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

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a) **Running title; Unique Effect of PGE1 on ICAM-1, B7.2 and CD40 Expression.**

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Abbreviations: cAMP, cyclic adenosine monophosphate; CD40L, CD40 ligand; CHO, Chinese hamster ovary; CMC, class-matched control ; EC,endothelial cells; 11-D-PGE$_1$, 11-deoxy-PGE$_1$; 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,

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nuclear factor-kappaB ; MHC, histocompatibility complex ; PBMC, peripheral blood mononuclear cells; PE, phycoerythrin; PGE1, prostagrandin 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

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ABSTRACT

In the present study, we examined the effects of Prostagrandin E_1 (PGE_1) 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 PGE_1 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, PGE_1 prevented IL-18-induced expression of ICAM-1, B7.2 and CD40. We examined the involvement of five subtypes of PGE_1 receptors (IP, EP_1, EP_2, EP_3 and EP_4) in the effect of PGE_1 on the expression of these adhesion molecules, using subtype-specific agonists. Among EP-receptor agonists, EP_2- and EP_4-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 PGE_1. The effect of a combination of ONO-1301 and 11-deoxy (D)-PGE_1 (EP_2/EP_4-receptor agonist) on ICAM-1, B7.2 and CD40 expression mimicked that of PGE_1. Moreover, PGE_1 inhibited the production of IL-12 and interferon (IFN)-γ in PBMC in the presence and absence of IL-18, whereas PGE_1 induced IL-10 production. In conclusion, IP-receptor in addition to EP_2/EP_4-receptor played an important role in the action of PGE_1 on the expression of adhesion molecules on monocytes and cytokine production.

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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 phosphatidyl-inositol 3 kinase (PI 3-K) in monocytes and T-cells (Matsumoto et al.,1997; Kojima et al.,1999).

PGE_1 is one of the prostanoids synthesized from linoleic acid in vivo and differs from the products of the arachidonate cascade. The major function of PGE_1 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, EP_1, EP_2, EP_3, EP_4) expressed in cultured Chinese hamster ovary (CHO) cells clearly showed that PGE_1 had high affinity for IP-receptor in addition to EP_1-, EP_2-, EP_3- and EP_4- receptors, whereas PGE_2 had a high affinity for FP-, EP_1-, EP_2-, EP_3- and EP_4- receptors (Narumiya et al.,1999). The EP_2- and EP_4-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

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couple with multiple signaling pathways including PI response and Ca^{2+} mobilization (Namba et al.,1994). In fact, PGI_2, an IP-receptor agonist, has been demonstrated to induce the elevation of free Ca^{2+} concentration in several cultured cell lines (Watanabe et al.,1991). Despite the clear difference in the receptor activation profile of PGE_1 and PGE_2, there is little information about the action characteristics of PGE_1 on particular immune responses. Previously, we reported that PGE_2 inhibited IL-18-induced expression of ICAM-1 and B7.2 on human monocytes through the stimulation of EP_2- and EP_4-receptors (Takahashi et al.,2002a). These effects of PGE_2 on adhesion molecules in turn modulated the production of IL-12, tumor necrosis factor (TNF)- α and IFN-γ in PBMC (Takahashi et al.,2002a), however, little is known about the pharmacological action of PGE_1 on adhesion molecules expression on monocytes, and the differences between the effects of PGE_1 and PGE_2 on the cell-cell interaction and cytokine production profiles.

In the present study, we examined the effect of PGE_1 on the expression of ICAM-1, B7.1, B7.2, CD40 and CD40L as well as the production of IL-12, IFN-γ and IL-10 in human PBMC in the presence or absence of IL-18 to clarify a functional role of PGE_1 and the differences between PGE_1 and PGE_2 using prostaglandin receptor subtype-selective agonists. Interestingly, we found that PGE_1 had a distinct action profile to that of PGE_2. We also found that the stimulation of IP-receptor had a unique effect on adhesion molecules expression and cytokines production.

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MATERIALS and METHODS

Reagents and drugs. Recombinant human (rh) IL-18 was purchased from MBL (Nagoya, Japan). PGE_1, ONO-1301, ONO-DI-004, ONO-AE1-259-01, ONO-AE-248, ONO-AE1-329 and 11-deoxy (D)-PGE_1 were kindly provided by Ono Pharmaceutical Co. Ltd. (Tokyo, Japan). For flow cytometric analysis, FITC-conjugated mouse IgG_1 mAb against ICAM-1/CD54 (6.5B5) and PE-conjugated anti-CD14 mAb were purchased from DAKO (Glostrup, Denmark). FITC-conjugated mouse IgG_1 mAb against B7.1 (MAB104) was purchased from IMMUNOTECH (Marseille, France). FITC-conjugated mouse IgG_1 mAb against B7.2 (2331FUN-1) and CD40 (5C3) were purchased from Pharmingen (SanDiego, CA). FITC-conjugated mouse IgG_1 mAb against CD40L/CD154 was purchased from Ancel (Bayport, MN). FITC-conjugated MOPC 21, an IgG_1 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)

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heat-inactivated fetal calf serum.

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^oC 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, PGE_1, IP- and EP-receptor agonists for 24 h at $37\,^oC$ in a 5% CO_2 / 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%

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NaN_3, 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 1 μg 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 ($1x10^6$ cells/ml) were incubated with PGE_1, PGE_2, IP- and EP-receptor agonists in the presence or absence of IL-18 for 24 hours at 37℃ in a humidified atmosphere of 5% CO_2 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.

