Unique Regulation Profile of PGE1 on Adhesion Molecules Expression and Cytokines Production in Human PBMC.

HIDEO KOHKA TAKAHASHI, HIROMI IWAGAKI, RYUJI TAMURA, DONG XUE, MASAHIRO SANO, SHUJI MORI, TADASHI YOSHINO, NORIAKI TANAKA, and MASAHIRO NISHIBORI

Department of Pharmacology (H.K.T.,S.M.,M.N.), Department of Tumour Biology (H.K.T.,H.I.,R.T.,D.X., N.T.) and Department of Pathology (T.Y.,M.S.), Okayama University Graduate School of Medicine and Dentistry, 2-5-1 Shikata-cho, Okayama 700-8558, Japan.
a) Running title; Unique Effect of PGE1 on ICAM-1, B7.2 and CD40 Expression.

b) Address correspondence and reprint requests to: Dr. Masahiro Nishibori,
Department of Pharmacology, Okayama University Graduate School of Medicine and Dentistry, 2-5-1 Shikata-cho, Okayama 700-8558, Japan.
Tel/FAX: +81-86-235-7140; e-mail: mbori@md.okayama-u.ac.jp

c) Text pages 31 pages
Tables 0
Figures 7
References 40
Abstract 246 words
Introduction 493 words
Discussion 1322 words

Abbreviations: cAMP, cyclic adenosine monophosphate; CD40L, CD40 ligand; CHO, Chinese hamster ovary; CMC, class-matched control; EC, endothelial cells; 11-D-PGE1, 11-deoxy-PGE1; fr., fraction; mAb, monoclonal Ab; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorting; FITC, fluorescein isothiocyanate; ICAM, intercellular adhesion molecule; IFN, interferon; IL, interleukin; LFA, lymphocyte function-associated antigen; NF-κB,
nuclear factor-kappaB ; MHC, histocompatibility complex ; PBMC, peripheral
blood mononuclear cells; PE, phycoerythrin; PGE1, prostaglandin E1; PI 3-K,
phosphatidyl-inositol 3 kinase; PKA, protein kinase A ; RA, rheumatoid arthritis;
rh, recombinant human ; TCR, T-cell receptor ; TNF, tumor necrosis factor

e) Inflammation & Immunopharmacology
ABSTRACT

In the present study, we examined the effects of Prostaglandin E1 (PGE1) on the expression of intercellular adhesion molecule (ICAM)-1, B7.1, B7.2, CD40 and CD40 ligand (CD40L) on peripheral blood mononuclear cells (PBMC) using FACS analysis as well as on cytokine production using ELISA. Whereas no inhibitor of spontaneous expression of adhesion molecules was reported, we found that PGE1 inhibited spontaneous ICAM-1, B7.2 and CD40 expression on monocytes in a concentration-dependent manner, but had no effect on the expression of B7.1 and CD40L. While interleukin (IL)-18 induced the expression of ICAM-1, B7.2, CD40 and CD40L, PGE1 prevented IL-18-induced expression of ICAM-1, B7.2 and CD40. We examined the involvement of five subtypes of PGE1 receptors (IP, EP1, EP2, EP3 and EP4) in the effect of PGE1 on the expression of these adhesion molecules, using subtype-specific agonists. Among EP-receptor agonists, EP2- and EP4-receptor agonists inhibited IL-18-elicited ICAM-1, B7.2 and CD40 expression. ONO-1301 (IP-receptor agonist) prevented the expression of ICAM-1, B7.2 and CD40 regardless of the presence of IL-18 with the same potency as PGE1. The effect of a combination of ONO-1301 and 11-deoxy (D)-PGE1 (EP2/EP4-receptor agonist) on ICAM-1, B7.2 and CD40 expression mimicked that of PGE1. Moreover, PGE1 inhibited the production of IL-12 and interferon (IFN)-γ in PBMC in the presence and absence of IL-18, whereas PGE1 induced IL-10 production. In conclusion, IP-receptor in addition to EP2/EP4-receptor played an important role in the action of PGE1 on the expression of adhesion molecules on monocytes and cytokine production.
The induction of an immune response requires a coordinated collective cell-cell interaction, including ICAM-1/LFA-1, B7/CD28 and CD40/CD40L (Durie et al., 1994; Ranger et al., 1996; Camacho et al., 2001). IL-18, a Th1-cytokine, plays a key role in regulating IFN-γ-production (Okamura et al., 1995). IL-18 augments T-cell activation in conjunction with cell-cell interaction through adhesion molecules (Takahashi et al., 2002a; Takahashi et al., 2002b), and therefore is capable of influencing the development of innate immune responses. It has been reported that IL-18-induced adhesion molecule expression was mediated through nuclear factor-kappaB (NF-κB) and phosphatidylinositol 3 kinase (PI 3-K) in monocytes and T-cells (Matsumoto et al., 1997; Kojima et al., 1999).

