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Synergistic effect of PGD_2 via prostanoid DP receptor on TNF-α-induced production of MCP-1 and IL-8 in human monocytic THP-1 cells

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Abstract

Prostaglandin (PG) D_2, a major cyclooxygenase metabolite generated predominantly from immunologically stimulated mast cells, is thought to contribute to the pathogenesis of allergic diseases via the two PGD_2 receptors, prostanoid DP receptor and chemoattractant receptor homologous molecule expressed on Th2 cells (CRTH2). Monocytes are known to express the prostanoid DP receptor, however, the role of it in inflammatory responses is still unclear. In the present study, to clarify the functional roles of prostanoid DP receptor on monocytes, we examined the effect of PGD_2 on the production of monocyte chemoattractant protein (MCP)-1 and interleukin (IL)-8 from a human monocytic cell line, THP-1. Single activation of prostanoid DP receptor hardly produced any cytokines or chemokines. However, activation with PGD_2 in the presence of tumor necrosis factor (TNF)-α mediated significant production of MCP-1 and IL-8, but not the other cytokines and chemokines, in comparison to single stimulation with TNF-α. In addition, the selective prostanoid DP receptor antagonist, pinagladin ((*Z*)-7-[(1*R*,2*R*,3*S*,5*S*)-2-(benzothiophen-3-ylcarbonylamide)-10-norpinan-3-yl]hept-5-enoic acid) inhibited the production of MCP-1 and IL-8 upon combined stimulation with PGD_2 and TNF-α. The synergistic production of MCP-1 and IL-8 by PGD_2 was mimicked by dibutyryl cAMP (db-cAMP) and was inhibited by a protein kinase A (PKA) inhibitor. Our findings suggest that activation of the prostanoid DP receptor on THP-1 cells enhances TNF-α-induced MCP-1 and IL-8 production via the cAMP/PKA signaling pathway.

Keywords: cAMP; PGD_2; Pinagladin; PKA; Prostanoid DP receptor; THP-1 cell; TNF-α

1. Introduction

Prostaglandin (PG) D_2 is a major cyclooxygenase metabolite produced mainly by mast cells responding to various allergic stimuli (Lewis et al., 1982). Local allergen challenge in patients with allergic rhinitis (Naclerio et al., 1983, 1985), bronchial asthma (Murray et al., 1986), allergic conjunctivitis (Proud et al., 1990), and atopic dermatitis (Charlesworth et al., 1991) has been shown to induce rapid elevation of PGD_2 in nasal and bronchial lavage fluids, tears and skin chamber fluids. PGD_2 has been reported to exert a variety of inflammatory responses such as increased vascular permeability in the conjunctiva and skin (Flower et al., 1976; Woodward et al., 1990), increased nasal airway resistance (Doyle et al., 1990), airway narrowing (Johnston et al., 1995), and eosinophil infiltration into the conjunctiva and trachea (Emery et al., 1989; Woodward et al., 1996).

So far, PGD_2 has been reported to bind to two different functional receptors, prostanoid DP receptor and chemoattractant receptor homologous molecule expressed on Th2 cells (CRTH2) (Hirai et al., 2001). Prostanoid DP receptor is a Gs-protein-coupled rhodopsin-type receptor with seven transmembrane domains. Various studies on signal transduction have demonstrated that the stimulation of prostanoid DP receptor activates adenylate cyclase, increases the level of cAMP (Hirata et al., 1994; Boie et al., 1995; Ito et al., 1989), activates protein kinase A (PKA), and then subsequently activates the intracellular signaling pathway. In prostanoid DP receptor-deficient mice, ovalbumin-induced airway hyperreactivity, accumulation of lymphocytes and eosinophils in the bronchial alveolar lavage fluid, and production of Th2 cytokines are reduced (Matsuoka

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et al., 2000). In addition, we have previously demonstrated that prostanoid DP receptor antagonist S-5751 dramatically inhibits early and late nasal responses and eosinophil infiltration in an allergic rhinitis model, plasma exudation in an allergic conjunctivitis model, and eosinophil infiltration into the lung in allergic asthma in guinea pigs (Arimura et al., 2001). These results suggest that the prostanoid DP receptor plays a critical role in allergic diseases.

The mRNA for the prostanoid DP receptor has been reported to be upregulated in the lung after ovalbumin challenge in mice (Fujitani et al., 2002). With resting cells, the prostanoid DP receptor is known to be expressed on eosinophils (Gervais et al., 2001), dendritic cells and monocytes (Gosset et al., 2003). An anti-apoptotic effect on eosinophils (Gervais et al., 2001), and an induction of Th2 polarization caused by the activation of dendritic cells, (Gosset et al., 2003) have been reported to be mediated via the prostanoid DP receptor. In contrast to eosinophils and dendritic cells, its functional role on monocytes remains unclear.

In the present study, we investigated the role of the prostanoid DP receptor on the production of chemokines and cytokines from the THP-1 monocytic cell line, and showed that PGD_2 enhanced production of MCP-1 and IL-8, but not the other cytokines or chemokines, in the presence of TNF-α via the prostanoid DP receptor. We also found that these enhancements were induced via the cAMP-PKA signaling pathway.