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RESULTS

Dose-response relationship of the effects of PGE_1 on ICAM-1, B7.1, B7.2, CD40 and CD40L expression on human monocytes. The effects of PGE_1 ($0\text{-}10^{-6}$ 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). PGE_1 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). IC_{50} values for the inhibitory effect of PGE_1 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. PGE_1 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). IC_{50} values for the inhibitory effect of PGE_1 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 PGE_1 receptor subtypes (IP, EP_1, EP_2, EP_3, EP_4) are involved in the effects of PGE_1 on ICAM-1, B7.1 B7.2, CD40 and CD40L expression, we examined the effects of EP-receptor agonists ($0\text{-}10^{-6}$ M) on ICAM-1, B7.1 B7.2, CD40 and CD40L expression on monocytes in the presence and

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absence of IL-18 (100 ng/ml) after 24 h incubation of PBMC (Fig. 2). ONO-DI-004 (EP_1-receptor agonist) (Suzawa et al.,2000; Kitagawa et al.,2001) and ONO-AE-248 (EP_3-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 (EP_2-receptor agonist) and ONO-AE1-329 (EP_4-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). IC_{50} 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). IC_{50} value for the inhibitory effect of ONO-1301 on the expression of ICAM-1 was estimated to be 3 nM (Fig. 2).

Effect of PGE_1, IP-, EP_2- and EP_4-agonist on ICAM-1 expression on isolated monocytes. The effects of PGE_1, IP-, EP_2- and EP_4-agonist (10^{-6} M) on the expression of ICAM-1 on isolated monocytes were examined (Fig. 3). ONO-1301 as well as PGE_1 prevented the expression of ICAM-1 in the presence and absence of IL-18 (100

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ng/ml). While EP_2- and EP_4-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-PGE_1 on ICAM-1, B7.2 and CD40 expression on human monocytes. We examined the effects of ONO-1301 and 11-D-PGE_1 (EP_2/EP_4-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-PGE_1 (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-PGE_1 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-PGE_1 (Fig. 4B).

Dose-response relationship of the effects of PGE_1 and PGE_2 on cytokine responses in PBMC. The effect of PGE_1 and PGE_2 (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, PGE_1 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. PGE_2 induced the production of IFN-γ without IL-12 production, but inhibited the production of IL-10. The same concentration of PGE_1 and PGE_2 inhibited IL-12 and IFN-γ production in IL-18-stimulated PBMC, but induced IL-10 production. IC_{50} value for the inhibitory

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effect of PGE_1 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-, EP_1-, EP_2-, EP_3- and EP_4-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 □□ 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 effect 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-PGE_1 on cytokine responses in PBMC. The effect of ONO-1301 and 11-D-PGE_1 on IL-18-induced IL-12, IFN-γand IL-10 production was investigated (Fig. 7). In the presence (10^{-8} M) and absence of ONO-1301, 11-D-PGE_1 (0-10^{-6} M) concentration-dependently inhibited IL-12 and IFN-γproduction, but induced IL-10 production. At the concentration (10^{-6} M) of ONO-1301, 11-D-PGE_1 did not affect the production of IL-12, IFN-γand IL-10 (Fig. 7A). In the presence (10^{-8} and 10^{-6} M) and absence of 11-D-PGE_1, ONO-1301 (0-10^{-6} M) inhibited IL-12 and IFN-γproduction (Fig. 7B).

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DISCUSSION

In vascular endothelial cells (EC), PGE_1 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 PGE_1 on the cell-cell interaction between monocytes and T-/NK-cells. In the present study, we found that PGE_1 concentration-dependently inhibited the spontaneous expression of ICAM-1, B7.2 and CD40 on monocytes 24 h after the start of incubation (Fig. 1A). PGE_1 also prevented the expression of ICAM-1, B7.2 and CD40 in the presence of IL-18 (Fig. 1B). Previously, we found that PGE_2 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 PGE_1 on the spontaneous expression of the three adhesion molecules were in contrast to those of PGE_2.

It was reported that PGE_1 binds to EP_2- and EP_4-receptor (Fan and Chapkin,1998), whereas earlier studies suggested the existence of distinct receptors for PGE_1 from those for PGE_2 (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 PGE_1 (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 (EP_2-receptor agonist) and ONO-AE1-329 (EP_4-receptor agonist) inhibited IL-18-induced ICAM-1,

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B7.2 and CD40 expression (Fig. 2), but ONO-DI-004 (EP_1-receptor agonist) and ONO-AE-248 (EP_3-receptor agonist) had no effect on the expression of adhesion molecules (data not shown). The affinity of PGE_1 for IP-receptor is higher than that for EP_2- and EP_4-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-PGE_1 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-PGE_1 (10^{-6} M). Therefore, the stimulation of IP-receptor might be involved in the effect of PGE_1 both in the presence and absence of IL-18, and that the stimulation of EP_2- and EP_4-receptor might be involved in the effect of PGE_1 in the presence of IL-18 as in the case of PGE_2 ((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 PGE_1 and PGE_2 on cytokine production in human PBMC stimulated with Con A or LPS were reported (Dooper et al.,2002). The production of TNF-α, IFN-γ and to a lesser extent IL-10 was inhibited by PGE_1 and PGE_2 in ConA-stimulated PBMC concomitant with unaffected IL-2 levels (Dooper et al.,2002).

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In LPS-stimulated PBMC, TNF-α production was inhibited by PGE_1 and PGE_2, 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 PGE_1 and PGE_2 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-, EP_2- and EP_4-receptor might contribute to the inhibition of IL-18-elicited cytokine production. In the experiment on the effect of ONO-1301 and 11-D-PGE_1 (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 PGE_1 might inhibit IL-18-initiated cytokine production through regulating the expression of ICAM-1, B7.2 and CD40 as suggested for PGE_2 action ((Takahashi et al.,2002a) and Fig. 5).