PGE1 is one of the prostanoids synthesized from linoleic acid in vivo and differs from the products of the arachidonate cascade. The major function of PGE1 have been known as vasodilatation and anti-platelet aggregation. The prostaglandin family plays important roles in the regulation of immune responses through various receptors. Receptor binding experiments to determine the affinity of prostaglandins for eight types of receptors (DP, IP, TP, FP, EP1, EP2, EP3, EP4) expressed in cultured Chinese hamster ovary (CHO) cells clearly showed that PGE1 had high affinity for IP-receptor in addition to EP1-, EP2-, EP3- and EP4-receptors, whereas PGE2 had a high affinity for FP-, EP1-, EP2-, EP3- and EP4- receptors (Narumiya et al., 1999). The EP2- and EP4-receptors are coupled to Gs and mediate the increase in cyclic adenosine monophosphate (cAMP) (Narumiya et al., 1999). The IP-receptor has also been found to stimulate adenylate cyclase, however, expression studies revealed that it may
couple with multiple signaling pathways including PI response and Ca\textsuperscript{2+} mobilization (Namba et al., 1994). In fact, PGI\textsubscript{2}, an IP-receptor agonist, has been demonstrated to induce the elevation of free Ca\textsuperscript{2+} concentration in several cultured cell lines (Watanabe et al., 1991). Despite the clear difference in the receptor activation profile of PGE\textsubscript{1} and PGE\textsubscript{2}, there is little information about the action characteristics of PGE\textsubscript{1} on particular immune responses. Previously, we reported that PGE\textsubscript{2} inhibited IL-18-induced expression of ICAM-1 and B7.2 on human monocytes through the stimulation of EP\textsubscript{2}- and EP\textsubscript{4}-receptors (Takahashi et al., 2002a). These effects of PGE\textsubscript{2} on adhesion molecules in turn modulated the production of IL-12, tumor necrosis factor (TNF)-\textalpha and IFN-\gamma in PBMC (Takahashi et al., 2002a), however, little is known about the pharmacological action of PGE\textsubscript{1} on adhesion molecules expression on monocytes, and the differences between the effects of PGE\textsubscript{1} and PGE\textsubscript{2} on the cell-cell interaction and cytokine production profiles.

In the present study, we examined the effect of PGE\textsubscript{1} on the expression of ICAM-1, B7.1, B7.2, CD40 and CD40L as well as the production of IL-12, IFN-\gamma and IL-10 in human PBMC in the presence or absence of IL-18 to clarify a functional role of PGE\textsubscript{1} and the differences between PGE\textsubscript{1} and PGE\textsubscript{2} using prostaglandin receptor subtype-selective agonists. Interestingly, we found that PGE\textsubscript{1} had a distinct action profile to that of PGE\textsubscript{2}. We also found that the stimulation of IP-receptor had a unique effect on adhesion molecules expression and cytokines production.
MATERIALS and METHODS

Reagents and drugs. Recombinant human (rh) IL-18 was purchased from MBL (Nagoya, Japan). PGE1, ONO-1301, ONO-DI-004, ONO-AE1-259-01, ONO-AE-248, ONO-AE1-329 and 11-deoxy (D)-PGE1 were kindly provided by Ono Pharmaceutical Co. Ltd. (Tokyo, Japan). For flow cytometric analysis, FITC-conjugated mouse IgG1 mAb against ICAM-1/CD54 (6.5B5) and PE-conjugated anti-CD14 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 (2331FUN-1) and CD40 (5C3) were purchased from Pharmingen (SanDiego, CA). FITC-conjugated mouse IgG1 mAb against CD40L/CD154 was purchased from Ancel (Bayport, MN). FITC-conjugated MOPC 21, an IgG1 class-matched control (CMC), was purchased from Sigma Chemical (St. Louis, MO).

