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Prostaglandin E2 Inhibits IL-18-Induced ICAM-1 and B7.2 Expression Through EP2/EP4 Receptors in Human Peripheral Blood Mononuclear Cells

Hideo K. Takahashi,† Hiromi Iwagaki,† Tadashi Yoshino,† Shuji Mori,† Toshihiko Morichika,† Hideyuki Itoh,† Minori Yokoyama,* Shinichiro Kubo,*† Eisaku Kondo,† Tadaatsu Akagi,‡ Noriaki Tanaka,† and Masahiro Nishibori‡*†

Costimulatory molecules play important roles in immune responses. In the present study we investigated the effects of PGE2 on the expression of ICAM-1, B7.1, and B7.2 on monocytes in IL-18-stimulated PBMC using FACS analysis. Addition of PGE2 to PBMC inhibited ICAM-1 and B7.2 expression elicited by IL-18 in a concentration-dependent manner. We examined the involvement of four subtypes of PGE2 receptors, EP1, EP2, EP3, and EP4, in the modulatory effect of PGE2 on ICAM-1 and B7.2 expression elicited by IL-18, using subtype-specific agonists. ONO-AE1-259-01 (EP2R agonist) inhibited IL-18-elicited ICAM-1 and B7.2 expression in a concentration-dependent manner with a potency slightly less than that of PGE2, while ONO-AE1-329 (EP4R agonist) was much less potent than PGE2. The EP2/EP4R agonist 1-deoxy-PGE1 mimicked the effect of PGE2 with the same potency. ONO-D1-004 (EP1R agonist) and ONO-AE-248 (EP3R agonist) showed no effect on IL-18-elicited ICAM-1 or B7.2 expression. These results indicated that EP2 and EP4Rs were involved in the action of PGE2. Dibutyryl cAMP and forskolin down-regulated ICAM-1 and B7.2 expression in IL-18-stimulated monocytes. As EP2 and EP4Rs are coupled to adenylate cyclase, we suggest that PGE2 down-regulates IL-18-induced ICAM-1 and B7.2 expression in monocytes via EP2 and EP4Rs by cAMP-dependent signaling pathways. The fact that anti-B7.2 as well as anti-ICAM-1 Ab inhibited IL-18-induced cytokine production implies that PGE2 may modulate the immune response through regulation of the expression of particular adhesion molecules on monocytes via EP2 and EP4Rs. The Journal of Immunology, 2002, 168: 4446–4454.

Interleukin-18 was originally described as IFN-γ-inducing factor (1). IL-18 requires the cleavage at its aspartic acid residue by IL-1β-converting enzyme/caspase-1 to become the active, mature protein (2, 3). APCs produce IL-18 during interactions with cognate T cells (4). IL-18 plays a role in the expression of Th1 responses (1, 5, 6). In an established Th1 clone, the levels of IFN-γ induced by IL-18 were higher than those induced by IL-12 (1). IL-18 acts in synergy with IL-12 to induce IFN-γ production in CD4+ cells by using different signaling pathways (5, 7), and both IL-12 and IL-18 are necessary for the full expression of Th1 responses. However, at early stages of T cell differentiation, IL-18 can promote either Th1 or Th2 responses, dependent on IL-12 or IL-4 (8). IL-18 enhances T cell and NK cell cytotoxicity and directly induces IFN-γ production by NK cells (6). The function of IL-18 is suggested to be functionally regulated by IL-18 binding protein, a recently described Ig-like binding factor that strongly suppresses the development of Th1 responses through IL-18 neutralization (9).