It is known that IP-receptor shows a high affinity for PGE_1, but not for PGE_2 (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

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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, PGE_1 suppressed the production of IFN-γ even in the absence of IL-18, while under the same condition PGE_2 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 PGE_1 on the production of IFN-γ in the absence of IL-18 might be depend on the stimulation of IP-receptor. PGE_1 is reported to stimulate cAMP production more effectively than PGE_2 (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 PGE_1 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 PGE_1 on IFN-γ production in the absence of IL-18 depends on the

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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 PGE_1 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 PGE_1 possesses anti-inflammatory properties and the ability to modulate vascular reactivity, PGE_1 might have some beneficial therapeutic effects on IL-18-initiated diseases. In conclusion, PGE_1 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 EP_2/EP_4-receptor. These results implicated that the changes by PGE_1 might result in the diminution of IFN-γ-dependent events irrespective of the presence of IL-18, and that the immunomodulatory effects of PGE_1 and PGE_2 might be distinct in cytokine production. We have compared the effect of autacoids such as PGE_2, 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. PGE_1 was shown to be more powerful than PGE_2 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, PGE_1 might have distinct biological activities from PGE_2 as well as histamine and epinephrine. Since the role of endogenous PGE_1 in immune response is not well understood, further effects of PGE_1 on immune response

should be examined.

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Figure legends

Fig. 1 Dose-response relationships for the effects of PGE_1 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 PGE_1 for 24 h. At the end of the culture, PBMC ($5x10^5$/ 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 PGE_1 for 24 h. The results are the means $\pm$ SEM of five donors. *$P < 0.05$, **$P < 0.01$ compared with the value in the absence of PGE_1.

Fig. 2 The effects of EP_2-, EP_4- and IP-receptor agonists on ICAM-1, B7.2 and CD40 expression on human monocytes.

PBMC ($1x10^6$/ ml) were incubated with increasing concentrations of ONO-AE1-259-01 (EP_2-receptor agonist), ONO-AE1-329 (EP_4-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 $\pm$ 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 PGE_1, IP-, EP_2- and EP_4-agonist on ICAM-1 expression on isolated monocytes.

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Isolated monocytes ($1x10^6$/ ml) were incubated with PGE_1, ONO-AE1-259-01 (EP_2-receptor agonist), ONO-AE1-329 (EP_4-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 $\pm$ SEM of five donors. *P < 0.05, **P < 0.01 compared with the corresponding value in the absence of PGE_1, ONO-AE1-259-01, ONO-AE1-329 or ONO-1301.

Fig. 4 The effect of ONO-1301 and 11-deoxy-PGE_1 on IL-18-induced ICAM-1, B7.2 and CD40 expression on human monocytes.

A) PBMC ($1x10^6$/ ml) were incubated with increasing concentrations of 11-D-PGE_1 (EP_2/ EP_4-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-PGE_1. The results are the means $\pm$ 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 PGE_1 and PGE_2 on cytokine responses in PBMC.

PBMC ($1x10^6$ cells / ml) were incubated with different concentrations (0, 10^{-9}, 10^{-8}, 10^{-7}

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and 10^{-6} M) of PGE_1 (A) or PGE_2 (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 EP_2-, EP_4- and IP-receptor agonists on cytokine production in human PBMC.

PBMC ($1x10^6$/ ml) were incubated with increasing concentrations of ONO-AE1-259-01 (EP_2-receptor agonist), ONO-AE1-329 (EP_4-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-PGE_1 on IL-18-induced cytokine production in human PBMC..

A) PBMC ($1x10^6$/ ml) were incubated with increasing concentrations of 11-D-PGE_1 (EP_2/ EP_4-receptor agonist) for 24 h in the presence of IL-18 (100 ng/ml) and two