Isolation of PBMC. Normal human PBMC were obtained from human volunteers with their oral informed consent. Samples of 50 ml of peripheral blood were withdrawn from a forearm vein. PBMC were isolated from the buffy coat of ten healthy volunteers by centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden), then washed three times in RPMI 1640 medium (Nissui Co. Ltd., Tokyo, Japan) supplemented with 10% (v/v) heat-inactivated FCS, 20 µg/ml of kanamycin and 100 µg/ml of streptomycin and penicillin (Sigma). PBMC were suspended at a final concentration of 1x10^6 cells/ml in RPMI 1640 medium supplemented with 10% (v/v)
Preparation of isolated monocytes. PBMC were prepared as described in Isolation of PBMC. Separation of monocytes from PBMC was conducted by counterflow centrifugal elutriation using the SRR6Y elutriation system and a rotor equipped with a 4.5 ml chamber (Hitachi Koki Co. Ltd, Tokyo, Japan). PBMC resuspended at 5 to 10x10^7 cells in 10 ml of PBS supplemented with 1% (v/v) FCS were injected at an initial flow rate of 10 ml/ min at 4°C with a rotor speed of 2,000 rpm. The flow rate was gradually increased and the cell fractions were collected serially as follows: fraction 1 (fr. 1), 200 ml at 10 ml/min; fr. 2, 200 ml at 12 ml/min; fr. 3, 200 ml at 14 ml/min; fr. 4, 200 ml at 16 ml/min; and fr. 5, 200 ml at 18 ml/min. The cell population of each fraction was determined by flow cytometry with FITC-conjugated anti-CD 14 Ab (monocytes), PE-conjugated anti-CD3 Ab (T-cells) and PE-conjugated anti-CD19 Ab (B-cells). Fr.2 contained 65% T-cells and 20% B-cells but less than 5% monocytes. Both fr. 3 and 4 contained 85% monocytes but less than 5% T- and B-cells. These two fractions were used as the monocyte-rich fractions. The other fractions contained less than 5% monocytes, T- and B-cells.

Flow cytometric analysis. PBMC and isolated monocytes (1x10^6 cells/ml) were incubated with IL-18, PGE1, IP- and EP-receptor agonists for 24 h at 37°C in a 5% CO2 / air mixture under different conditions. The cells (5x10^5 cells/sample) were washed once with washing buffer (PBS supplemented with 2.5% normal horse serum, 0.1%
NaN3, and 0.01M HEPES, pH7.3). The changes in expression of human leukocyte antigens (ICAM-1, B7.1, B7.2, CD40 and CD40L) on monocytes were examined by double-labeling flow cytometry using combination of anti-CD14 Ab with anti-ICAM-1Ab, anti-B7.1 Ab, anti-B7.2 Ab, anti-CD40 Ab or anti-CD40L Ab. Then, the cells were incubated with 1µg of FITC-conjugated anti-ICAM-1Ab, anti-B7.1 Ab, anti-B7.2 Ab, anti-CD40 Ab or anti-CD40L Ab or CMC, and PE-conjugated anti-CD14 Ab for 20 min at 4°C. After washing, the cells were fixed with 2% paraformaldehyde and analyzed with a FACSCalibur (Becton Dickinson, Biosciences, San Jose, CA), and data were processed using the CELL QUEST program (Becton Dickinson Biosciences). The data are expressed as the relative fluorescence intensities against CMC. The results are the means±SEM of five donors.

**Cytokine assay.** PBMC (1x10⁶ cells/ml) were incubated with PGE1, PGE2, IP- and EP-receptor agonists in the presence or absence of IL-18 for 24 hours at 37°C in a humidified atmosphere of 5% CO₂ in air. After culture, the cell-free supernatant fractions were assayed for IL-12 (p70), IFN-γ and IL-10 protein as described previously (Takahashi et al., 2002a; Takahashi et al., 2002b).

**Statistical analysis.** The statistical significance of differences was evaluated by analysis of variance followed by Tukey test. P< 0.05 was considered statistically significant.
RESULTS

Dose-response relationship of the effects of PGE1 on ICAM-1, B7.1, B7.2, CD40 and CD40L expression on human monocytes. The effects of PGE1 (0-10^{-6} M) on the changes in expression of ICAM-1, B7.1 B7.2, CD40 and CD40L on monocytes in the presence and absence of IL-18 (100 ng/ml) were determined by double-staining flow cytometry after 24 h the incubation of PBMC (Fig. 1A). PGE1 concentration-dependently inhibited the spontaneous expression of ICAM-1, B7.2 and CD40 on monocytes (Fig. 1A), but had no effect on the expression of B7.1 and CD40L (data not shown). IC50 values for the inhibitory effect of PGE1 on the expression of ICAM-1, B7.2 and CD40 were estimated to be 10, 3 and 7 nM, respectively. IL-18 (100 ng/ml) up-regulated the expression of ICAM-1, B7.2, CD40 and CD40L on monocytes. PGE1 inhibited IL-18-induced ICAM-1, B7.2 and CD40 expression in a concentration-dependent manner (Fig. 1B), but had no effect on the expression of B7.1 and CD40L (data not shown). IC50 values for the inhibitory effect of PGE1 on the expression of ICAM-1, B7.2 and CD40 induced by IL-18 were estimated to be 5, 3 and 3 nM, respectively.

