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Enhanced Expression of B7.2 (CD86) in Patients with Atopic Dermatitis: A Potential Role in the Modulation of IgE Synthesis

Oratthai Jirapongsananuruk,* Michäel F. Hofer,* Anne E. Trumble,* David A. Norris,† and Donald Y. M. Leung2*‡

Recent studies have suggested that the accessory molecules B7.1 (CD80) and B7.2 (CD86) differ in their capacity to generate Th1 vs Th2 responses. Atopic dermatitis (AD) is a chronic allergic skin disease associated with increased IgE synthesis. To determine the potential role of B7.2 molecules in AD, the present study was conducted to compare the expression of B7.1 vs B7.2 on B cells from patients with AD vs normal subjects or patients with psoriasis. The expression of B7.2 on B cells of AD patients (53.67 ± 3.10%) was significantly higher than normals (38.02 ± 4.95%; p = 0.02) and psoriasis patients (40.19 ± 2.70%; p = 0.006). In contrast, there was no significant difference in B7.1 expression among the three subject groups. Interestingly, total serum IgE from patients with AD vs normal subjects or patients with psoriasis. The expression of B7.2 on B cells of AD patients (53.67 ± 3.10%) was significantly higher than normals (38.02 ± 4.95%; p = 0.02) and psoriasis patients (40.19 ± 2.70%; p = 0.006). Anti-human B7.2, but not B7.1, mAb significantly (p = 0.04) decreased IgE production by PBMC stimulated with IL-4 and anti-CD40 mAb. Furthermore, B7.2+ B cells had a significantly higher level of IL-4R and CD23 expression than B7.1+ B cells. These data demonstrate the predominant expression of B7.2 in AD, but not psoriasis, and a novel role for this molecule in IgE synthesis. The Journal of Immunology, 1998, 160: 4622–4627.

T and B cells play a critical role in the pathogenesis of allergic diseases (1). Activation of resting lymphocytes require at least two signals (2, 3). In addition to the engagement of the TCR with the MHC plus peptide complex expressed on B cells, the interaction between CD40 molecules on B cells and the TCR with the MHC plus peptide complex expressed on T cells is a second important costimulatory signal required for switch recombination to IgE synthesis in the presence of IL-4 (4–6). The engagement of B7 on APCs with CD28/CTLA-4 on T cells is another well-characterized costimulatory pathway for T and B cell activation (7).

At least two members of the B7 family of costimulatory ligands, B7.1 (CD80) and B7.2 (CD86), have now been identified. B7.1 is normally expressed at low levels on “professional” APCs, such as dendritic cells and macrophages, and up-regulated on these APCs as well as on B cells following activation by soluble factors (e.g., cytokines) or ligation of cell surface molecules (e.g., MHC class II and CD40). B7.2 is constitutively expressed on dendritic cells and is rapidly induced on B cells following activation by cross-linking of the Ig receptor or the addition of various cytokines (8). Several studies have demonstrated distinct kinetics and interaction sites between the B7.1/B7.2 and the CD28/CTLA-4 receptor-counter

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Materials and Methods

Patients and control subjects

We obtained blood from eight patients (three males and five females, aged 27 to 45 yr) with moderate to severe AD (skin involvement more than 20%) and serum IgE ranging from 269 to 797 U/mL. None of the patients had previously used systemic corticosteroids. Topical corticosteroids were withheld for greater than 48 h before blood collection. Eight normal healthy adults (four males and four females, aged 27–36 yr) who were skin

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3 Abbreviations used in this paper: CD40L, CD40 ligand; AD, atopic dermatitis; PerCP, peridinin chlorophyll-a protein; PE, phycoerythrin.

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without anti-CD40 mAb (10^6 purified B cells), and cultured in the presence of rIL-4 (400 U/ml) with or without anti-human B7.2 mAb and the mouse anti-human B7.1 mAb were used at 1:1 mixture. In these studies, the mouse IgG control, mouse IgG1 control; FITC, PE, PerCP) and G2CL (mouse IgG2 control; FITC, PE, PerCP). The anti-B7.2 PE-conjugated mAb was obtained from Ancell (Bayport, MN). The anti-CD23 FITC conjugated mAb was obtained from PharMingen.

Cell preparation and cell cultures

PBMC were isolated from heparinized venous blood from study subjects by density gradient centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden), and washed three times in HBSS (Life Technologies, Grand Island, NY).