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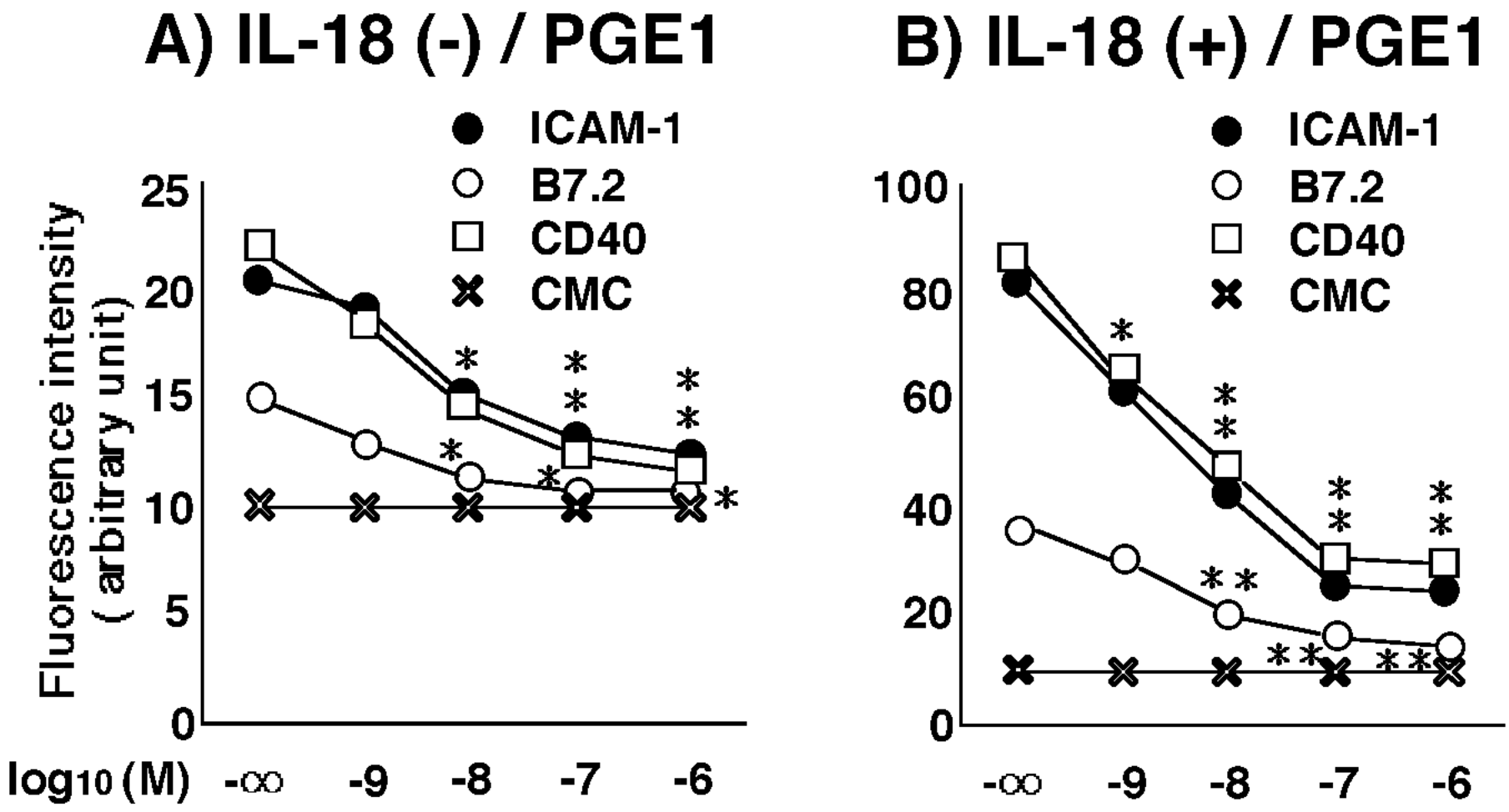
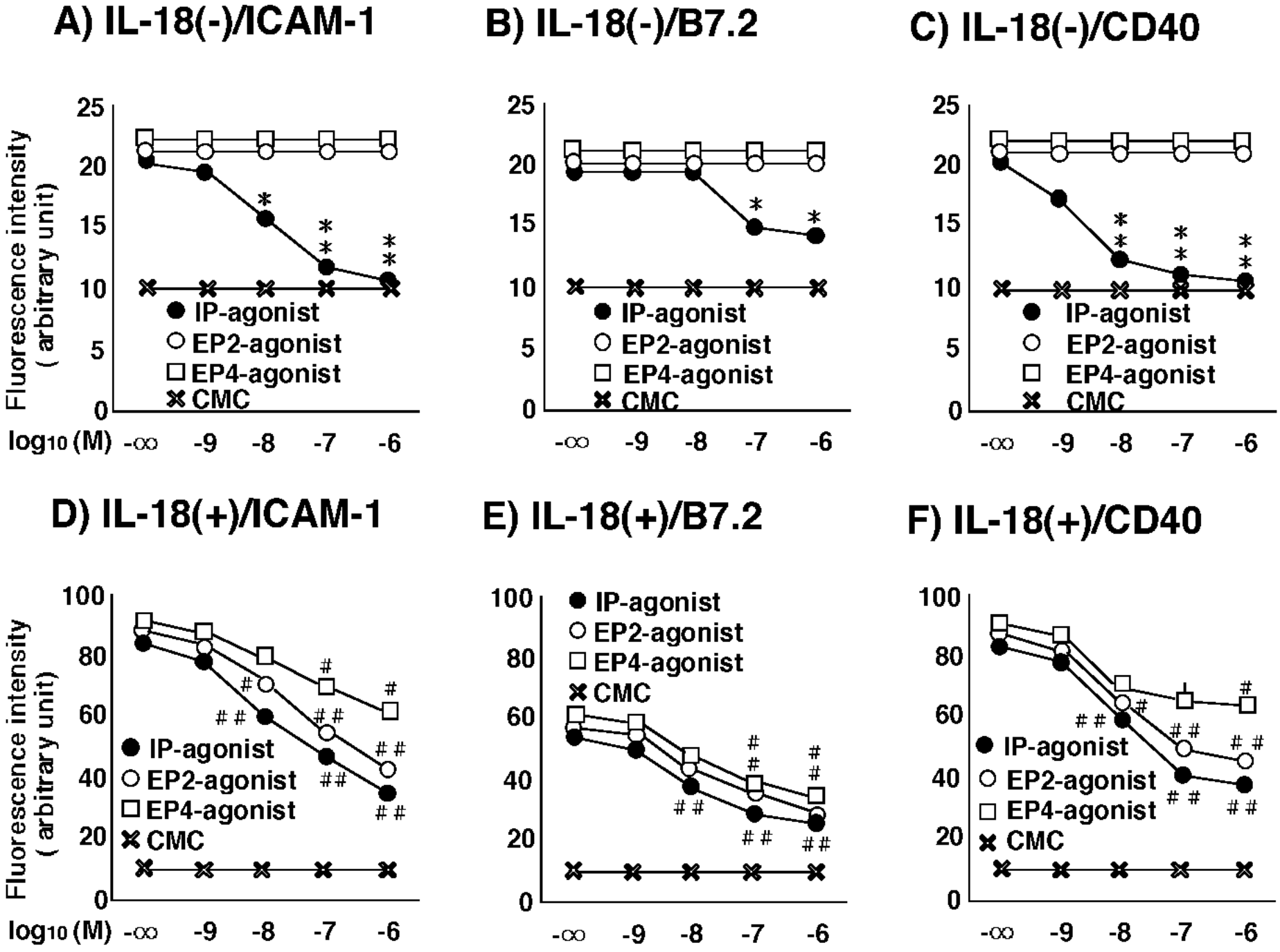
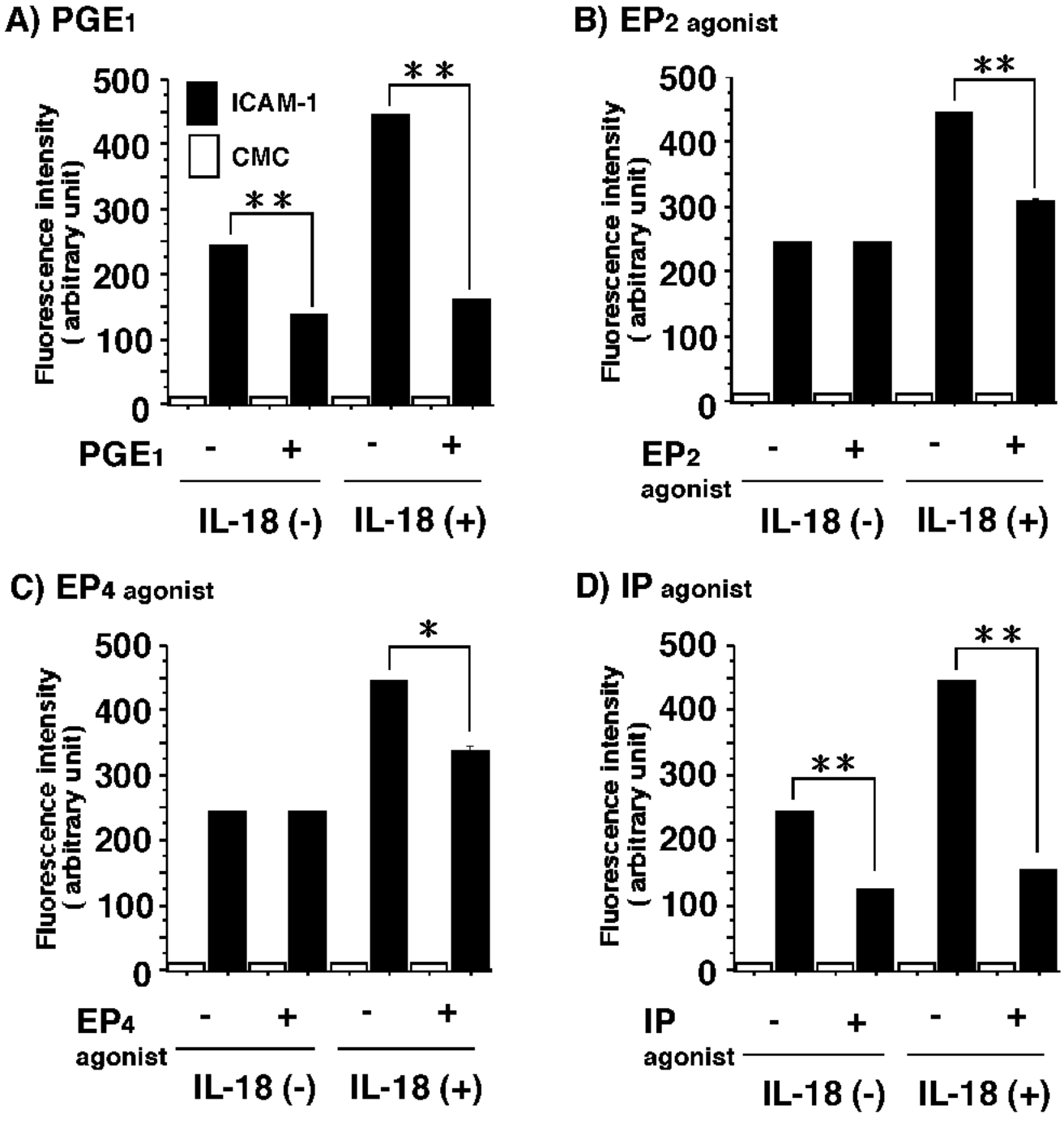
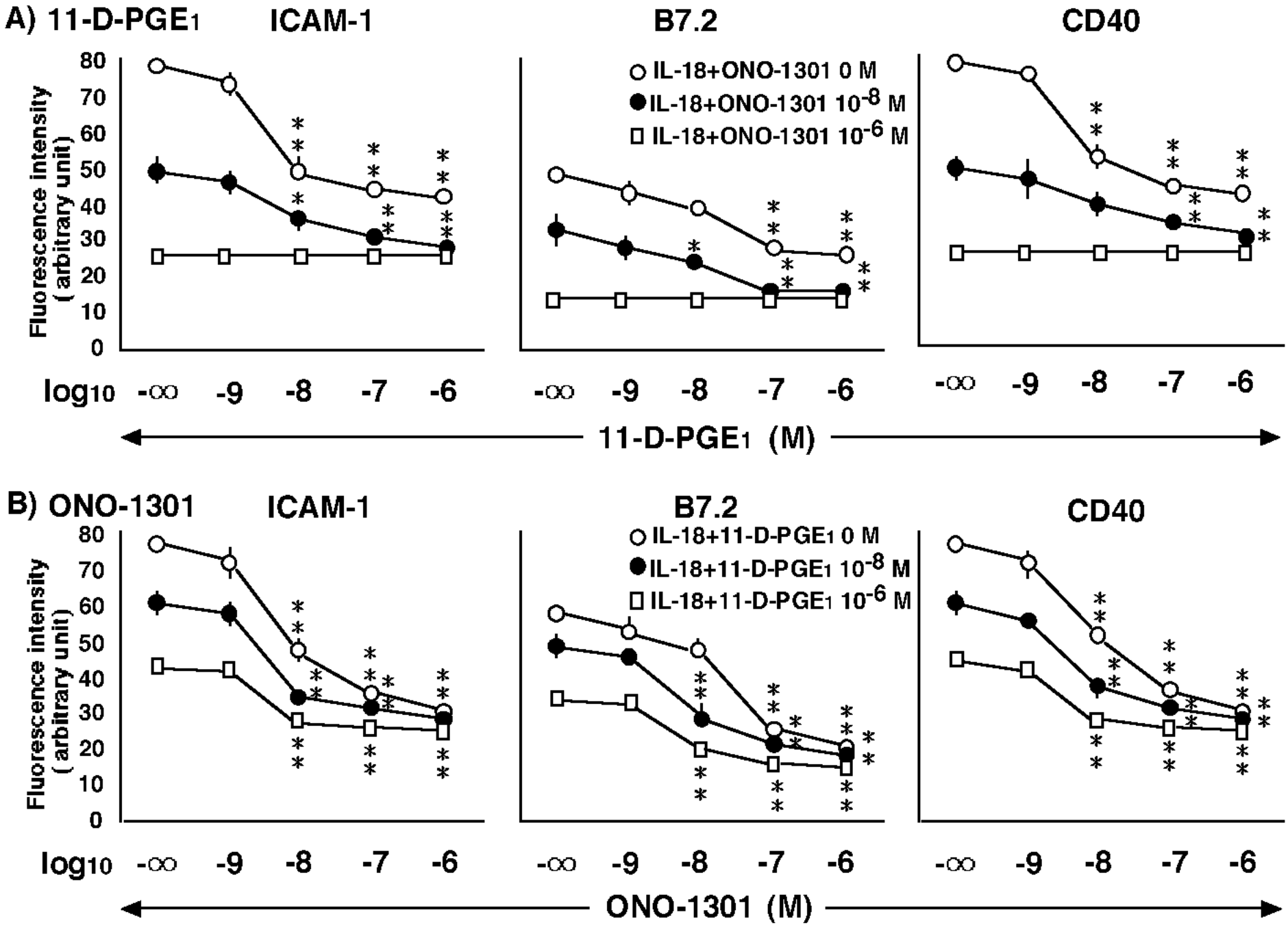