The acquired immune response requires the engagement of T cells with APC, mediated by interaction of the TCR with antigenic peptides presented by MHC molecules on the APC (10). These recognition and signaling events just described are now known to be effected by a dynamic structure known as the immunological synapse, which is assembled upon T cell-APC contact (11, 12). For T cell activation, the costimulatory signals are required from accessory receptors (13); the interaction of LFA-1 (CD11a/CD18) with its ligand, ICAM-1/CD54, and the binding of CD28 to B7 (B7.1/CD80 or B7.2/CD86) play important roles in cell signaling as well as cell-cell adhesion. The binding of CD28 on the T cell surface to B7 present on APC determines the direction of T cell responses, activation or anergy, after antigenic stimulation (14, 15). The interaction of LFA-1 with ICAM-1 is important in the activation of T cells by lowering the concentration of Ag required for stimulation of T cells and promoting sustained signaling from the TCR in a CD28-independent manner (16, 17). Recently, we found that IL-18 up-regulated the expression of ICAM-1 in a monocyte population in human PBMC as well as KG-1, a human myelomonocytic cell line, while the expression of CD11a, CD18, CD29, CD44, and CD62 ligand in monocytes and that of ICAM-1, CD11a, CD18, CD29, CD44, and CD62 ligand in T cells were not influenced by IL-18 (18, 19). IL-18 in the same concentration range stimulated the production of IL-12, TNF-α, and IFN-γ in cultures of human PBMC. However, IL-18-induced
expression of ICAM-1 in monocytes was independent of IL-18-elicited IL-12, TNF-α, and IFN-γ production (18). Anti-ICAM-1 and anti-LFA-1 Abs not only prevented IL-18-induced aggregation of PBMC, but also down-regulated IL-18-induced production of IL-12, IFN-γ, and TNF-α (18). These findings strongly indicated that the ICAM-1/LFA-1 interaction induced by IL-18 is important for the enhanced cytokine induction. However, little is known about the effect of IL-18 on the expression of other costimulatory molecules, such as B7.1 and B7.2, on monocytes, although these costimulatory molecules are intimately involved in IL-2 production and clonal expansion in T cells during Ag presentation (20, 21). Some cytokines that induce Th1/Th2 immune responses exert differential effects on B7 isoform expression on resting monocytes (22). IL-4 and IL-10 down-regulated B7.2 and moderately up-regulated B7.1 expression. IFN-γ enhanced the expression of both B7.1 and B7.2 isoforms. TNF-α, which elicited both Th1 and Th2 characteristics depending on experimental conditions, down-regulated B7.2, but did not alter B7.1 expression. None of the other cytokines, including IL-1α, IL-1β, IL-2, IL-5, IL-6, and IL-12, modulated the expression of B7 isoforms (22). B7.1 and B7.2 have different affinities for their binding partners on T cells, CD28 and CTLA-4, and the differential roles of B7.1 and B7.2 have been suggested for the initial, sustained T cell activation or termination of T cell activation (20, 21). Previously, we demonstrated that histamine regulated the IL-18-initiating cytokine cascade through down-regulation of ICAM-1 expression on monocytes (23). The changes may result in a shift of Th1/Th2 balance toward Th2 dominance (23, 24). Thus, many kinds of chemical mediators in inflammation may modulate immune responses by regulation of the expression of adhesion molecules.

PGE₂, the major arachidonic acid metabolite released from APC, primes naive human T cells for enhanced production of anti-inflammatory cytokines and inhibition of proinflammatory cytokines through cyclooxygenase 2 (COX-2) (25, 26). The actions of PGE₂ are expected to be exerted via stimulation of the four PGE₂ receptor subtypes, EP1, EP2, EP3, and EP4, alone or in combination. EP1 and EP3 are coupled to a Gₛ protein, phosphatidylinositol production, and increased intracellular Ca²⁺ concentration (23). EP2 and EP4 are coupled to a Gₛ protein, adenylyl cyclase activation, and cAMP production (26). EP2Rs directly inhibit T cell proliferation, while EP2 and EP4Rs regulate APC functions (27). There are at least three variants of EP3, and these variants can cause increased intracellular Ca²⁺ concentration or the activation or inhibition of adenylate cyclase (28). While little is known about EP3 receptor signaling, the cAMP formation induced by the activation of endogenous EP4Rs is strongly blocked by EP1R or EP3R stimulation in hamster ovary cells and human embryonic kidney 293 cells (29, 30). EP4Rs can also be distinguished pharmacologically from EP1 and EP3 receptors (26, 27). EP2Rs play an important role in uterine implantation (31, 32) and a relaxant role in bronchioles (26, 28, 32) and salt-sensitive hypertension (31, 32). A particular role for the EP4R was suggested in regulated perinatal closure of the pulmonary ductus arteriosus (33).