T cells were purified from PBMC by magnetic bead separation as previously described (29). Briefly, PBMC were incubated with mAb anti-CD3 (Becton Dickinson) at 4°C for 30 min, then washed twice in HBSS and incubated with magnetic beads coated with anti-mouse IgG (Dynabeads M-450, Dynal, Oslo, Norway) at 4°C for 60 min. Ab-coated to the beads were separated from non-T cells using a magnet (Advanced Magnetics, Cambridge, MA). Both T cells and non-T cells were incubated in culture medium (RPMI plus 10% FCS) overnight at 37°C to detach T cells from the magnetic beads (subsequently removed by a magnet), and to separate monocytes/macrophages from B cells by plastic adherence. T cell purity was 96.5% by this separation method. Purified CD19~+~ B7.2~+~ B cells were isolated from B cell preparations by fluorescence-activated cell sorting on an EPICS 752 cell sorter (Coulter, Hialeah, FL) using the anti-CD19 FITC and B7.2-PE mAbs, as previously described (30). Purification by cell sorting resulted in 95.8% of CD19~+~, and 91.2% of CD19~+~ B7.2~+~ cells.

For cell culture studies, PBMC (at 1 × 10^6 cells/ml) or purified B cells (1 × 10^5 cells/ml) were cultured in Iscove’s modified Dulbecco’s medium (Life Technologies) supplemented with 10% heat-inactivated FCS (Life Technologies), 0.5% BSA, 50 μg/ml human transferrin, 5 μg/ml bovine insulin, and 1 μg/ml each of oleic, linolenic, and palmitic acid (Sigma) as previously described (31, 32) in round-bottom 96-well plates (Costar, Cambridge, MA) at 37°C in a humidified 5% CO_2 atmosphere. Purified T cells at 1 × 10^5 cells/ml were added to microculture wells containing purified B cells, and cultured in the presence of rIL-4 (400 U/ml) with or without anti-CD40 mAb (1 μg/ml) to induce IgE or IgG synthesis. In selected experiments, the effects of anti-B7.1 or anti-B7.2 mAb on IgE synthesis was studied. In these studies, the mouse IgG control, mouse anti-human B7.2 mAb, and the mouse anti-human B7.1 mAb were used at a concentration of 10 μg/ml. Culture supernatants were collected after 14 days and stored at −20°C until assayed.

ELISA for Ig determination

The IgE assay was conducted as previously described (33). Ninety-six-well microtiter plates (Dynatech, Chantilly, VA) were coated with 0.1 ml of a 1:1 mixture of purified monoclonal anti-human IgE (4.15 and 7.12, a kind gift from Dr. A. Saxen, University of California, Los Angeles, CA) diluted in 0.1 M NaHCO_3, at pH 9.6, at a concentration of 20 μg/ml after overnight incubation at 4°C. The wells were blocked with 0.1% gelatin in 0.1 M NaHCO_3 at room temperature for 1 h. Serial dilutions of culture supernatants were incubated in duplicate for 2 h at room temperature and overnight at 4°C, with parallel human IgE standard controls (Pharmacia). The plates were then washed, a 1:100 dilution of affinity-purified biotinylated goat anti-human IgE (Vector, Burlingame, CA) was added, and plates were incubated for 90 min at 37°C. After a subsequent wash, wells were incubated with a 1:1500 dilution of streptavidin-alkaline phosphatase (Tago, Burlingame, CA) for 90 min at 37°C. The wells were then developed with 2 mM p-nitrophenyl phosphate substrate (Sigma) and the OD was read at 405 nm on an Emax microplate reader ( Molecular Devices, Menlo Park, CA). The concentrations of IgE in the supernatants were read from an IgE standard curve. The lower limit of sensitivity of this assay was 0.50 ng/ml.

The protocol for IgG assay (33) was identical to that for IgE except that the initial capture Ab was an affinity-purified polyclonal goat anti-human IgG Ab (Tago) diluted in 0.1 M NaHCO_3 at a concentration of 10 μg/ml. The second Ab was biotinylated goat-anti-human IgG (Vector). The IgG standards were obtained from Sigma. The lower limit of sensitivity of this assay was 1 ng/ml.

Cell staining and flow cytometric analysis

Five parameter analysis was performed using a FACSCalibur flow cytometer (Becton Dickinson) with the three-color parameters. Immunofluorescence staining for this multiparameter analysis and methods of cytometer set up and data acquisition were performed as described previously (30). List mode multiparameter data files (each file with forward scatter, side scatter, and three fluorescent parameters) were analyzed by using the Cell Quest MacIntosh program (Becton Dickinson). Analysis was 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 Abs.