Effects of IP- and EP-receptor agonists on ICAM-1, B7.1, B7.2, CD40 and CD40L expression on human monocytes. To determine which PGE1 receptor subtypes (IP, EP1, EP2, EP3, EP4) are involved in the effects of PGE1 on ICAM-1, B7.1 B7.2, CD40 and CD40L expression, we examined the effects of EP-receptor agonists (0-10^{-6} M) on ICAM-1, B7.1 B7.2, CD40 and CD40L expression on monocytes in the presence and
absence of IL-18 (100 ng/ml) after 24 h incubation of PBMC (Fig. 2). ONO-DI-004 (EP1-receptor agonist) (Suzawa et al., 2000; Kitagawa et al., 2001) and ONO-AE-248 (EP3-receptor agonist) (Suzawa et al., 2000; Kitagawa et al., 2001), in the concentration range from $10^{-9}$ to $10^{-6}$ M, had no effect on the expression of these five adhesion molecules regardless the presence of IL-18 (data not shown). ONO-AE1-259-01 (EP2-receptor agonist) and ONO-AE1-329 (EP4-receptor agonist) (Suzawa et al., 2000; Kitagawa et al., 2001) also had no effect on the expression of these five adhesion molecules in the absence of IL-18 (Figs. 2A, 2B and 2C). ONO-AE1-259-01 and ONO-AE1-329 inhibited ICAM-1, B7.2 and CD40 expression on monocytes in the presence of IL-18 (Figs. 2D, 2E and 2F), but had no effect on the expression of B7.1 and CD40L (data not shown). IC50 value for the inhibitory effect of ONO-AE1-259-01 on the expression of ICAM-1 was estimated to be 100 nM (Fig. 2).

Moreover, we found that ONO-1301 (IP-receptor agonist) (Hayashi et al., 1997; Imawaka and Sugiyama, 1998) strongly prevented the expression of ICAM-1, B7.2 and CD40 in the presence and absence of IL-18 (Fig. 2), but had no effect on the expression of B7.1 and CD40L (data not shown). IC50 value for the inhibitory effect of ONO-1301 on the expression of ICAM-1 was estimated to be 3 nM (Fig. 2).

**Effect of PGE1, IP-, EP2- and EP4-agonist on ICAM-1 expression on isolated monocytes.** The effects of PGE1, IP-, EP2- and EP4-agonist ($10^{-6}$ M) on the expression of ICAM-1 on isolated monocytes were examined (Fig. 3). ONO-1301 as well as PGE1 prevented the expression of ICAM-1 in the presence and absence of IL-18 (100
ng/ml). While EP2- and EP4-agonist inhibited the expression of ICAM-1 in the presence of IL-18, these two agonists did not in the absence of IL-18.

**Effect of ONO-1301 and 11-deoxy-PGE1 on ICAM-1, B7.2 and CD40 expression on human monocytes.** We examined the effects of ONO-1301 and 11-D-PGE1 (EP2/EP4-receptor agonist) on IL-18-induced ICAM-1, B7.2 and CD40 expression (Fig. 4). In the presence (10^{-8} M) and absence of ONO-1301, 11-D-PGE1 (0-10^{-6} M) concentration-dependently suppressed the expression of ICAM-1, B7.2 and CD40. At the concentration (10^{-6} M) of ONO-1301, 11-D-PGE1 had no effect on the expression of these adhesion molecules (Fig. 4A). On the other hand, ONO-1301 (0-10^{-6} M) inhibited the expression of ICAM-1, B7.2 and CD40 in the presence (10^{-8} and 10^{-6} M) and absence of 11-D-PGE1 (Fig. 4B).

**Dose-response relationship of the effects of PGE1 and PGE2 on cytokine responses in PBMC.** The effect of PGE1 and PGE2 (0-10^{-6} M) on the production of IL-12, IFN-γ and IL-10 in PBMC treated with and without IL-18 was determined by ELISA after 24 h of culture (Fig. 5). In the absence of IL-18 stimulus, PGE1 prevented the spontaneous production of IFN-γ and IL-10 in a concentration-dependent manner, but had no effect on the production of IL-12. PGE2 induced the production of IFN-γ without IL-12 production, but inhibited the production of IL-10. The same concentration of PGE1 and PGE2 inhibited IL-12 and IFN-γ production in IL-18-stimulated PBMC, but induced IL-10 production. IC50 value for the inhibitory
effect of PGE₁ on the production of IL-12 induced by IL-18 was estimated to be 5 nM.