In the present study, we designed experiments to examine whether IL-18 affects the expression of B7.1 and/or B7.2 on human monocytes, in addition to ICAM-1, using FACS analysis. Second, we investigated the effects of exogenous PGE₂ on the IL-18-induced changes in the expression of ICAM-1, B7.1, and B7.2 and characterized the receptor subtypes involved in the action of PGE₂ using selective receptor agonists. We found that PGE₂ regulated the expression of ICAM-1 and B7.2 on monocytes through stimulation of specific receptor subtypes.

### Materials and Methods

#### Reagents and drugs

Recombinant human IL-18 was purchased from MBL (Nagoya, Japan). PGE₂ was purchased from Sigma-Aldrich (St. Louis, MO). ONO-D1-004, ONO-AE1-259-01, ONO-AE-248, ONO-AE-329, and 11-deoxy-PGE₁ were provided by Ono Pharmaceutical (Tokyo, Japan). Dibutylryl cAMP and forskolin were purchased from WAKO (Tokyo, Japan). For flow cytometric analysis, FITC-conjugated mouse IgG1 mAb against CD54 (6.8B5) and PE-conjugated anti-CD3, anti-CD14, or anti-CD19 mAb were purchased from DAKO (Glostrup, Denmark). FITC-conjugated mouse IgG1 mAb against B7.1 (MAb104) was purchased from Immunotech (Marseille, France). FITC-conjugated mouse IgG1 mAb against B7.2 (3331FUN-1) was purchased from BD Pharmingen (San Diego, CA). FITC-conjugated MOPC 21, an IgG1 class-matched control (CMC), was purchased from Sigma-Aldrich.

#### Isolation and culture of PBMC

Normal human PBMC were obtained from human volunteers after they gave their oral informed consent. Samples of 20–50 ml peripheral blood were withdrawn from a forearm vein. PBMC were isolated from the buffy coat of 10 healthy volunteers by centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden), then washed three times in RPMI 1640 (Nissui, Tokyo, Japan) supplemented with 10% (v/v) heat-inactivated FCS, 20 μg/ml kanamycin, and 100 μg/ml streptomycin and penicillin (Sigma-Aldrich). PBMC were suspended at a final concentration of 1 × 10⁶ cells/ml in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated FCS.

#### Flow cytometric analysis

PBMC (1 × 10⁶ cells/ml) were incubated with IL-18, PGE₂, EPR agonists, dibutylryl cAMP, and/or forskolin for 24 h at 37°C in a 5% CO₂/air mixture under different conditions. The cells (5 × 10⁵ cells/sample) were washed once with washing buffer (PBS supplemented with 2.5% normal horse serum, 0.1% NaN₃, and 0.01 M HEPES, pH 7.3). Then the cells were incubated with 1 μg FITC-conjugated anti-ICAM-1Ab, anti-B7.1 Ab, anti-B7.2 Ab or CMC, or PE-conjugated anti-CD3 Ab, anti-CD14 Ab, or anti-CD19 Ab for 20 min at 4°C. After washing, the cells were fixed with 2% paraformaldehyde and analyzed with a FACS Calibur (BD Biosciences, San Jose, CA), and data were processed using the CellQuest program (BD Biosciences). The data are expressed as the relative fluorescence intensities against CMC. The results are the mean ± SEM of five donors.

#### Cytokine assay

PBMC (1 × 10⁶ cells/ml) were incubated with IL-18 in the presence or the absence of anti-ICAM-1, anti-B7.1 (MAB104), and/or anti-B7.2 (3331FUN-1) Ab for 24 h at 37°C in a humidiﬁed atmosphere of 5% CO₂/air mixture in air. After culture, the cell-free supernatant fractions were assayed for IL-12, TNF-α, IFN-γ, and IL-10 protein as described previously (23). ELISA for IL-12 detected p70 protein.

#### Statistical examination

The statistical significance of differences was evaluated by ANOVA, followed by Student’s two-tailed t test. A value of p < 0.05 was considered statistically significant.

