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Beraprost Enhances the APC Function of B Cells by Upregulating CD86 Expression Levels

Jini Kim,* Chan-Sik Park,† Chan-Hum Park,‡ Doo-Il Jeoung,§ Young-Myeong Kim,¶ and Jongseon Choe*

Lipid mediators are emerging as important regulators of the immune system. Based on our previous result that shows strong expression of prostacyclin synthase in the germinal center, we investigated whether prostacyclin would regulate the APC function of B cells. Owing to the very short half-life of prostacyclin in experimental conditions, we used a more stable analog, beraprost. Beraprost increased the amounts of the costimulatory molecule CD86 but not CD80 on the surface of activated B cells in time- and dose-dependent manners. However, the enhancing effect of beraprost was not observed on memory B cells, centroblasts, and centrocytes. Beraprost required BCR and CD40 signals to upregulate CD86 expression levels. Other prostanoids such as PGE2, 6-keto-PGF1α, and PGF2α failed to alter CD86 expression levels, whereas other prostacyclin analogs were as potent as beraprost. Results carried out with receptor antagonists revealed that beraprost enhanced CD86 levels by binding to prostacyclin receptor IP and by increasing intracellular cAMP concentrations. Beraprost-treated B cells potently stimulated allogeneic T cells, which was significantly abolished by CD86 neutralization. Our data imply an unrecognized cellular and molecular mechanism about the germinal center reactions. The Journal of Immunology, 2011, 186: 000–000.

The germinal center (GC) of the secondary lymphoid tissues is the culminating microanatomic site for the generation of high-affinity Abs. It is histologically compartmentalized by the dark, light, and follicular mantle zones (4). There is a differential distribution of the costimulatory molecules in the GC; CD80 is preferentially expressed in the dark zone, whereas the expression of CD86 is strong on centrocytes of the light zone where Th2 cells are occasionally populated (5–7). Follicular dendritic cells (FDCs) are important stromal cells of the GC and play central roles in B cell differentiation into memory and plasma cells (8–10). FDCs are also thought to control activation and differentiation of T cells in the GC. However, the cellular and molecular mechanisms of the regulatory role of FDC are unclear.

We have recently reported that prostacyclin synthase (PGIS) is expressed in the GC and that FDC-like cells produce prostacyclin (11). Because FDCs are known to regulate important functions of B cells in the GC, we investigated whether prostacyclin modulates the APC function of B cells. Beraprost, the first orally active prostacyclin analog, was used throughout the experiments due to the very short half-life of prostacyclin (3–5 min) (12, 13). Beraprost shows a high binding affinity for the human IP receptor (39 nM), an improved elimination half-life (1 h), and a similar activity to prostacyclin in terms of inhibition of platelet aggregation (IC50 of 19 versus 5 nM, respectively) (13). Our results show that beraprost binds to the prostacyclin receptor IP and enhances CD86 expression levels on activated B cells. Furthermore, beraprost-treated B cells displayed an augmented APC activity that was significantly abrogated by CD86 neutralization. Based on these results we propose a novel regulatory pathway through which FDC controls T cell activation and differentiation.

Materials and Methods

Abs and other reagents

Anti-IgM (DA4.4, IgG1) and anti-CD40 (G28.5, IgG1) were obtained from the American Type Culture Collection, Neutralizing anti-CD80 (2D10) and anti-CD86 (B1/63) were purchased from Biosource and Ancell, respectively. Other mAbs were FITC-conjugated anti-IgD (Sigma-Aldrich), anti-CD20, anti-CD27, anti-CD54, anti-CD80, and anti-CD86, PE-conjugated.
anti-CD3 and anti-CD28, and streptavidin-conjugated anti-CD184 (CXCR4) (BD Biosciences). PGE2, prostacyclin, beraprost, 6-keto-PGF1α, iloprost, treprostinil, RO1118452, AH6809, and AH23848 were purchased from Cayman Chemical. Dibutyryl-cAMP was obtained from Alexis, and IL-2 was from Hoffmann-La Roche. Recombinant IL-4 and IL-10 were prepared in our laboratory (14).

