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

Orathai 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 AD patients and normal subjects correlated significantly with B7.2 expression on B cells (r = 0.68; p = 0.004), suggesting a role for B7.2+ B cells in IgE synthesis. Indeed, purified B7.2+ B cells produced significantly more IgE than B7.2− B cells in vitro (p = 0.04). Anti-human B7.2, but not B7.1, mAb significantly (p < 0.05) 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 APCs, the interaction between CD40 molecules on B cells and CD40 ligand (CD40L) on T cells has been identified as an 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

ligand system (9–12). Such differential binding may have unique signaling properties that affect T cell activation and subsequent Th1/Th2 development. Studies in mice have suggested that the generation of Th2 cells depends mainly on the interaction of CD28 with B7.2 (13). In vitro studies of human T cells reported that B7.2 transfectants preferentially activate Th2-type cytokines whereas B7.1 transfectants skewed responses toward the production of Th1-type cytokines (14). Other reports, however, have not confirmed that B7.2 are involved in Th2 responses. These studies found that both B7.1 and B7.2 were able to costimulate IL-4 and IFN-γ production from murine T cells (15, 16).

Atopic dermatitis (AD) is a prototypic allergic skin disease associated with elevated IgE synthesis and increased B cell expression of CD23 (17–19). The IgE molecule is thought to play an important role in allergen-driven responses and allergen presentation to CD23-positive B cells in AD (20). Skin infiltrating and peripheral blood T cells from patients with AD express high levels of IL-4 and IL-5, but not IFN-γ, consistent with the Th2-type cytokine pattern of synthesis (21–26). The relevance of B7.2 in Th2-predominant human allergic diseases such as AD has not been previously examined. In the current report, we therefore studied the expression of B7.1 vs B7.2 in AD and normal subjects as well as patients with psoriasis, a skin disease known to be associated with Th1-mediated responses (27, 28). We demonstrate an abnormally high expression of B7.2 on B cells from patients with AD, and a potential role for B7.2 in the induction of IgE synthesis.

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 9797 IU/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
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 these experiments, purified B7.2+ B cells from AD patients (20.3 ± 6 SEM) had serum IgE levels that were significantly higher than cultures of the same subjects. Nonparametric data differences compared between 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% ) 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

Cell staining and flow cytometric analysis

Five parameter analysis was performed using a FACScanIFL flow cytometer (Becton Dickinson) using FITC, PE, and PerCP as the three fluorescent parameters. Immunofluorescence staining for this multiparameter analysis and methods of cytotherapy 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. 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.
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 IL-4 and anti-human CD40 mAb. As shown in Figure 3 (left), IgE production by PBMC cultured with anti-B7.2 mAb (8.44 ± 2.94 ng/ml) synthesized significantly lower levels of IgE than PBMC cultured with the mouse IgG isotype control (34.35 ± 17.04 ng/ml; p = 0.01). In contrast, IgE production from cultures with anti-human B7.1 mAb (46.86 ± 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+ B cells, 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 ± 6.46%) was significantly higher than on B7.1+ B cells (34.83 ± 6.30%; p = 0.02). Similarly, the expression of IL-4R on B7.2+ B cells in normal subjects (46.23 ± 7.03%) was also significantly higher than on B7.1+ B cells (23.49 ± 4.39%; p = 0.009).

In Figure 4 (lower panel), the expression of CD23 on B7.2+ B cells in AD patients (55.55 ± 3.69%) was studied and found to be significantly higher than on B7.1+ B cells (18.48 ± 2.80%; p < 0.0001). In normal subjects, the expression of CD23 on B7.2+ B cells (40.61 ± 2.56%) was also higher than on B7.1+ B cells (15.10 ± 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 and B7.2-transfected Chinese hamster ovary cells were able to murine T cells. Natesan et al. (16) demonstrated that both B7.1 and B7.2 on 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, preactivated T cell clones, and IL-4, IgE but not IgG production was inhibited in a dose response fashion. Recently, Gause et al. (38) 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).

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**Table I. IgE and IgG production by B7.2 B vs B7.2 B 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>
</tr>
</thead>
<tbody>
<tr>
<td>B7.2 B</td>
<td>–</td>
<td>IL-4</td>
<td>&lt;0.50</td>
<td>2020.0</td>
</tr>
<tr>
<td>B7.2 B</td>
<td>–</td>
<td>IL-4</td>
<td>&lt;0.50</td>
<td>2386.5</td>
</tr>
<tr>
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<td>+</td>
<td>IL-4</td>
<td>56.7</td>
<td>2635.0</td>
</tr>
<tr>
<td>B7.2 B</td>
<td>+</td>
<td>IL-4</td>
<td>25.3</td>
<td>1848.5</td>
</tr>
<tr>
<td>B7.2 B</td>
<td>–</td>
<td>IL-4 + anti-CD40</td>
<td>22.0</td>
<td>1916.0</td>
</tr>
<tr>
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<td>IL-4 + anti-CD40</td>
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<tr>
<td>B7.2 B</td>
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<td>IL-4 + anti-CD40</td>
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<td>1693.0</td>
</tr>
<tr>
<td>B7.2 B</td>
<td>+</td>
<td>IL-4 + anti-CD40</td>
<td>138.0</td>
<td>1831.5</td>
</tr>
</tbody>
</table>

* B7.2 B 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).

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**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.
results in CD40L expression and IL-4 secretion (47), which induce CD23 expression on B cells (48). Recently, Macaulay et al. (49) also demonstrated that Ag-specific B cells preferentially induce CD4+ T cells to secrete IL-4, and that the interaction between CD40 and CD40L is important for IL-4 production by T cells.

Based on these observations, we propose the following model: after Ag exposure, elevated IgE expression and CD23 on 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