### Results

#### Effects of PGE₂ on IL-18-induced ICAM-1, B7.1 and B7.2 expression on human monocytes

The effects of IL-18 (100 ng/ml) and/or PGE₂ (10⁻⁶ M) on changes in the expression of human leukocyte Ags (ICAM-1/CD54, B7.1/CD80, and B7.2/CD86) were examined by double-staining flow cytometry using combinations of anti-CD14, anti-CD3, and anti-CD19 Abs after 24 h of incubation of PBMC. IL-18 (100 ng/ml) produced up-regulation of ICAM-1 and B7.2 specifically on monocytes, but not on T or B cells (Fig. 1). In contrast, IL-18 (100 ng/ml) showed no effect on B7.1 expression.

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1 Abbreviations used in this paper: COX, cyclooxygenase; CMC, class-matched control.
on any of these cell types (Fig. 1). PGE₂ (10⁻⁶ M) inhibited ICAM-1 and B7.2 expression induced by IL-18 (100 ng/ml; Fig. 1). The same concentration of PGE₂ alone did not influence ICAM-1 or B7.2 expression on monocytes.

**Time course of IL-18-induced ICAM-1, B7.1, and B7.2 expression on human monocytes**

The time courses of changes in the expression of ICAM-1, B7.1, and B7.2 on monocytes induced by IL-18 were examined 0, 2, 6, 18, and 24 h after the start of incubation. IL-18 (100 ng/ml) upregulated ICAM-1 and B7.2 expression on monocytes in a time-dependent manner (Fig. 2). The effect of IL-18 on ICAM-1 expression was significant at 12 and 24 h, whereas that on B7.2 was significant at 24 h. IL-18 did not induce the expression of B7.1 at any time point examined.

**Dose-response relationship of the effects of IL-18 on ICAM-1, B7.1, and B7.2 expression on human monocytes**

We examined the effects of different concentrations of IL-18 on the expression of ICAM-1, B7.1, and B7.2 by flow cytometry after 24 h of incubation of PBMC (Fig. 3). IL-18 (0.1–100 ng/ml) upregulated the expression of ICAM-1 and B7.2, but not that of B7.1,
on monocytes in a concentration-dependent manner (Fig. 3). The threshold concentrations for the significant effects of IL-18 on ICAM-1 and B7.2 expression were 10 and 100 ng/ml, respectively, compared with the buffer control.

Dose-response relationship of the effects of PGE2 on IL-18-induced ICAM-1, B7.1, and B7.2 expression on human monocytes

The effects of PGE2 on the changes in IL-18 (100 ng/ml)-induced expression of ICAM-1, B7.1, and B7.2 were determined 24 h after the start of culture (Fig. 4). PGE2 (10⁻⁹–10⁻⁵ M) had no effect on ICAM-1, B7.1, or B7.2 expression on monocytes without IL-18 treatment. However, PGE2 down-regulated IL-18 (100 ng/ml)-induced ICAM-1 and B7.2 expression on monocytes in a concentration-dependent manner (Fig. 4). The IC₅₀ values for the inhibitory effect of PGE2 on the expression of ICAM-1 and B7.2 induced by IL-18 were estimated to be the same, 50 nM.

Effects of selective EPR agonists on IL-18-induced ICAM-1, B7.1, and B7.2 expression on human monocytes

To determine the PGE₂ receptor subtypes involved in the effects of PGE₂ on ICAM-1 and B7.2 expression, we examined the effects of EPR agonists (10⁻⁹–10⁻⁵ M) on ICAM-1, B7.1, and B7.2 expression on monocytes induced by IL-18 (100 ng/ml) after 24-h incubation of PBMC. ONO-D1-004 (EP1R agonist) (34, 35), and ONO-AE-248 (EP3R agonist) (33–35), in the concentration range from 10⁻⁹ to 10⁻⁵ M, had no effect on ICAM-1, B7.1, or B7.2 expression induced by IL-18 (Fig. 5A). On the other hand, ONO-AE1-259-01 (EP2R agonist) (34–36) inhibited ICAM-1 and B7.2 expression on IL-18-treated monocytes in a concentration-dependent manner (Fig. 5). ONO-AE1-329 (EP4R agonist) (34–36) also inhibited ICAM-1 and B7.2 expression on monocytes with IL-18; however, the potency of the inhibitory effect of ONO-AE1-329 on ICAM-1 expression...
was lower than that of ONO-AE1-259-01. IC_{50} values for the inhibitory effect of ONO-AE1-259-01 on the expression of ICAM-1 was estimated to be 100 nM (Fig. 5). Moreover, we confirmed that 11-deoxy-PGE_1 (EP2R/EP4R agonist) (36, 37), inhibited IL-18-induced ICAM-1 and B7.2 expression in a concentration-dependent manner (Fig. 6). Thus, EP2 and EP4Rs are