Inhibition of IL-18-induced cytokine responses in PBMC by IP- and EP-receptor agonists. We examined the effect of IP-, EP₁-, EP₂-, EP₃- and EP₄-receptor agonist on the production of IL-12, IFN-γ and IL-10 in PBMC in the presence and absence of IL-18 (Fig. 6). While ONO-AE1-259-01 and ONO-AE1-329 induced the production of IFN-γ in the absence of IL-18, ONO-1301 inhibited it. ONO-1301, ONO-AE1-259-01 and ONO-AE1-329 inhibited IL-10 production, but did not affect on IL-12 production. In IL-18-treated PBMC, ONO-1301, ONO-AE1-259-01 and ONO-AE1-329 prevented the production of IL-12 and IFN-γ, but induced IL-10 production. ONO-DI-004 and ONO-AE-248 had no effect on the expression of these cytokines regardless the presence of IL-18 (data not shown).

Effect of ONO-1301 and 11-deoxy-PGE₁ on cytokine responses in PBMC. The effect of ONO-1301 and 11-D-PGE₁ on IL-18-induced IL-12, IFN-γ and IL-10 production was investigated (Fig. 7). In the presence (10⁻⁸ M) and absence of ONO-1301, 11-D-PGE₁ (0-10⁻⁶ M) concentration-dependently inhibited IL-12 and IFN-γ production, but induced IL-10 production. At the concentration (10⁻⁶ M) of ONO-1301, 11-D-PGE₁ did not affect the production of IL-12, IFN-γ and IL-10 (Fig. 7A). In the presence (10⁻⁸ and 10⁻⁶ M) and absence of 11-D-PGE₁, ONO-1301 (0-10⁻⁶ M) inhibited IL-12 and IFN-γ production (Fig. 7B).
DISCUSSION

In vascular endothelial cells (EC), PGE₁ suppressed TNF-α-induced ICAM-1 and VCAM-1 expression, leading to the inhibition of interaction between leukocytes and EC (Weiss et al., 1995; Natori et al., 1997; Iwata et al., 1999). However, little is known about the effect of PGE₁ on the cell-cell interaction between monocytes and T-/NK-cells. In the present study, we found that PGE₁ concentration-dependently inhibited the spontaneous expression of ICAM-1, B7.2 and CD40 on monocytes 24 h after the start of incubation (Fig. 1A). PGE₁ also prevented the expression of ICAM-1, B7.2 and CD40 in the presence of IL-18 (Fig. 1B). Previously, we found that PGE₂ inhibited the IL-18-induced expression of ICAM-1 and B7.2, but had no effect on the expression of ICAM-1, B7.1 and B7.2 in the absence of IL-18 (Takahashi et al., 2002a). The effects of PGE₁ on the spontaneous expression of the three adhesion molecules were in contrast to those of PGE₂.

It was reported that PGE₁ binds to EP₂- and EP₄-receptor (Fan and Chapkin, 1998), whereas earlier studies suggested the existence of distinct receptors for PGE₁ from those for PGE₂ (Datta-Ray et al., 1983; Kanba et al., 1991). The IP-receptor selective agonist ONO-1301, whose affinity for IP-receptor expressed in CHO cells was reported to be almost the same as that of PGE₁ (Narumiya et al., 1999). In the present study, we found that ONO-1301 (IP-receptor agonist) suppressed the expression of ICAM-1, B7.2 and CD40 in the absence of IL-18, however EP-receptor agonists had no effect on these adhesion molecules expression (Fig. 2). ONO-1301, ONO-AE1-259-01 (EP₂-receptor agonist) and ONO-AE1-329 (EP₄-receptor agonist) inhibited IL-18-induced ICAM-1,
B7.2 and CD40 expression (Fig. 2), but ONO-DI-004 (EP1-receptor agonist) and ONO-AE-248 (EP3-receptor agonist) had no effect on the expression of adhesion molecules (data not shown). The affinity of PGE1 for IP-receptor is higher than that for EP2- and EP4-receptor (Narumiya et al., 1999). The inhibitory effect of ONO-1301 (10^{-6} M) on the expression of ICAM-1 showed the significant difference from that of ONO-AE1-259-01 (10^{-6} M) and ONO-AE1-329 (10^{-6} M) (Tukey test). As shown in Fig. 4, increasing concentrations of 11-D-PGE1 had no additive inhibitory effect on the expression of adhesion molecules in the presence of ONO-1301 (10^{-6} M), while ONO-1301 additively inhibited the expression of ICAM-1, B7.2 and CD40 in the presence of 11-D-PGE1 (10^{-6} M). Therefore, the stimulation of IP-receptor might be involved in the effect of PGE1 both in the presence and absence of IL-18, and that the stimulation of EP2- and EP4-receptor might be involved in the effect of PGE1 in the presence of IL-18 as in the case of PGE2 ((Takahashi et al., 2002a) and Fig. 2).

