establishment of antigen-driven immune responses.¹ Antigen-specific immune responses are initiated after the interaction between T cells and antigen-presenting cells such as B cells. T-cell activation occurs after two signals. The first signal is the interaction between the T-cell receptor and major histocompatibility complex class II molecules. The second signal involves accessory molecules such as CD28 and B7. CD28 has been shown to provide a necessary signal, independent of T-cell receptor signaling, that costimulates T-cell responses by inducing IL-2 gene transcription.¹² This signal is initiated after interaction with its ligand, either B7.1 or B7.2, both of which costimulate to induce T-cell proliferation, production of IL-2, and expression of the

TABLE II. IFN- γ and IL-12 do not alter B7.1 and B7.2 expression on human PBMCs cultured with IL-4

Cytokines added to culture	Surface molecule on B cells (%)	
	B7.1	B7.2
None	8.26 $\pm$ 2.97	28.2 $\pm$ 5.97
IL-4	8.65 $\pm$ 1.64	45.88 $\pm$ 4.90
IL-4 + IFN- γ	8.25 $\pm$ 1.54	50.65 $\pm$ 6.49
IL-4 + IL-12	7.69 $\pm$ 0.66	42.64 $\pm$ 1.22

IL-4 increased B7.2 but not B7.1 expression compared with medium alone ($p = 0.02$). The addition of IFN- γ versus IL-12 to cells cultured with IL-4 did not modify the expression of B7.1 or B7.2 on B cells ($p > 0.05$).

IL-2 receptor.¹³ The relevance of the B7/CD28 pathway in allergic response is supported by recent observations demonstrating that anti-CD28 antibodies block IgE, but not IgG, responses by tonsillar B cells cultured in the presence of T cells and IL-4.¹⁴

Because both B7.1 and B7.2 interact with CD28 to provide costimulatory signals, it has been proposed that they may deliver different signals to T cells and thereby activate different pathways of T-helper cell development. Indeed, Kuchroo et al.¹⁵ reported that treatment of mice with anti-B7.2 resulted in the production of effector cells with a T_{H1} phenotype and with anti-B7.1 in the generation of T_{H2} cells. The T_{H2} cells were able to prevent and even abrogate the occurrence of experimental allergic encephalomyelitis, whereas T_{H1} cells increased disease severity. In a subsequent study Freeman et al.¹⁶ suggested that B7.2 directs the immune response toward T_{H2} or T_{H0} cells. However, B7.1 has a more neutral differentiation signal. Using transfected Chinese hamster ovary cells, Natesan et al.¹⁷ reported that both B7 molecules could costimulate IL-4 production by anti-CD3-activated murine T cells, with a similar quantitative effect. These latter observations provided evidence against the hypothesis of a specific effect of B7.2 on the direction of T_{H2} development.

Additional studies with transfection of B7 molecules also could not confirm the earlier reports that B7.1 and B7.2 directed differential development of T_{H1} versus T_{H2} cells. A study by Lanier et al.¹⁸ demonstrated that both B7.1 and B7.2 could efficiently costimulate anti-CD3 antibody-induced proliferation, as well as the secretion of IL-2 and IFN- γ by murine T cells. In human beings, De Boer et al.¹⁹ has shown that B7.1 could act as a costimulatory molecule for IL-4 production by T lymphocytes activated by monoclonal antibodies directed at the T-cell receptor. Nevertheless, it has been shown in *in vitro* and *in vivo* studies with transgenic mice that CD28 costimulation is important for the differentiation of T_{H2} cells.²⁰⁻²³

Although these experimental models do not support a clear-cut dichotomy in the ability of B7.1 versus B7.2 molecules to generate T_{H1} versus T_{H2} responses, it is

perhaps more important to determine whether these molecules are differentially used in naturally occurring human disease states where distinct antigen-driven T_{H2} responses arise. To our knowledge, there have been no previous studies delineating the role of B7.1 and B7.2 in human allergic diseases such as asthma, which has now been demonstrated to involve a prototypic T_{H2} -mediated immune response.⁷

In this study we demonstrate for the first time that the B7.2 molecule is selectively upregulated in allergic patients with asthma, but only when these patients are exposed to allergen *in vivo*. Our results suggest that this upregulation is mediated by IL-4 and IL-13 because both cytokines increase the expression of B7.2 and it is well established that allergens induce the expansion of IL-4- and IL-13-secreting T_{H2} cells.²⁴ In contrast, we found that two T_{H1} -like cytokines (i.e., IFN- γ and IL-12) failed to alter the expression of B7.1 or B7.2 on B cells. In contrast, IFN- γ and IL-12 significantly inhibited the induction of CD23 on B cells (Table I).