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

**FIGURE 5.** Effects of EPR agonists (EP1, EP2, EP3, and EP4) on IL-18-induced ICAM-1, B7.1, and B7.2 expression on human monocytes. PBMC (1 × 10^6/ml) were incubated with increasing concentrations of ONO-D1-004 (EP1R agonist), ONO-AE1-259-01 (EP2R agonist), ONO-AE-248 (EP3R agonist), or ONO-AE1-329 (EP4R agonist) for 24 h in the presence or the absence of IL-18 and were stained with Abs (ICAM-1, B7.1, and B7.2) or CMC. ○ and □, results obtained with anti-ICAM-1 Ab, anti-B7.1 Ab, or anti-B7.2 Ab after incubation in the presence or the absence of IL-18, respectively. ○ and □. Fluorescence intensity obtained with CMC after incubation in the presence or the absence of IL-18, respectively. The results are the mean ± SEM of five donors. *, p < 0.05; **, p < 0.01 (compared with the corresponding value in the absence of EPR agonists). Where error bars are not shown, they were smaller than the symbol.

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

**FIGURE 6.** The effects of 11-deoxy-PGE_1 on IL-18-induced ICAM-1, B7.1, and B7.2 expression on human monocytes. PBMC (1 × 10^6/ml) were incubated with increasing concentrations of 11-deoxy-PGE_1 (EP2R/EP4R agonist) for 24 h in the presence or the absence of IL-18 and were stained with Abs (ICAM-1, B7.1, and B7.2) or CMC. ○ and □, Results obtained with anti-ICAM-1 Ab, anti-B7.1 Ab, or anti-B7.2 Ab after incubation in the presence or the absence of IL-18, respectively. ○ and □. Fluorescence intensity obtained with CMC after incubation in the presence or the absence of IL-18, respectively. The results are the mean ± SEM of five donors. ***, p < 0.01 (compared with the corresponding value in the absence of 11-deoxy-PGE_1). Where error bars are not shown, they were smaller than the symbol.
involved in the down-regulation of IL-18-elicited ICAM-1 and B7.2 expression by PGE₂. The IC₅₀ values for the inhibitory effect of 11-deoxy-PGE₁ on the expression of ICAM-1 and B7.2 were estimated to be the same, 50 nM. At 10⁻⁶ and 10⁻⁵ M, 11-deoxy-PGE₁ blocked the expression of ICAM-1 and B7.2 completely.

Effects of dibutyryl cAMP and forskolin on IL-18-induced ICAM-1 and B7.2 expression on human monocytes
To investigate the mechanism by which PGE₂ inhibited ICAM-1 and B7.2 expression elicited by IL-18, the effects of dibutyryl cAMP, a membrane-permeable cAMP analog, and forskolin, an adenylyl cyclase activator, on the IL-18-induced expression of ICAM-1 and B7.2 on monocytes were examined (Fig. 7). Dibutyryl cAMP and forskolin (10⁻⁷–10⁻⁴ M) down-regulated IL-18-induced ICAM-1 and B7.2 expression in a concentration-dependent manner. The IC₅₀ values of dibutyryl cAMP and forskolin in ICAM-1 and B7-2 expression were the same, 5 μM. These results indicated that an increase in intracellular cAMP level can mimic the effects of EP2/4R stimulation.