Although the expression of IP-receptor on human monocytes was observed (Li et al., 1997), it remained unclear whether the direct stimulation of IP-receptors on monocytes caused the change in adhesion molecules expression on monocytes in PBMC preparation. In the present study, we found for the first time that the stimulation of IP-receptor on isolated monocytes suppressed the expression of ICAM-1 (Fig. 3).

The effects of exogenous PGE1 and PGE2 on cytokine production in human PBMC stimulated with Con A or LPS were reported (Dooper et al., 2002). The production of TNF-\(\alpha\), IFN-\(\gamma\) and to a lesser extent IL-10 was inhibited by PGE1 and PGE2 in ConA-stimulated PBMC concomitant with unaffected IL-2 levels (Dooper et al., 2002).
In LPS-stimulated PBMC, TNF-α production was inhibited by PGE1 and PGE2, whereas IL-6 remained unaffected and IL-10 production was increased (Dooper et al., 2002). In the previous (Takahashi et al., 2002a) and the present study (Fig. 5), both PGE1 and PGE2 inhibited IL-18-induced IL-12 and IFN-γ production, but induced IL-10 production. In IL-18-treated PBMC, ONO-1301, ONO-AE1-259-01 and ONO-AE1-329 suppressed the production of IL-12 and IFN-γ (Fig. 6), while ONO-DI-004 and ONO-AE-248 did not (data not shown). Therefore, the stimulation of IP-, EP2- and EP4-receptor might contribute to the inhibition of IL-18-elicited cytokine production. In the experiment on the effect of ONO-1301 and 11-D-PGE1 (Fig. 7), ONO-1301 showed a dominant effect on IL-18-elicited cytokine production as well as adhesion molecules. Anti-ICAM-1 and anti-B7.2 antibodies inhibited IL-18-induced IL-12 and IFN-γ production, but induced IL-10 production (Takahashi et al., 2002a). In addition, anti-CD40 antibody had no effect on these cytokines production (data not shown), suggesting that PGE1 might inhibit IL-18-initiated cytokine production through regulating the expression of ICAM-1, B7.2 and CD40 as suggested for PGE2 action ((Takahashi et al., 2002a) and Fig. 5).

It is known that IP-receptor shows a high affinity for PGE1, but not for PGE2 (Narumiya et al., 1999). The biological effects of IP-receptor stimulation included anti-thrombosis (Murata et al., 1997) and vasodilator actions, which have been targeted therapeutically to treat pulmonary hypertension (Tuder et al., 1999; Hoeper et al., 2000). The expression of IP-receptor mRNA has been shown in various mouse organs including neurons, megakaryocytes and the smooth muscles of arteries (Oida et
al., 1995). However, the function of IP-receptor in monocytes remains unknown. The IP-receptor is coupled to Gs and Gq proteins, leading to not only a rise in cAMP levels but also PI responses in CHO cells (Namba et al., 1994). The elevation of cAMP inhibits NF-κB activation in the human monocytic cell line THP-1 (Delgado and Ganea, 2001). Dibutyryl cAMP, a membrane-permeable cAMP analog, inhibited the expression of ICAM-1 and B7.2 on IL-18-treated monocytes, however it had no effect on the expression of ICAM-1 and B7 in the absence of IL-18 (Takahashi et al., 2002a). Thus, there might be another IP-receptor signaling than the regulation of activation of NF-κB by cAMP in the absence of IL-18.

On the other hand, PGE₁ suppressed the production of IFN-γ even in the absence of IL-18, while under the same condition PGE₂ stimulated the production of IFN-γ (Fig. 5). ONO-1301 also inhibited the spontaneous production of IFN-γ, whereas ONO-AE1-259-01 and ONO-AE1-329 induced the production of IFN-γ (Fig. 6), suggesting that the effect of PGE₁ on the production of IFN-γ in the absence of IL-18 might be depend on the stimulation of IP-receptor. PGE₁ is reported to stimulate cAMP production more effectively than PGE₂ (Knudson et al., 1986; Salvatori et al., 1992). Dibutyryl cAMP induced the production of IFN-γ in the absence of IL-18 (data not shown). These results suggested that the regulation of production of IFN-γ by PGE₁ might be in a cAMP independent-manner. Because anti-ICAM-1, anti-B7.2 and anti-CD40 antibodies had no effect on cytokines production in the absence of IL-18 (data not shown), it is not unclear whether the inhibitory effect of ONO-1301 or PGE₁ on IFN-γ production in the absence of IL-18 depends on the
suppression of ICAM-1, B7.2 and CD40 expression.