Our data therefore suggests that B7.2 is likely to be the relevant costimulatory molecule on B cells interacting with CD28 on T cells to induce allergen-driven T-cell responses in human beings. Consistent with our current finding, Tsuyuki et al.²⁵ has recently reported that airway allergen challenge upregulates the expression of B7.2 on B cells from the lung and airway administration of anti-B7.2 monoclonal antibody-inhibited local IgE production in a mouse model of asthma. Similarly, Krinzman et al.²⁶ found that blockade of costimulation with CTLA4-Ig (a fusion protein known to prevent costimulation by blocking CD28/B7 interactions) inhibits airway hyperresponsiveness, inflammatory cell infiltration, and allergen-specific responsiveness of thoracic T cells in a murine model of asthma. Finally, Gause et al.²⁷ demonstrated that the increased serum IgE response to goat anti-mouse IgD was blocked in CD28 knockout mice.

We also found a potential link between B7.2 upregulation by IL-4 and the low affinity Fc receptor for IgE (CD23) in that both receptors were induced primarily on the same B cell. This may be of considerable interest because CD23 expression on B cells plays an important role in the stimulation of IgE responses in atopic disease and is also upregulated by IL-4.²⁸⁻³⁰ Previous studies have demonstrated that binding of allergen-specific IgE to CD23 allows nonspecific B cells to capture specific allergens and present very low quantities of allergen to antigen-specific T cells.^{11, 31, 22} Thus CD23 is thought to facilitate allergen capture and allergen presentation by B cells.

The effectiveness of antigen-specific B cells to act as antigen-presenting cells has been found to depend on both antigen uptake and the upregulation of B7.2.³² Our observation that both B7.2 and CD23 are selectively induced by IL-4 to be expressed on the same B cell may represent a significant amplification loop leading to the enhancement of allergic responses. Thus we propose that after allergen exposure, IL-4 and IL-13 production is initially released from mast cells, basophils, or T_{H2}