Inhibition of IL-18-induced cytokine responses in PBMC by anti-ICAM-1, anti-B7.1, or anti-B7.2 Ab
We investigated the effects of anti-ICAM-1, anti-B7.1, or anti-B7.2 Ab on IL-18-induced cytokine production to evaluate the possible involvement of ICAM-1, B7.1, or B7.2 in the response (Fig. 8). Fig. 8 shows that anti-ICAM-1 Ab or anti-B7.2 Ab down-regulated IL-18-induced IL-12, TNF-α, and IFN-γ production, whereas both Abs up-regulated IL-10 production in a concentration-dependent manner. Anti-B7.1 Ab had no effect on these cytokine responses. The maximal effects obtained with anti-ICAM-1 Ab or anti-B7.2 Ab were 60 and 30%, respectively, regardless of the kinds of cytokines produced by IL-18. The combination of anti-ICAM-1 and anti-B7.2 Ab produced ~80% inhibition of the cytokine responses induced by IL-18. The class-matched nonrelevant Ab at a concentration of 100 μg/ml had no effect on IL-18-induced cytokine responses.

Discussion
CD28 and LFA-1 are the two main costimulatory molecules in immune responses, and the engagement of these molecules by their respective ligands, B7 and ICAM-1, on APC along with the TCR signaling play important roles in Ag recognition and optimal T cell responses (38, 39). Previously, we found that IL-18 up-regulated the expression of ICAM-1 in a monocyte population in human PBMC (18). The IL-18-induced expression of ICAM-1 on monocytes, in turn, induced the interaction of ICAM-1 and LFA-1 on APC along with the TCR (18). Therefore, the IL-18-initiated cytokine cascade was suggested to be dependent on the up-regulation of ICAM-1 on monocytes and subsequent interaction of ICAM-1 on monocytes and LFA-1 on T/NK cells (18). In the present study we also demonstrated that IL-18 up-regulated B7.2, but not B7.1, on monocytes in addition to ICAM-1 (Figs. 1–3). The fact that anti-B7.2 Ab, but not anti-B7.1 Ab, inhibited the production of IL-12, TNF-α, and IFN-γ (Fig. 8) suggested that the interaction of B7.2 with CD28 is also involved in cytokine cascade induced by IL-18 in addition to
ICAM-1/LFA-1 interaction. The extent of the inhibition of IL-18-induced cytokine response by anti-B7.2 was smaller than that by anti-ICAM-1. Therefore, it is likely that the ICAM-1/LFA-1 interaction is relatively important for IL-18 action in human PBMC. The costimulatory molecules on APC, including B7.2, have been suggested to be involved in the diversity of T cell responses (21). B7.2/CD28 interaction was suggested to be required for initial T cell costimulation during Ag presentation because of the constitutive expression and relatively rapid induction of B7.2 on APC, whereas B7.1/CD28 interaction may be involved in the sustained T cell activation because of the slow induction of B7.1 expression with low expression levels in the resting T cells (20, 21). In this context, IL-18 may be able to facilitate the initial T cell response through the up-regulation of B7.2 expression on monocytes. Although the functional profile of IL-18 appears to be similar to that of IL-12, the up-regulating effect of IL-18 on B7.2 was distinct from that of IL-12 (22).