IL-18 has been considered as a mediator of inflammatory disease such as allo-rejection after organ transplantation, RA or hepatitis (Saha et al., 1999; Affleck et al., 2001; Yumoto et al., 2002). Using a mouse model, recent studies reported that PGE1 reduced ischemia-reperfusion injury following lung transplantation (de Perrot et al., 2001), collagen induced arthritis (Moriuchi-Murakami et al., 2002) and LPS-induced liver injury (Mokuno et al., 1999). In addition to the fact that PGE1 possesses anti-inflammatory properties and the ability to modulate vascular reactivity, PGE1 might have some beneficial therapeutic effects on IL-18-initiated diseases. In conclusion, PGE1 is a potent inhibitor of ICAM-1, B7.2 and CD40 expression as well as IFN-γ production in the presence and absence of IL-18 through the stimulation of IP- and EP2/EP4-receptor. These results implicated that the changes by PGE1 might result in the diminution of IFN-γ-dependent events irrespective of the presence of IL-18, and that the immunomodulatory effects of PGE1 and PGE2 might be distinct in cytokine production. We have compared the effect of autacoids such as PGE2, histamine and epinephrine (Takahashi et al., 2002a; Takahashi et al., 2002b; Takahashi et al., 2003; Nishibori et al., 2003) on the expression of adhesion molecules. PGE1 was shown to be more powerful than PGE2 in exerting anti-inflammatory effects in a rat adjuvant arthritis model (Zurier et al., 1977) and mouse Lupus model (Zurier, 1982). Taking the present findings along with these results, PGE1 might have distinct biological activities from PGE2 as well as histamine and epinephrine. Since the role of endogenous PGE1 in immune response is not well understood, further effects of PGE1 on immune response
should be examined.
Acknowledgments: This study was supported in part by a grant for Promotion of Research from Okayama University (No. 21 to M.N.), a grant from Okayama Medical Foundation (to H.K.T.) and grants from Grant-in-Aid for Scientific Research (C) (15590467 to H.K.T. and 15590228 to M.N.). The authors thank Ono Pharmaceutical Co. (Tokyo, Japan) for generously gifts of PGE1, ONO-1301, ONO-DI-004, ONO-AE1-259-01, ONO-AE-248, ONO-AE1-329 and 11-deoxy-PGE1. The authors thank Ms. Yumiko Shiotani and Ms. Yuki Onoda for excellent technical assistance.
REFERENCES


Knudson PJ, Dinarello CA and Strom TB (1986) Prostaglandins post-transcriptionally


Nature 388:678-682.


Ranger AM, Das MP, Kuchroo VK, and Glimcher LH (1996) B7-2 (CD86) is essential for the development of IL-4-producing T cells. Int Immunol 10:1549-1560.


Figure legends

**Fig. 1** Dose-response relationships for the effects of PGE1 on ICAM-1, B7.2 and CD40 expression on human monocytes.

A) PBMC (1 × 10^6/ml) were incubated with different concentrations (0, 10^{-9}, 10^{-8}, 10^{-7} and 10^{-6} M) of PGE1 for 24 h. At the end of the culture, PBMC (5 × 10^5/ml) were double-stained with antibodies (CD14, ICAM-1, B7.2, CD40 or CMC) as described in Materials and Methods. B) PBMC were incubated with IL-18 (100 ng/ml) and PGE1 for 24 h. The results are the means ± SEM of five donors. *P < 0.05, **P < 0.01 compared with the value in the absence of PGE1.

**Fig. 2** The effects of EP2-, EP4- and IP-receptor agonists on ICAM-1, B7.2 and CD40 expression on human monocytes.

PBMC (1 × 10^6/ml) were incubated with increasing concentrations of ONO-AE1-259-01 (EP2-receptor agonist), ONO-AE1-329 (EP4-receptor agonist) and ONO-1301 (IP-receptor agonist) for 24 h in the presence and absence of IL-18 (100 ng/ml) and stained with antibodies (ICAM-1, B7.2, CD40 or CMC). The results are the means ± SEM of five donors. *P < 0.05, **P < 0.01 compared with the corresponding value in the medium. *P < 0.05, **P < 0.01 compared with the corresponding value in the presence of IL-18 alone. The error bars smaller than the symbol are not shown.