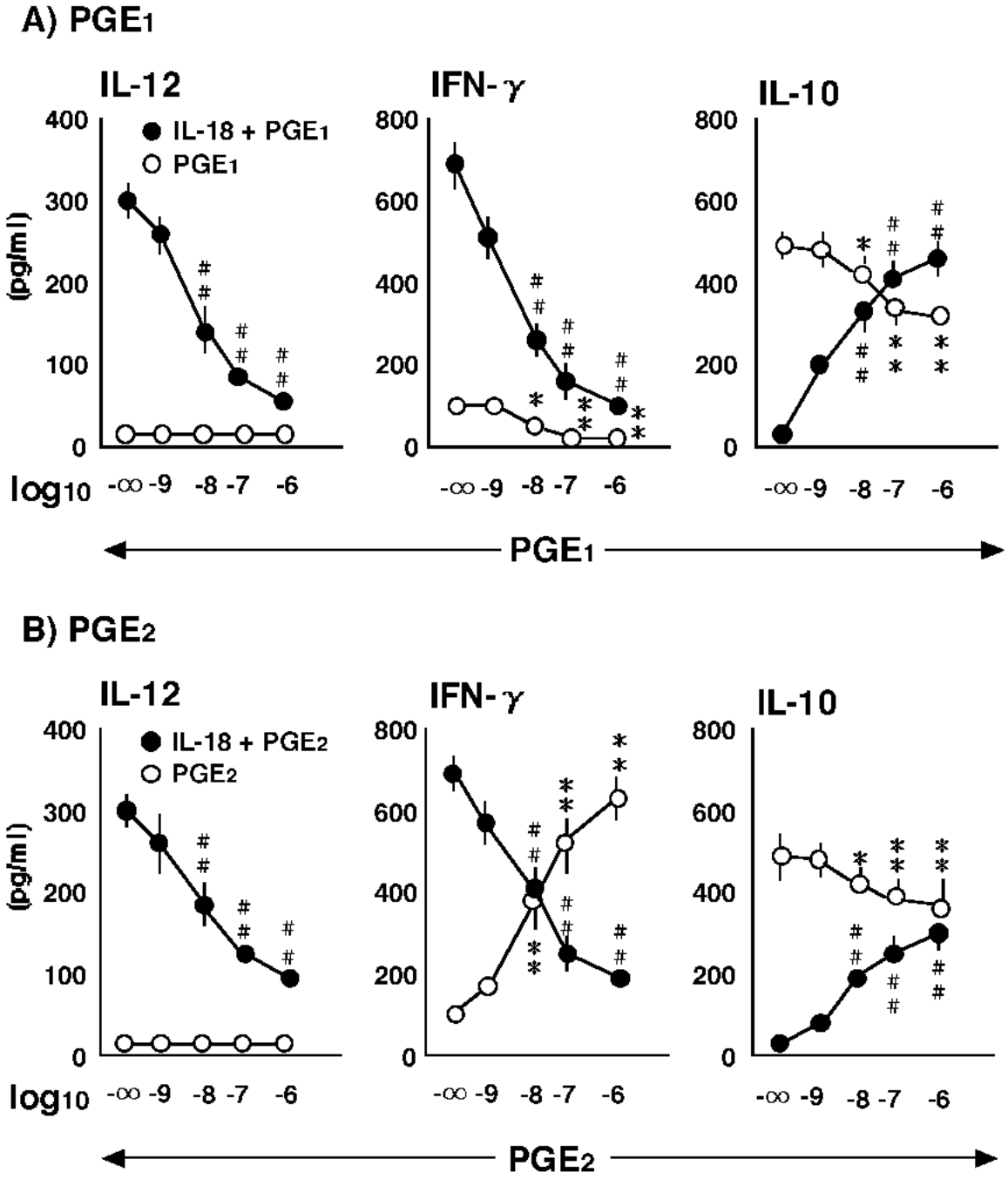
different concentrations (0, 10^{-8}, 10^{-6} M) of ONO-1301 (IP-receptor agonist). At the end of the culture, the levels of IL-12 (p70), IFN-γ and IL-10 in the conditioned media were determined by ELISA. B) PBMC were incubated with increasing concentrations of ONO-1301 for 24 h in the presence of IL-18 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.