In the present study, we clearly demonstrated inhibitory effects of PGE2 on the expression of ICAM-1 and B7.2 on monocytes elicited by IL-18 (Figs. 1, 3, and 4). These modulatory effects were similar to those of histamine (23), another important chemical mediator in inflammatory and immune responses. To investigate the receptor subtypes involved in the action of PGE2, we used selective agonists for respective receptors (34). ONO-AE1-259 and ONO-AE-1-329 were demonstrated to be highly selective for mouse EP2 and EP4 receptors, respectively, using a receptor binding assay in Chinese hamster ovary cells transfected with each EP cDNA (34). Suzuwa et al. (34) reported that the selective EP1R–EP4R agonists used in the present study were highly selective for their respective receptors. For example, the EP2R agonist ONO-AE1-259 had at least 700-fold higher affinity for EP2Rs compared with other receptor agonists (34). In the present study the IC50 values for the inhibitory effects of ONO-AE1-259 and ONO-AE-1-329 on the expression of ICAM-1 on monocytes induced by IL-18 were similar (50 and 100 nM, respectively; Fig. 5). Therefore, these findings indicated that EP2 and EP4 receptor agonists stimulated respective receptors and both EP2 and EP4 receptors were repeatedly demonstrated to be coupled with adenylate cyclase (26). As shown in Fig. 7, the findings that forskolin and dibutyryl cAMP mimicked the effects of PGE2 on ICAM-1 and B7.2 expression were consistent with the intracellular signaling pathway stimulated by EP2 and EP4 receptors. Monocytes/macrophages are known to express COX-1 and COX-2 (40, 41). When stimulated with LPS,
zymosan, or polymerized bovine albumin (40, 41), the expres-
sion of COX-2 was specifically up-regulated, leading to en-
hanced production of PGE<sub>2</sub>. LPS stimulates monocytes/macrophages through CD14R/Toll-like receptor 4 and activates multiple signal transduction pathways, including IL-1R-associated kinase-mediated activation of NF-κB and c-Jun N-terminal kinase. Some of the pathways appear to be shared by IL-1R signaling (42), because Toll-like receptor 4 and IL-18R have homologous IL-1R-like intracellular domains. However, IL-18 did not induce PGE<sub>2</sub> production in human PBMC (43), but, rather, inhibited PGE<sub>2</sub> production in an IFN-γ-dependent manner. Therefore, it is likely that the endogenous production of PGE<sub>2</sub> in PBMC did not occur under the present conditions. Previously, we demonstrated that IFN-γ production induced by IL-18 in human PBMC was totally dependent on the interaction between ICAM-1 on monocytes and LFA-1 on NK/T cells (18). Thus, IFN-γ-dependent inhibition of PGE<sub>2</sub> production by IL-18 may facilitate IFN-γ production by IL-18.

The recent development of mouse lines with targeted mutations of genes encoding enzymes and receptors in the prostanoid pathway has enabled evaluation of the functional roles of prostanoids in complex inflammatory and immune responses (27, 44, 45). Using mouse lines with disrupted genes encoding each of the four EPRs, Nataraj et al. (27) demonstrated that EP2Rs and EP4Rs play major roles in the inhibitory effects of PGE<sub>2</sub> on cell proliferation as well as cytokine release in the MLR with EP2 dominance, whereas EP1 and EP3 did not alter the inhibitory response to PGE<sub>2</sub>. These profiles of the involvement of EPR subtypes in cellular immune responses were quite similar to the regulatory effects of EPR agonists on ICAM-1 and B7.2 expression in the present study. Therefore, it is possible that some of the inhibitory effects of PGE<sub>2</sub> in MLR in wild-type responder cells may be due to inhibition of the expression of ICAM-1 and B7.2, leading to reduction of costimulatory signals in responding T cells. Further studies are necessary to clarify this point.

In patients with injury, infection, or cancer, elevated PGE<sub>2</sub> levels contribute to the inhibition of cellular immunity through cAMP production (46, 47). On the other hand, in transplantation, PGE<sub>2</sub>-induced elevation of cAMP exerts a beneficial effect on allograft survival by modulating T cell function (48). Enhanced PGE<sub>2</sub> production reduces Th1 cytokine levels, independently of those of Th2 cytokines, in the local graft environment after donor-specific blood transfusion, which induces donor-specific intragraft suppressor factors, accompanied by reduced local and systemic immune activation (49). Therefore, stable PGE<sub>2</sub> analogs may be useful for the treatment of Th1-mediated inflammatory diseases. PGE<sub>2</sub>-induced down-regulation of IL-18-induced ICAM-1 and B7.2 expression may result in the control of inflammatory and immune responses. In conclusion, the results of the present study suggested that PGE<sub>2</sub> down-regulated ICAM-1 and B7.2 expression on IL-18-stimulated monocytes through a cAMP-dependent mechanism via stimulation of EP2R/EP4Rs, leading to modulation of immune responses by changing cell to cell interactions through ICAM-1/ LFA-1 and B7.2/CD28.

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