**Fig. 3** The effect of PGE1, IP-, EP2- and EP4-agonist on ICAM-1 expression on isolated monocytes.
Isolated monocytes (1x10⁶/ ml) were incubated with PGE₁, ONO-AE1-259-01 (EP2-receptor agonist), ONO-AE1-329 (EP4-receptor agonist) and ONO-1301 (IP-receptor agonist) (10⁻⁶ M) in the presence and absence of IL-18 (100 ng/ml) for 24 h. The cells were stained with anti-ICAM-1 Ab or CMC. The results are the means ± SEM of five donors. *P < 0.05, **P < 0.01 compared with the corresponding value in the absence of PGE₁, ONO-AE1-259-01, ONO-AE1-329 or ONO-1301.

Fig. 4  The effect of ONO-1301 and 11-deoxy-PGE₁ on IL-18-induced ICAM-1, B7.2 and CD40 expression on human monocytes.

A) PBMC (1x10⁶/ ml) were incubated with increasing concentrations of 11-D-PGE₁ (EP2/EP4-receptor agonist) for 24 h in the presence of IL-18 (100 ng/ml) and three different concentrations (0, 10⁻⁸, 10⁻⁶ M) of ONO-1301 (IP-receptor agonist) and were stained with antibodies (ICAM-1, B7.2 and CD40) or CMC. B) PBMC were incubated with increasing concentrations of ONO-1301 (IP-receptor agonist) for 24 h in the presence of IL-18 and two different concentrations (0, 10⁻⁸, 10⁻⁶ M) of 11-D-PGE₁. The results are the means ± SEM of five donors. *P < 0.05, **P < 0.01 compared with the corresponding value in the presence of IL-18 alone. The error bars smaller than the symbols are not shown.

Fig. 5  The dose-response relationship for the effects of PGE₁ and PGE₂ on cytokine responses in PBMC.

PBMC (1x10⁶ cells / ml) were incubated with different concentrations (0, 10⁻⁹, 10⁻⁸, 10⁻⁷
and $10^{-6}$ M) of PGE1 (A) or PGE2 (B) in the presence and absence of IL-18 (100 ng/ml) for 24 h. At the end of the culture, the levels of IL-12 (p70), IFN-γ and IL-10 in the conditioned media were determined by ELISA. The results are the means ± SEM of five donors. *P < 0.05, **P < 0.01 compared with the corresponding value in the medium. *P < 0.05, **P < 0.01 compared with the corresponding value in the presence of IL-18 alone. The error bars smaller than the symbols are not shown.

**Fig. 6** The effects of EP2-, EP4- and IP-receptor agonists on cytokine production in human PBMC.

PBMC (1x10⁶/ml) were incubated with increasing concentrations of ONO-AE1-259-01 (EP2-receptor agonist), ONO-AE1-329 (EP4-receptor agonist) and ONO-1301 (IP-receptor agonist) for 24 h in the presence and absence of IL-18 (100 ng/ml). At the end of the culture, the levels of IL-12 (p70), IFN-γ and IL-10 in the conditioned media were determined by ELISA. The results are the means ± SEM of five donors. *P < 0.05, **P < 0.01 compared with the corresponding value in the medium. *P < 0.05, **P < 0.01 compared with the corresponding value in the presence of IL-18 alone. The error bars smaller than the symbols are not shown.

**Fig. 7** The effect of ONO-1301 and 11-deoxy-PGE1 on IL-18-induced cytokine production in human PBMC.

A) PBMC (1x10⁶/ml) were incubated with increasing concentrations of 11-D-PGE1 (EP2/EP4-receptor agonist) for 24 h in the presence of IL-18 (100 ng/ml) and two
different concentrations (0, 10^{-8}, 10^{-6} M) of ONO-1301 (IP-receptor agonist). At the end of the culture, the levels of IL-12 (p70), IFN-γ and IL-10 in the conditioned media were determined by ELISA. B) PBMC were incubated with increasing concentrations of ONO-1301 for 24 h in the presence of IL-18 and three different concentrations (0, 10^{-8}, 10^{-6} M) of 11-D-PGE1. The results are the means ± SEM of five donors. *P < 0.05, **P < 0.01 compared with the corresponding value in the presence of IL-18 alone. The error bars smaller than the symbols are not shown.
A) PGE$_1$

B) EP$_2$ agonist

C) EP$_4$ agonist

D) IP agonist