Isolation of B and T cells

Tonsillar B cells were prepared as described previously (15). Tonsillar mononuclear cells were subject to two rounds of depletion of T cells by rosetting with SRBC: the resulting cells contained >95% CD20+ B cells as analyzed by FACScan (BD Biosciences, Sunnyvale, CA). B cells were further separated according to their density using a discontinuous gradient of Percoll consisting of four layers (80, 60, 50, and 30%) of Percoll solutions in a 15-ml conical tube. B cells (1 × 10^7) were laid at the top of the gradient and centrifuged for 10 min at 2000 × g at 20°C. B cells recovered at 60–80% Percoll interface were referred to as high-density B cells, those at 50–60% as mid-density, and those at 30–50% as low-density B cells. Naïve B cells were further purified from high-density B cells by magnetic cell separation (Miltenyi Biotec) as described previously (15). In brief, high-density B cells were incubated with anti-IgD (H9, IgG1) for 15 min, washed, and then incubated with rat anti-mouse IgG1 microbeads for 15 min on ice. After the final wash, the cells were loaded on a magnetic cell separation column and positive selection of IgD+ naive B cells. The purity was 98% by quantifying the expression of IgD. Memory B cells were isolated by depleting IgD+ and CD38+ B cells from the high-density B cells by magnetic cell separation as described previously (16). The high-density B cells were incubated with OKT10 (anti-CD38) for 15 min, washed, and incubated with rat anti-mouse IgG1 microbeads for 15 min on ice. The cells were loaded on a magnetic cell separation column and the negative fraction was collected and subjected to another cycle of magnetic cell separation using H9 (anti-IgD). The purity of memory B cells was >95% by quantitating the IgD-CD38− population. The CD27+ cells were >70%, suggesting that the population contained both CD27+ and CD27− memory cells (17, 18). GC B cells were obtained from low-density B cells by depleting CD44+ cells as described before (15). The purity of GC B cells was >98% by estimating CD20+CD38− cells in FACScan analysis. All the purification steps were performed at 4°C to prevent death of GC B cells, except centrifugation of cells using Ficoll or Percoll, which was carried out at room temperature. Centroblasts and centrocytes were sorted by a FACSaria II (BD Biosciences) from GC B cells or low-density B cells on the basis of CXCR4 expression (19). T cells were isolated from peripheral blood by rosetting with SRBC; the resulting cells contained <2% CD19+ T cells as analyzed by a FACScalibur (BD Biosciences). T cell preparations contained 0–20% CD56− NK cells depending on blood donors.

Culture of B cells

The culture medium was RPMI 1640 (Irvine Scientific) containing 10% FCS (HyClone), 2 mM l-glutamine (Life Technologies), 100 U/ml penicillin G (Sigma-Aldrich), and 100 μg/ml streptomycin (Invitrogen). B cells were plated into a 24-multwell plate in the presence of anti-IgM (1 μg/ml), anti-CD40 Ab (1 μg/ml), and IL-4 (50 U/ml) with or without IL-2 (30 U/ml) and IL-10 (50 ng/ml). CD154-transfected L cells (American Type Culture Collection) were used after treatment with 50 μg/ml mitomycin C (Sigma-Aldrich).

Allostimulation of T cells

Tonsillar B cells were cultured with anti-IgM, anti-CD40 Abs, and IL-4 in the presence or absence of beraprost for 72 h, followed by treatment with mitomycin C. Peripheral blood T cells were cocultured with beraprost-treated B cells at various B cell/T cell ratios. In CsFSE dilution experiments, isolated T cells were labeled with a CsFSE kit (Invitrogen) according to the manufacturer’s instructions. The degree of T cell proliferation was measured by a FACScalibur.

Flow cytometry and cell sorting

Single-cell suspensions were double stained with FITC- or PE-conjugated mAb (IgD, CD20, CD38, CD80, or CD86). Flow cytometric analysis was carried out on a FACScalibur with CellQuest software (BD Biosciences). Cell sorting was conducted using a FACSaria II with FACSDivia software (BD Biosciences).

Statistical analysis

Statistical analysis and graphic presentation were carried out with GraphPad Prism 5.03 (GraphPad Software). Results are presented as means of at least three independent experiments plus SEM. The statistical significance of differences was determined by a Student t test; p < 0.05 was considered significant.