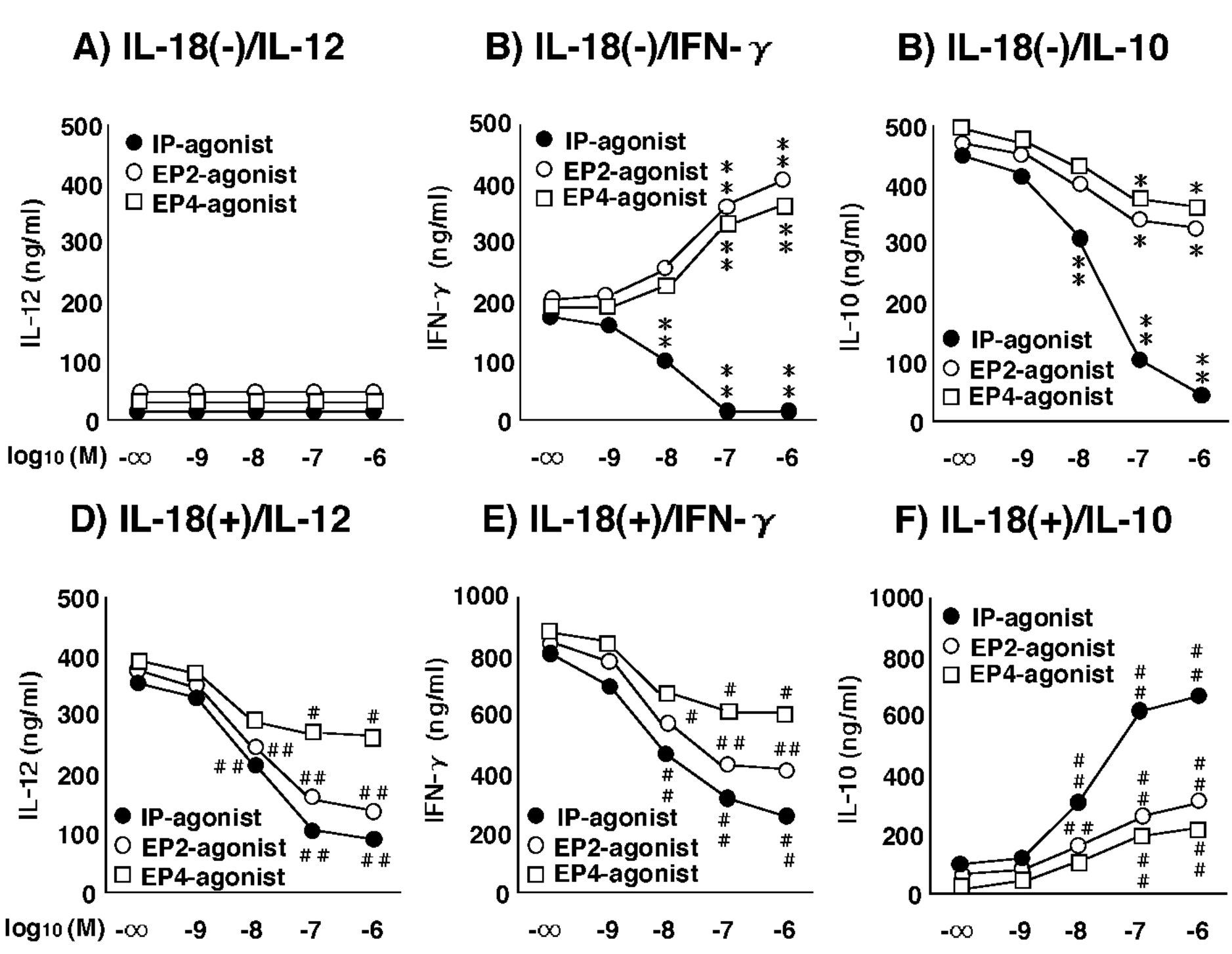

A) IL-18 (-) / PGE1
B) IL-18 (+) / PGE1
ICAM-1
B7.2
CD40
CMC
Fluorescence intensity
(arbitrary unit)
25
20
15
10
5
0
100
80
60
40
20
0
log10 (M)
-∞
-9
-8
-7
-6