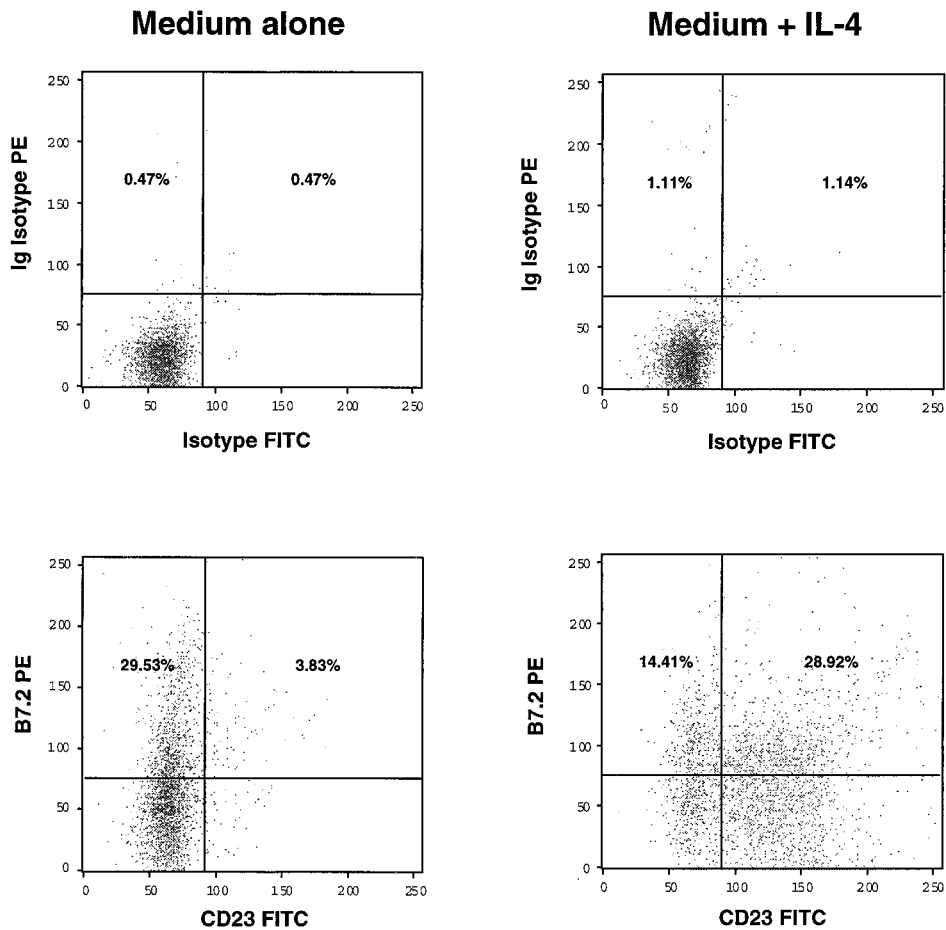


FIG. 4. B7.2 immunofluorescence is increased on CD23⁺, but not CD23⁻, B cells after IL-4 stimulation. PBMCs from normal subject were cultured in RPMI and 10% fetal calf serum with 400 U/ml rIL-4 (*right panels*) or with medium alone (*left panels*) for 48 hours. Cells were harvested and stained with CD23-FITC, B7.1-PE, or B7.2-PE and CD19-PerCP. The staining characteristics of CD19⁺ B cells with regard to B7.2 versus CD23 are indicated in the profiles, shown with the quadrants delineating the cutoffs for positive and negative staining. The percentages of B7.2⁺ CD23⁺ or B7.2⁺ CD23⁻ cells are given in the upper right and left corners of each profile, respectively. After IL-4 stimulation, B7.2 immunofluorescence increased in the CD23⁺, but not the CD23⁻, B cells.

TABLE III. IL-4 upregulates the expression of B7.2 on CD23⁺, but not CD23⁻, B cells from normal control subjects

Surface expression	Positive B cells after incubation (%)							
	Experiment 1		Experiment 2		Experiment 3		Mean ± SEM	
	Medium	IL-4	Medium	IL-4	Medium	IL-4	Medium	IL-4
B7.2 ⁺	30.2	54.4	26.5	39.5	23.4	62.7	26.7 ± 2.0	52.3 ± 6.7*
CD23 ⁺ B7.2 ⁺	3.1	35.7	4.3	22.4	4.8	48.5	3.7 ± 0.3	35.3 ± 7.5*
CD23 ⁻ B7.2 ⁺	27.2	18.7	22.3	17.1	18.7	14.3	22.7 ± 2.3	16.3 ± 1.2*

Results are shown as percentage of B7.2⁺ B cells, percentage of CD23⁺B7.2⁺ B cells, and percentage of CD23⁻B7.2⁺ B cells. Individual results and the mean ± SEM of three experiments are shown.

**p* < 0.05.

cells. This leads to enhanced CD23 expression on B cells and upregulation of B7.2 expression on CD23⁺ B cells. IgE binds to CD23 allowing nonspecific B cells to become potent allergen-presenting cells acting in con-

cert with the costimulatory molecule B7.2.³³ As a result of these double-positive B cells, T_{H2}-like cells may expand and induce more B cells to switch to IgE, with a subsequent overproduction of IL-4 and IgE. This ampli-

fication loop provides an explanation for the observation that certain allergic patients, sensitive to only one allergen, develop a polyclonal IgE response.

In summary, this study supports the concept that B7.2 acts as an important costimulatory molecule in human allergic responses and may act in concert with the CD23 molecule to enhance IgE responses. Experimental animal models demonstrating that antibodies blocking B7.2 action can interfere with the development of T_{H2} cells¹⁵ support the concept that B7.2 may be a candidate for future therapeutic approaches in the management of allergic disease.

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