Results

Beraprost enhances CD86 expression levels of activated B cells

To examine the response of B cell subpopulations to beraprost, tonsillar B cells were fractionated into three compartments according to Percoll density (i.e., high-, mid-, and low-density B cells). The low-density B cells comprised largely CD20+CD38+IgD− GC B cells, whereas CD20+CD38+IgD+ were the major cellular component of high-density B cells, as shown in a representative result (Fig. 1A). CD20+CD38+IgD+ is the phenotype of naive or recently activated B cells (20). The expression levels of these markers were in-between in the mid-density B cells.

The expression levels of CD54, CD80, and CD86 on the surface of the three subsets were measured after a culture under the optimal conditions in the presence or absence of beraprost. These adhesion and costimulatory molecules play important roles in the APC function (2, 21, 22). The addition of beraprost to unfractionated B cells resulted in 2- and 3-fold increases of CD54 and CD86 expression levels, respectively (Fig. 1C, 1E). These increases were reproduced when beraprost was added to high-density and mid-density B cells. Highly purified IgD+ naive B cells also exhibited a similar response to beraprost treatment (Supplemental Fig. 1A–C). However, the presence of beraprost did not lead to a modulation of CD54 and CD86 in low-density B cells. The enhancing effect of beraprost on high- and mid-density B cells was specific to CD54 and CD86 because CD20 and CD80 levels did not change by the addition of beraprost (Fig. 1B, 1D).

The effect of beraprost was obvious in kinetics and dose-response experiments. In a typical kinetics experiment, both CD54 and CD86 levels on high- and mid-density B cells were upregulated at 24 h in the culture and then slightly decreased. However, the high- and mid-density B cells cultured in the presence of beraprost continuously increased CD54 and CD86 levels up to 72 h (Fig. 2A, 2C). Low-density B cells exhibited higher levels of these molecules before culture but lost the expression levels during the culture period probably due to the apoptosis of GC B cells. CD80 levels on different B cell subsets were not modulated by the presence of beraprost throughout the culture period examined (Fig. 2B). The increase of CD54 and CD86 in response to beraprost was dose-dependent, with the optimal concentration being ~1–10 μM (Fig. 2D, 2F). In contrast, B cells were unresponsive to a wide range of beraprost concentrations in the expression of CD80 (Fig. 2E).

Distinct from other B cell subsets, GC B cells are highly prone to spontaneous apoptosis when placed in a culture (23). Because low-density B cells failed to enhance CD54 and CD86 expression levels in response to beraprost, we thought that the failure might have been derived from the absence of the essential survival molecule CD154 for GC B cells in the culture stimuli. We replaced the anti-CD40 Ab with CD154-transfected L cells to ensure the survival and growth of GC B cells. As shown in Fig. 3, the expression levels of CD80 as well as CD54 and CD86 were increased by ~5-fold after culture on CD154-transfected L cells, indicating the potent stimulatory activity of CD154 compared with anti-CD40 Ab (Fig. 1). The addition of beraprost resulted in a significant enhancement of CD54 and CD86 expression levels. For example, beraprost increased CD86 expression levels on both high-density and mid-density B cells by 1.8-fold (Fig. 3C). Unlike the culture system supported by anti-CD40 Ab, beraprost enhanced CD86 expression of low-density B cells in the CD154− B cell system (Figs. 1E, 3C). However, the CD86 increased fold on
low-density B cells was much lower than those on high- 
and mid-density B cells (∼1.4- versus 1.8 fold), which prompted us to 
examine the effect of beraprost on purified GC B cells. GC B cells 
did not upregulate CD54 and CD86 expression in response to 
beraprost treatment (Fig. 3A, 3C).

Next, because high-density B cells comprise memory B cells, 
and GC B cells are composed of centroblasts and centrocytes, we 
purified these cell subsets to examine the effects of beraprost on 
CD86 expression levels on them. Freshly purified CXCR4+ cen-

Tonsillar B cells were fractionated into three sub-
sets according to Percoll density. B cells 
were stained by FITC-conjugated anti-
CD20 or anti-IgD Ab and PE-conjugated 
anti-CD38 Ab to be analyzed by flow 
cytometry. The numbers represent per-
centages in each quadrant. B–E, B cells 
(1 × 10^6 cells) were stimulated with 
anti-IgM, anti-CD40, IL-2, IL-4, and IL-
10 in the presence or absence of beraprost (10^{-9} M) for 72 h and then 
expression levels of indicated markers 
were analyzed using a flow cytometer. 
The relative levels of each marker were 
calculated by comparing with that of 
B cells cultured in the presence of etha