Statistical analysis

Data are expressed as individual values or the mean for each subject group. Statistical comparisons were made using an unpaired Student t test to compare different groups of study subjects, and a paired Student t test to compare the results from the same subjects. Nonparametric data differences between groups of paired t test and serum IgE level for Pearson correlation were log transformed before being tested. Differences between groups were considered significant at a p value < 0.05.

Results

Elevated B7.2 and CD23 expression on B cells of AD patients

Freshly isolated PBMC from patients with AD and psoriasis, and from normal subjects were analyzed by flow cytometry for the expression of B7.2, B7.1, and CD23 on their B cells. As shown in Figure 1, B7.2 expression was significantly higher on B cells from AD patients (mean ± SEM = 53.67 ± 3.10%) as compared with normal subjects (38.02 ± 4.95%; p = 0.02) or psoriasis patients (40.19 ± 2.7%; p = 0.006). Similarly, CD23 expression was significantly higher on B cells from AD patients (80.33 ± 2.67%) as compared with normal subjects (65.48 ± 4.17%; p = 0.01) and psoriasis patients (66.55 ± 4.5%; p = 0.02). In contrast, B7.1 expression on B cells from AD patients (15.44 ± 2.09%), although lower than the other two study groups, did not show any significant differences compared with normal subjects (20.69 ± 3.99%; p = 0.26) and psoriasis patients (20.3 ± 5.27%; p = 0.41).

Serum IgE correlates with B7.2 expression

Serum IgE levels were analyzed from AD patients and normal subjects, and compared with B7.2 expression on B cells from freshly isolated PBMC of these subjects. As shown in Figure 2, B7.2 expression on B cells correlated significantly with log serum IgE (r = 0.68, p = 0.004). In contrast, B7.1 expression on B cells did not demonstrate this correlation (r = 0.08, p = 0.76). These data suggested a possible role for the B7.2 molecule in the induction of IgE synthesis.

B7.2~+~ B cells synthesize more IgE than B7.2~−~ B cells

In these experiments, purified B7.2~+~ vs B7.2~−~ B cells from normal subjects, isolated by cell sorting, were cultured with rIL-4 in the presence and absence of anti-CD40 or T cells as described in Table I. Culture supernatants were analyzed for IgE and IgG synthesis after 14 days of culture. Following stimulation with IL-4 plus anti-human CD40 mAb, IgE production was significantly higher in cultures containing T cells plus B7.2~+~ B cells (line 7) than cultures...
with T cells plus B7.2\(^ {−} \) B cells (line 8) in three of three experiments \((p = 0.04)\). In the absence of T cells, the overall IgE production was lower but did show a higher level of IgE synthesis by B7.2\(^ {+} \) (line 5) as compared with B7.2\(^ {−} \) B cells (line 6) used in experiments 1 and 2 (Table I). In contrast, no significant differences were observed in the level of IgG synthesis by B7.2\(^ {+} \) vs B7.2\(^ {−} \) cells under all culture conditions examined.

Anti-human B7.2 but not anti-human B7.1 mAb decreased in vitro IgE production from normal PBMC

To directly test the hypothesis that the B7.2 molecule played a role in the induction of IgE synthesis, we examined whether mAb to B7.2, as compared with B7.1, inhibited IgE synthesis following stimulation of PBMC from eight normal subjects with rIL-4 and anti-human CD40 mAb. As shown in Figure 3 (left), IgE production by PBMC cultured with anti-B7.2 mAb (8.44 \( \pm \) 2.94 ng/ml) synthesized significantly lower levels of IgE than PBMC cultured with the mouse IgG isotype control (34.35 \( \pm \) 17.04 ng/ml; \( p = 0.01 \)). In contrast, IgE production from cultures with anti-human B7.1 mAb (46.86 \( \pm \) 18.64 ng/ml) was not significantly different from the mouse IgG isotype control. Furthermore, IgE production in cultures with anti-B7.2 mAb demonstrated significantly lower levels of IgE synthesis than cultures with anti-B7.1 mAb (\( p = 0.004 \)). IgG production (see Fig. 3, right) was not significantly affected by the addition of these different Abs (\( p < 0.05 \)).

Increased expression of IL-4R and CD23 on B7.2\(^ {+} \) B cells

The observation in Table I that IL-4 stimulated a higher level of IgE synthesis in purified B7.2\(^ {+} \), but not B7.2\(^ {−} \), B cells suggested a differential expression of the IL-4R in these two cell types. To address this possibility more directly, we analyzed freshly isolated PBMC from AD patients and normal subjects for the expression of IL-4R and CD23 on their B7.1\(^ {+} \) vs B7.2\(^ {+} \) B cells. In Figure 4 (upper panel), IL-4R expression on B7.2\(^ {+} \) B cells in AD patients (53.28 \( \pm \) 6.46\%) was significantly higher than on B7.1\(^ {+} \) B cells (34.83 \( \pm \) 6.30\%; \( p = 0.02 \)). Similarly, the expression of IL-4R expression on B7.2\(^ {+} \) B cells in normal subjects (46.23 \( \pm \) 7.03\%) was also significantly higher than on B7.1\(^ {+} \) B cells (23.49 \( \pm \) 4.39\%; \( p = 0.009 \)).