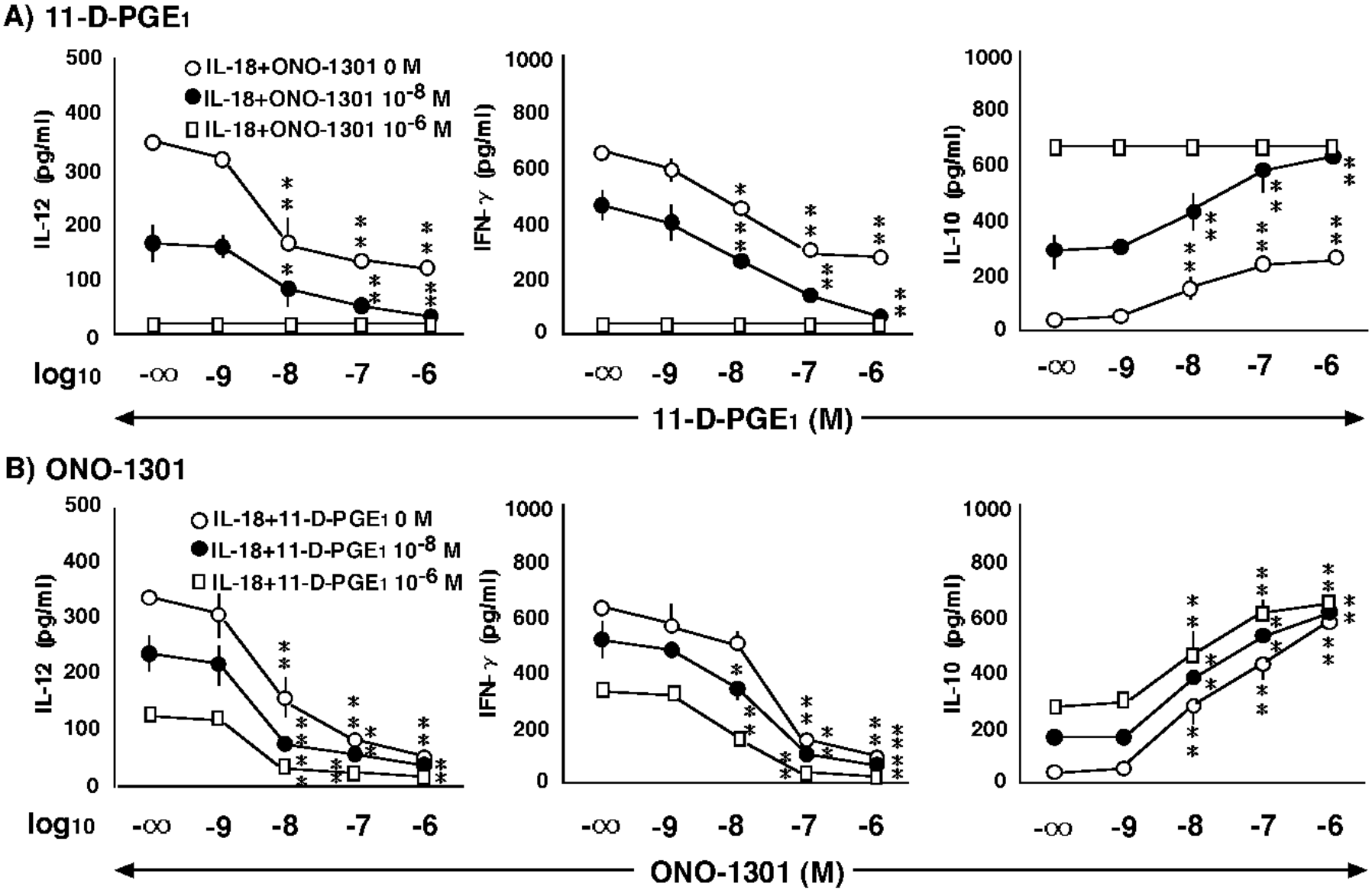

A) IL-18(-)/ICAM-1
B) IL-18(-)/B7.2
C) IL-18(-)/CD40
D) IL-18(+)/ICAM-1
E) IL-18(+)/B7.2
F) IL-18(+)/CD40
Fluorescence intensity
(arbitrary unit)
log10 (M)
-∞
-9
-8
-7
-6
IP-agonist
EP2-agonist
EP4-agonist
CMC


A) PGE1
ICAM-1
CMC
Fluorescence intensity (arbitrary unit)
500
400
300
200
100
0
**
**
PGE1 - + - +
IL-18 (-) IL-18 (+)
B) EP2 agonist
**
EP2 agonist - + - +
IL-18 (-) IL-18 (+)
C) EP4 agonist
*
EP4 agonist - + - +
IL-18 (-) IL-18 (+)
D) IP agonist
**
**
IP agonist - + - +
IL-18 (-) IL-18 (+)


A) 11-D-PGE1
ICAM-1
B7.2
CD40
IL-18+ONO-1301 0 M
IL-18+ONO-1301 10-8 M
IL-18+ONO-1301 10-6 M
Fluorescence intensity (arbitrary unit)
80
70
60
50
40
30
20
10
0
log10 -∞ -9 -8 -7 -6
11-D-PGE1 (M)
B) ONO-1301
ICAM-1
B7.2
CD40
IL-18+11-D-PGE1 0 M
IL-18+11-D-PGE1 10-8 M
IL-18+11-D-PGE1 10-6 M
Fluorescence intensity (arbitrary unit)
log10 -∞ -9 -8 -7 -6
ONO-1301 (M)


A) PGE1
IL-12
IFN-γ
IL-10
IL-18 + PGE1
PGE1
(pg/ml)
400
300
200
100
0
800
600
400
200
0
log10
-∞ -9 -8 -7 -6
PGE1
B) PGE2
IL-12
IFN-γ
IL-10
IL-18 + PGE2
PGE2
(pg/ml)
log10
-∞ -9 -8 -7 -6
PGE2


A) IL-18(-)/IL-12
B) IL-18(-)/IFN-γ
B) IL-18(-)/IL-10
D) IL-18(+)/IL-12
E) IL-18(+)/IFN-γ
F) IL-18(+)/IL-10
IP-agonist
EP2-agonist
EP4-agonist
IL-12 (ng/ml)
IFN-γ (ng/ml)
IL-10 (ng/ml)
log10 (M)
-∞
-9
-8
-7
-6
0
100
200
300
400
500
600
800
1000
*
#


A) 11-D-PGE1
IL-18+ONO-1301 0 M
IL-18+ONO-1301 10-8 M
IL-18+ONO-1301 10-6 M
IL-12 (pg/ml)
IFN-γ (pg/ml)
IL-10 (pg/ml)
log10 -∞ -9 -8 -7 -6
11-D-PGE1 (M)
B) ONO-1301
IL-18+11-D-PGE1 0 M
IL-18+11-D-PGE1 10-8 M
IL-18+11-D-PGE1 10-6 M
IL-12 (pg/ml)
IFN-γ (pg/ml)
IL-10 (pg/ml)
log10 -∞ -9 -8 -7 -6
ONO-1301 (M)