![FIGURE 1.](https://example.com/figure1.png)

**FIGURE 1.** Beraprost enhances CD54 and CD86 expression levels on high- 
and mid-density B cells. A, Tonsillar B cells were fractionated into three sub-
sets according to Percoll density. B cells 
were stained by FITC-conjugated anti-
CD20 or anti-IgD Ab and PE-conjugated 
anti-CD38 Ab to be analyzed by flow 
cytometry. The numbers represent per-
centages in each quadrant. B–E, B cells 
(1 × 10^6 cells) were stimulated with 
anti-IgM, anti-CD40, IL-2, IL-4, and IL-
10 in the presence or absence of beraprost (10^{-9} M) for 72 h and then 
expression levels of indicated markers 
were analyzed using a flow cytometer. 
The relative levels of each marker were 
calculated by comparing with that of 
B cells cultured in the presence of etha

![FIGURE 2.](https://example.com/figure2.png)

**FIGURE 2.** Beraprost increases the CD54 and CD86 levels in time- and dose-dependent manners. A–C, B cells (1 × 10^6 cells) were cultured with anti-
IgM, anti-CD40, and IL-4 in the presence or absence of beraprost (10^{-5} M). Expression levels of indicated molecules were measured before and at 24, 48, 
and 72 h after culture by flow cytometry. D–F, High-density B cells were cultured with various concentrations of beraprost in the presence of the above 
stimuli. After 72 h, the expression levels of CD54, CD80, and CD86 were measured with a flow cytometer. Means ± SEM of three independent 
experiments are depicted. *p < 0.05, **p < 0.01 compared with control.
FIGURE 3. Beraprost augments expression of CD54 and CD86 levels on activated B cells but not GC B cells. B cells (1 × 10^6 cells) were cultured with anti-IgM, IL-2, IL-4, IL-10, and CD154-expressing L cells (1 × 10^3 cells) in the presence or absence of beraprost (10^{-6} M). After 72 h, expression levels of CD54 (A), CD80 (B), and CD86 (C) were measured by flow cytometry. Means ± SEM of three independent experiments are depicted. D, High-density B cells (HD), GC B cells (GC), centroblasts (CB), and centrocytes (CC) were cultured under the same conditions as described above. High-density B cells cultured in the presence of anti-CD40 Ab instead of CD154-expressing L cells were included as a positive control (the second HD). Representative data (means ± SEM of duplicate) of three reproducible experiments are shown. *p < 0.05, **p < 0.01.

compared with CXCR4^- centrocytes (Supplemental Fig. 2). Unlike high-density B cells that responded to beraprost by increasing CD86 levels, memory B cells, centroblasts, and centrocytes did not upregulate CD86 levels in response to beraprost (Supplemental Figs. 1D, 3D). Although both centroblasts and centrocytes failed to respond to beraprost, these populations upregulated the CD86 levels in response to CD40 engagement. The downregulation of CD86 on centroblasts observed in Fig. 3 was not reproduced in the other two experiments (data not shown).

To determine the signals that are necessary for beraprost to upregulate CD86 levels on B cells, we analyzed the effect of anti-IgM Ab, anti-CD40 Ab, IL-4, or IL-2 plus IL-4 plus IL-10. These stimuli were added to B cells individually or in combination in the presence of beraprost. None of these stimuli did promote the enhancing effect individually. However, the enhancing effect of beraprost appeared in the culture containing anti-IgM and anti-CD40 Abs where beraprost significantly increased the CD86 levels by 1.7-fold (Fig. 4A). Addition of either IL-4 alone or IL-2 plus IL-4 plus IL-10 to anti-IgM and anti-CD40 gave rise to a further increase of CD86 levels. These results suggest that both BCR and CD40 signals are required for beraprost to enhance CD86 expression.

Finally, the timing of beraprost requirement of B cells was addressed by adding beraprost at 24-h intervals and then measuring its effect on CD86 expression levels. When beraprost was added simultaneously with anti-IgM, anti-CD40, and IL-4 to high-density B cells at the beginning of culture, it increased CD86 levels by 1.8-fold. A 24-h delayed addition resulted in a 2.3-fold increase of CD86 levels. However, a 48-h delayed addition failed to yield a significant increase of CD86 expression levels (Fig. 4B), indicating that only B cells at the early activation stage are responding to beraprost.