In Figure 4 (lower panel), the expression of CD23 on B7.2\(^ {+} \) B cells in AD patients (55.55 \( \pm \) 3.69\%) was studied and found to be significantly higher than on B7.1\(^ {+} \) B cells (18.48 \( \pm \) 2.80\%; \( p < 0.0001 \)). In normal subjects, the expression of CD23 on B7.2\(^ {+} \) B cells (40.61 \( \pm \) 2.56\%) was also higher than on B7.1\(^ {+} \) B cells (15.10 \( \pm \) 2.42\%; \( p < 0.0001 \)).

Discussion

Several studies support the concept that B7.1 and B7.2 have distinct functions in the generation of Th1 and Th2 responses. In the
Culture supernatants were analyzed for IgE and IgG after 14 days of culture. IgE production by T cells plus B7.2 costimulate IL-4 production and up-regulate IFN-γ. B7.2-transfected Chinese hamster ovary cells were able to costimulate murine T cells. Natesan et al. (16) demonstrated that both B7.1 and anti-human B7.1 (10 μg/ml) mAb inhibits IgE synthesis. PBMC from eight normal subjects were cultured for 14 days in IL-4-induced proliferation as well as IL-2 and IFN-γ. Inhibition studies indicated that both B7.1 and B7.2 could costimulate anti-CD3 Ab-induced proliferation. However, several studies could not confirm differential effects of B7.1 vs B7.2. A study by Lanier et al. (15) demonstrated that CD40 on APCs with anti-B7.1 and anti-B7.2 mAb resulted in Th2- and Th1-type response, respectively (34). However, several studies could not confirm differential effects of B7.1 vs B7.2. A study by Lanier et al. (15) indicated that both B7.1 and B7.2 could costimulate anti-CD3 Ab-induced proliferation as well as IL-2 and IFN-γ production by murine T cells. Natesan et al. (16) demonstrated that both B7.1- and B7.2-transfected Chinese hamster ovary cells were able to costimulate IL-4 production and up-regulate IFN-γ mRNA.

Considering these conflicting results, it is important to determine whether B7.1 and B7.2 molecules are differentially up-regulated in human allergic disease. In this report, we have studied AD as a prototypic allergic disease known to be associated with a predominant Th2 response and markedly elevated serum IgE levels (1, 21–26). The current study is the first to demonstrate the predominant expression of B7.2 in AD patients compared with normal subjects or patients with psoriasis, a Th1-mediated skin disease. Furthermore, we found a significant correlation between B7.2, but not B7.1, expression on B cells with the magnitude of serum IgE levels suggesting a potential role for this molecule in the induction of IgE synthesis.

To study the potential role of B7.2 in IgE synthesis, we investigated whether B7.2+ B cells produced higher levels of IgE than B7.2− B cells. As shown in Table I, following stimulation with IL-4 and anti-CD40 mAb, B7.2+ B cells produced more IgE than B7.2− B cells, particularly in the presence of T cells. This is explained in part by our current observation that B7.2+ B cells express significantly higher IL-4R and CD23 than B7.1+ B cells. Both of these surface molecules are known to have an important role in promoting IgE synthesis (35). Our observation that IL-4 and anti-CD40 mAb induce higher levels of IgE synthesis in the presence of T cells is consistent with previous observations that CD40 plus IL-4 stimulation provides better signaling for IgE production in PBMC than IL-4 alone (36). In the absence of T cells, B7.2+ B cells produced less IgE than in the presence of T cells following the stimulation of IL-4 and anti-CD40 mAb, but still showed a higher level of IgE production from B7.2+ B cells than B7.2− B cells. However, only two of our three experiments demonstrated IgE production in the absence of T cells following IL-4 and anti-CD40 mAb. The observation that not all individuals respond to IL-4 and anti-CD40 mAb has also been reported by Zhang et al. (36).

A role for CD28/B7 interactions in IgE synthesis has been previously suggested by other investigators. In a study by Life et al. (37) using anti-CD28 in cultures containing tonsillar B cells, activated T cell clones, and IL-4, IgE but not IgG production was inhibited in a dose response fashion. Recently, Gause et al. (38)...