Taken together, these results suggest that beraprost specifically augments CD86 expression of activated B cells except GC B cells.

*The enhancing effect is specific to prostacyclin receptor agonists*

We found that PGE2 synthase in addition to PGIS was expressed in the GC (11). Therefore, we examined whether PGE2 as well as beraprost might regulate CD54 and CD86 expression levels on activated B cells. Unlike beraprost, PGE2 had only minor, if any, effect on the expression levels of CD54, CD80, and CD86 at various concentrations from 10^{-5} to 10^{-10} M (Fig. 5, Supplemental Fig. 3). The stable metabolite of prostacyclin, 6-keto-PGF1a, did not display the enhancing effect either (Fig. 5). Because the enzyme responsible for the production of PGF2α was expressed in the GC, we examined this molecule for the modulatory effect. PGF2α did not change CD54, CD80, and CD86 levels on B cells (data not shown).

We next asked whether other prostacyclin analogs would share the enhancing effect with beraprost. Iloprost and treprostinil are well-known prostacyclin agonists widely used to treat pulmonary arterial hypertension (12). At the same concentrations, these agonists were comparable to beraprost in their ability to increase CD54 and CD86 expression. For example, both iloprost and treprostinil increased the CD86 levels by 3-fold over vehicle (Fig. 6A–C). Prostacyclin also enhanced CD86 expression levels, but it displayed much weaker activity than these analogs (Fig. 6D).

**Beraprost upregulates CD86 expression levels through the IP receptor**

Prostanoids exert various effects via their specific receptors. For example, prostacyclin binds to the IP receptor whereas PGE2 has four distinct receptors, EP1–4 (24). To identify the receptor through which beraprost stimulates B cells to increase CD86 levels, we examined the effect of IP and EP antagonists. RO1138452 is against IP, AH6809 is against EP1–3, and AH23848 is against EP4. When B cells were cultured in the presence of these reagents, only RO1138452 abrogated the effect of beraprost almost to background levels (Fig. 7A). In the absence of beraprost, RO1138452 did not modulate the expression levels of CD86. Because the engagement of IP by cognate ligands leads to a transient increase of intracellular cAMP (25), we thought the addition of cell-permeable cAMP analog dibutyryl (db)-cAMP would result in CD86 upregulation, mimicking the effect of beraprost. Indeed, db-cAMP increased the surface levels of CD86 but not CD80 (Fig. 7B). Collectively, these results lead to a conclusion that beraprost enhances CD86 expression in B cells by binding to the IP receptor.

**Beraprost enhances the APC ability of B cells by upregulating the costimulating molecule CD86**

The upregulation of CD86 on beraprost-stimulated B cells prompted us to compare their APC ability with that of control B cells. The APC ability was measured by allostimulation of
T cells. B cells were cultured in the presence or absence of beraprost for 3 d and then treated with mitomycin C. Varying numbers of these B cells were further incubated with fixed numbers of allogeneic T cells for 6 d. Mitomycin C-treated B cells, irrespective of beraprost treatment, died out during the culture period, and most viable cells at the end of culture were T cells as analyzed by flow cytometry. When we compared the viability of vehicle- or beraprost-treated B cells after mitomycin C treatment, both populations underwent a rapid cell death and <20% of both populations were viable after 24 h. Both populations did not show a significant difference in the viability rates until 120 h when few viable cells were detectable (data not shown). The addition of control B cells to T cells at a 4:1 ratio resulted in 36% recovery of viable cells, whereas beraprost-stimulated B cells increased the recovery to 110%, 3-fold higher recovery (Fig. 8A). The superior allostimulating activity of beraprost-stimulated B cells to control B cells was maintained at the lower B cell/T cell ratios up to 1:10, which was dose-dependent. The higher recovery of viable T cells could have resulted from either enhanced proliferation or better survival of T cells in the presence of beraprost-treated B cells. To differentiate between these possibilities, we carried out the CFSE dilution experiment. About half of the viable T cells cultured with beraprost-stimulated B cells turned out to be divided cells, whereas 23% of T cells incubated with control B cells were divided cells (Fig. 8B), indicating more potent T cell proliferation by beraprost-treated B cells than by control cells. We next investigated whether the increased levels of CD86 on beraprost-stimulated B cells indeed contributed to the T cell stimulation by using function-blocking Abs. The culture conducted in the presence of anti-CD86 Ab gave rise to a significant reduction (∼50%) of the beraprost effect (Fig. 8C). In contrast, anti-CD80 Ab did not modulate T cell proliferation by beraprost-stimulated B cells, indicating that the increased CD86 molecules on B cells mediated a potent costimulatory effect on allogeneic T cells.

**Discussion**
Prostanoids are lipid mediators derived from the cell membrane phospholipids in response to various stimuli. In addition to the established roles of prostanoids as inflammatory and vascular mediators (25, 26), they are emerging as important immune modulators (27–29). Discrepant results about the impact of prostanoids on the immune system (30) warrant extensive examinations in various experimental models. Based on the expression of PGIS in the GC, in this study, the effect of a prostacyclin analog on the APC activity of human B cells was examined. Our findings show that beraprost stimulates the receptor IP on B cells to increase expression levels of CD86, which contributes to a potent stimulation of allogeneic T cells.

**FIGURE 4.** Beraprost augments CD86 expression levels of activated B cells. A, Mid-density B cells (1 × 10⁶ cells) were stimulated with the indicated stimuli in the presence or absence of beraprost (10⁻⁶ M). After 72 h, flow cytometric analysis was performed to assess CD86 expression levels. The relative increase of mean fluorescence intensity (MFI) by the addition of beraprost is shown. Means ± SEM of four independent experiments are depicted. Il-2, IL-4, and α-IgM, anti-CD40, and IL-4 in the presence or absence of beraprost (10⁻⁵ M), which was added at the indicated time points. Cells were cultured for 72 h after the addition of beraprost, followed by a flow cytometric analysis to measure CD86 expression. Means ± SEM of four independent experiments are presented. *p < 0.05, **p < 0.01 compared with control.

B, High-density B cells (1 × 10⁶ cells) were cultured with anti-IgM, anti-CD40, and IL-4 in the presence or absence of beraprost (10⁻⁵ M), which was added at the indicated time points. Cells were cultured for 72 h after the addition of beraprost, followed by a flow cytometric analysis to measure CD86 expression. Means ± SEM of four independent experiments are depicted. *p < 0.05, **p < 0.01 compared with control.
Mature human B cells include naive, early activated, centroblast, centrocyte, and memory B cells (20). What, then, is the target subset of beraprost in our experimental system? High-, mid-, and low-density B cells responded to beraprost by enhancing CD86 on the surface membrane, but centroblasts, centrocytes, and memory B cells did not (Fig. 3). Most high- and mid-density B cells increased CD86 expression (Fig. 5). Highly purified IgD+ cells responded to beraprost, and the percentages of CD23-expressing cells were higher in high- and mid-density cells than in low-density cells (data not shown). These results indicate that early activated B cells are the responder. Because FDCs appear to be the major producer of prostacyclin in the GC (11), GC founder B cells that migrate after the initial contact with T cells in the extrafollicular region (31, 32) may receive prostacyclin signals that upregulate CD54 and CD86 to prepare for the contact with and stimulation of T cells. The results that the effect of beraprost on CD86 is expressed in the presence of anti-BCR plus anti-CD40 signals imply that prostacyclin in vivo may affect B cells that are activated by T cell-dependent Ags. It has been demonstrated that coligation of BCR and CD40 on naive B cells generates a blast population with GC B cell phenotype (33, 34).

In this study, beraprost and prostacyclin increased CD54 and CD86 expression levels from $10^{-8}$ to $10^{-5}$ M, respectively. This result raises the question of physiologic relevance. Considering that the plasma prostacyclin concentrations under physiologic conditions are between $10^{-11}$ and $10^{-8}$ M (35, 36), and those in inflammatory exudates range from $10^{-9}$ to $10^{-7}$ M (37), the prostacyclin levels required to exhibit the enhancing effect may be achieved at the actual production sites of GC microenvironment.

We detected enzymes that are required for the production of prostacyclin, PGE2, and PGF2α in the GC. The upregulation of CD86 on the surface of B cells was, however, achieved by prostacyclin agonists but not by PGE2 and PGF2α. The inability of PGE2 and PGF2α to enhance CD86 may be due to lack of the receptor expression in B cells. This possibility is implausible because tonsillar B cells express EP1, 2, 3 and 4 as well as IP, although quantitative differences among different subsets are observed (J. Kim and J. Choe, unpublished observations). For the same reason the refractory response of GC B cells to beraprost cannot be explained by differential receptor expression. Inasmuch as cAMP regulates CD86 expression (38), why B cells at different differentiation stages display differential response to distinct PG species needs further investigation. It is also intriguing to observe that B cells at the early but not late activation stages upregulate CD86 levels in response to beraprost.