### Table I. IgE and IgG production by B7.2+ vs B7.2− B cells

<table>
<thead>
<tr>
<th>B cell type</th>
<th>Addition of T cells</th>
<th>Stimulants</th>
<th>IgE (ng/ml)</th>
<th>IgG (ng/ml)</th>
<th>IgE (ng/ml)</th>
<th>IgG (ng/ml)</th>
<th>IgE (ng/ml)</th>
<th>IgG (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B7.2+</td>
<td>−</td>
<td>IL-4</td>
<td>&lt;0.50</td>
<td>2020.0</td>
<td>&lt;0.50</td>
<td>1032.5</td>
<td>&lt;0.50</td>
<td>1026.5</td>
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<tr>
<td>B7.2+</td>
<td>−</td>
<td>IL-4</td>
<td>&lt;0.50</td>
<td>2386.5</td>
<td>&lt;0.50</td>
<td>829.0</td>
<td>&lt;0.50</td>
<td>851.5</td>
</tr>
<tr>
<td>B7.2+</td>
<td>+</td>
<td>IL-4</td>
<td>56.7</td>
<td>2635.0</td>
<td>&lt;0.50</td>
<td>798.5</td>
<td>&lt;0.50</td>
<td>1540.0</td>
</tr>
<tr>
<td>B7.2+</td>
<td>+</td>
<td>IL-4</td>
<td>25.3</td>
<td>1848.5</td>
<td>&lt;0.50</td>
<td>629.5</td>
<td>&lt;0.50</td>
<td>1553.0</td>
</tr>
<tr>
<td>B7.2−</td>
<td>−</td>
<td>IL-4 + anti-CD40</td>
<td>22.0</td>
<td>1916.0</td>
<td>4.2</td>
<td>1070.0</td>
<td>&lt;0.50</td>
<td>1613.0</td>
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<td>1217.0</td>
<td>1.2</td>
<td>654.0</td>
<td>&lt;0.50</td>
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<td>IL-4 + anti-CD40</td>
<td>339.8</td>
<td>1693.0</td>
<td>14.8</td>
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</tr>
<tr>
<td>B7.2−</td>
<td>+</td>
<td>IL-4 + anti-CD40</td>
<td>138.0</td>
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<td>10.9</td>
<td>1307.5</td>
<td>22.6</td>
<td>2155.0</td>
</tr>
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</table>

* B7.2+ and B7.2− B cells, isolated by cell sorting, were cultured in the presence and absence of T cells with rIL-4 (400 U/ml) alone or plus anti-human CD40 mAb (1 μg/ml). Culture supernatants were analyzed for IgE and IgG after 14 days of culture. IgE production by T cells plus B7.2+ B cells in the presence of IL-4 and anti-CD40 mAb (line 7) is significantly higher than by B7.2− B cells under the same culture conditions (line 8) in all three of the experiments (p < 0.04).

![Image](http://www.jimmunol.org/)

**FIGURE 3.** Anti-human B7.2, but not anti-human B7.1, mAb inhibits IgE synthesis. PBMC from eight normal subjects were cultured for 14 days in IL-4 (400 U/ml) and anti-CD40 mAb (1 μg/ml) in the presence of either mouse IgG control (10 μg/ml), mouse anti-human B7.2 (10 μg/ml), or mouse anti-human B7.1 (10 μg/ml). IgE (left) and IgG (right) were determined in culture supernatants as described in Materials and Methods. Results from eight individual subjects were expressed as mean ± SEM.
patients and normal subjects was significantly higher on B7.2. It has been previously reported that Ag-specific B cells facilitate Ag presentation to T cells (20). T cells are activated by cognate (primary signal) and noncognate interactions (costimulatory signals) resulting in IL-4 secretion and Th2-type cell responses. CD40 engagement, as a costimulatory signal, up-regulates IL-4 production on T cells (49), and IL-4R expression (45) on B cells, subsequently leading to enhanced IgE synthesis. CD40 also up-regulates B7.2 expression (42), and B7.2, as an important costimulatory signal, increases CD40L expression and IL-4 secretion (47). This results in a significant amplification loop that augments IgE production. Our current data of higher expression of B7.2, CD23, and IL-4R on B cells of AD patients than of normal subjects are consistent with this model and point to a novel mechanism for high level IgE synthesis in AD patients. The predominant expression of IL-4R and CD23 on B7.2+ B cells, and the capacity of anti-B7.2 Ab to block IgE synthesis suggests B7.2 may be a therapeutic target in allergic diseases.

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References