Beraprost-treated B cells exhibited a superior allostimulating activity to control B cells. The greater number of viable T cells obtained was caused by significantly augmented T cell proliferation. Consistent with the well-recognized role for CD86 as a costimulatory molecule (21), CD86 neutralizing Ab potently deleted the beraprost effect, indicating that CD86 was the major molecule involved in T cell costimulation. This result suggests a novel pathway through which FDCs control T cell differentia-
tion in the GC. FDC may potentiate the APC activity of B cells by releasing prostacyclin. Because prostacyclin selectively upregulates CD86 but not CD80 on B cells, after T cell–B cell interactions, the resulting T cell differentiation may be favored toward Th2 type. Several groups demonstrated the dominant role for CD86 in Th2 activation and differentiation (2, 3). In other words, FDCs regulate T cell differentiation indirectly by controlling the APC potential of B cells in the GC. Because B cells were incubated with beraprost before the further culture with T cells, one may suspect that the residual beraprost might have influenced the enhanced proliferation. This is unlikely according to our previous results that showed a suppression of T cell proliferation by beraprost (11, 39). Considering the fact that GC T cells do not normally proliferate and produce IL-2 in response to Ag stimulation (40–42), there must be a balance between positive and negative signals for cellular proliferation. In this balance control, FDCs appear to have a dominating role by providing prostacyclin and PGE2 (11).

It is noteworthy that two CD28 counterreceptors, CD80 and CD86, exhibited different signal requirements for their upregulation on the surface of B cells. CD86 was induced on the surface in response to anti-IgM and anti-CD40 stimuli, which was further augmented by the additional presence of either beraprost or db-cAMP (Figs. 1, 7). In contrast, these stimuli failed to induce CD80 expression. More potent stimulation of B cells with CD154-transfected L cells instead of anti-CD40 mAb led to induction of both CD80 and CD86 but did not abolish the preferential enhancing effect of beraprost on CD86 (Fig. 3). Upregulation of CD80 and CD86 after CD154 treatment was also observed with murine B cells (43). However, unlike murine B cells whose CD80 surface expression was enhanced by the combined addition of anti-IgM and db-cAMP (44), human CD80 was not induced by anti-IgM and db-cAMP (Fig. 7), implying another difference between human and murine immune systems. Despite this difference, our preliminary results on the in vivo effect of beraprost in the murine system are supportive of the in vitro data presented in this study (data not shown).

In summary, we present evidence for a novel function of a prostacyclin analog. Beraprost enhances the expression levels of CD86 on B cells via the IP receptor and cAMP. A. Mid-density B cells (1 × 10^6 cells) were preincubated for 30 min with anti-IgM, anti-CD40, IL-2, IL-4, and IL-10 in the presence of IP antagonist (RO1138452, 2 × 10^-6 M), EP1–3 antagonist (AH6809, 10^-5 M), or EP4 antagonist (AH23848, 10^-5 M), followed by the addition of vehicle or beraprost for a further culture for 72 h. B. B cells were cultured for 72 h with the above stimuli in the presence of vehicle, db-cAMP (10^-5 M), or beraprost. At the end of the culture, expression levels of CD80 or CD86 were measured by flow cytometry. The relative levels of each marker were calculated by comparing with that of B cells cultured in the presence of vehicle control. Means ± SEM of independent experiments are depicted. *p < 0.05, **p < 0.01.

anti-IgM and db-cAMP (Fig. 7), implying another difference between human and murine immune systems. Despite this difference, our preliminary results on the in vivo effect of beraprost in the murine system are supportive of the in vitro data presented in this study (data not shown).

In summary, we present evidence for a novel function of a prostacyclin analog. Beraprost enhances the expression levels of CD86 on B cells via the IP receptor and then increased CD86 contributes to the APC capability of B cells. These findings imply another interaction pathway among FDCs, B cells, and T cells in the GC. Prostacyclin analogs may be used as effective adjuvants in vaccine development.

Disclosures
The authors have no financial conflicts of interest.

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