2. Materials and methods

2.1. Reagents

Pinagladin was synthesized at Shionogi & Co., LTD. (Osaka, Japan). PGD_2 and 3-isobutyl-1-methylxanthine (IBMX) were obtained from Sigma-Aldrich (St Louis, MO, USA). Human rTNF-α (Peprotech EC, London, UK), (4*S*)-(3-[(3*R*,*S*)-3-cyclohexyl-3-hydroxypropyl]-2,5-dioxo)-4-imidazolidineheptanoic acid (BW245C; Cayman Chemicals, Ann Arbor, MI, USA), *N*-[2-[*P*-Bromocinnamylamino]-ethyl)-5-isoquinolinesulfonmide (H89; Wako Pure Chemical Industries, Osaka, Japan), Fluo-3/AM (Dojindo Laboratories, Kumamoto, Japan) and F-127 (AnaSpec, San Jose, CA, USA) were purchased.

2.2. Cell culture

THP-1 cells, a human monocytic leukemia cell line, were obtained from the American Type Culture Collection (Manassas, VA, USA). The cells were cultured in culture medium (RPMI 1640 supplemented with 10 mM HEPES, 1.0 mM sodium pyruvate, supplemented with 0.05 mM 2-mercaptoethanol, 100 U/ml penicillin, 100 μg/ml streptomycin and 10% heat-inactivated fetal calf serum) at 37 °C in a humidified atmosphere of 5% CO_2. For the experiments, THP-1 cells were washed twice and resuspended in culture medium, then dispensed into 48-well plates at 4×10^5 cells/well. To stimulate THP-1 cells, various stimulants were added to the culture medium in the presence or absence of pinagladin or H89. After 24 h incubation at 37 °C in a humidified atmosphere of 5% CO_2, the cell supernatants were collected and stored in a −20 °C freezer until biological assay.

2.3. cAMP assay of THP-1 cells for prostanoid DP receptors

THP-1 cells were pretreated with 0.5 mM IBMX for 5 min, and then treated with 10 μM pinagladin. After incubation for 10 min, THP-1 cells were stimulated with PGD_2, or BW245C (an agonist for prostanoid DP receptor) for 10 min. The reaction was stopped by the addition of 1 N HCl, and the cells were disrupted with 1.2% Triton X (Nacalai Tesque, Kyoto, Japan). The supernatants were collected, and the amount of cAMP was measured by cAMP femto 2 (Cisbio International, Bagnols/Ceze, France).

2.4. Reverse transcriptase (RT)-PCR

Prostanoid DP receptor gene expression in THP-1 cells was detected by RT-PCR. Total RNA was obtained by using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) and reverse transcribed using random hexamer primers in the presence (negative control reaction) of superscript reverse transcriptase III (Invitrogen Life Technologies). cDNAs synthesized by RT were amplified with Takara Ex Taq (Takara Bio, Shiga, Japan) and oligonucleotide primers specific for human prostanoid DP receptor (sense, 5′-CTGGCTGCCTACGCTCAGA-3′, and anti-sense, 5′-AGCCCAAAGAAGGACATGAAGA-3′) and human β-actin (sense, 5′-CACGGCATCGTCACCAACT-3′, and anti-sense, 5′-TGATCTGGGTCATCTTCTCGC-3′). The PCR included a denaturation step (98 °C for 1 min) and 40 cycles for prostanoid DP receptor, or 30 cycles for β-actin (denaturation, 98 °C for 10 s; annealing, 58 °C for 45 s; extension, 72 °C for 1 min) and a extension step (72 °C for 5 min) using DNA Engine Tetrad (GMI, Ramsey, MN, USA). As a positive control, reaction of prostanoid DP receptor primer set for prostanoid DP receptor cDNAs in pcDNA3.1+expression vectors (constructed at Shionogi) were amplified by PCR.

2.5. Protein array

Supernatants of stimulated THP-1 cells were collected 24 h after incubation. The amounts of IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 p70, IL-13, IL-15, IL-17, eotaxin, FGF basic, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF bb, RANTES, TNF-α, and VEGF were determined with the Bio-Plex Multiplex Human Cytokine 27-plex assay kit (Bio-Rad Laboratories, Hercules, CA, USA) and the Cytokine Reagent kit (Bio-Rad Laboratories), according to the manufacturer's protocol. Briefly, 50 μl of the culture supernatants or cytokine standards were plated in a 96-well filter plate coated with a multiplex of beads coupled to antibodies, and incubated for 30 min at room temperature. After a series of washings, a mixture of biotinylated detection antibodies was added to the reaction. Streptavidin-phycoerythrin was then added to bind to the biotinylated detection antibodies on the bead surface. The data from the reaction were

collected and analyzed by using the Bio-Plex suspension array system (Bio-Rad Laboratories). The concentration of TNF-α was excluded from the graph, because TNF-α was used as stimulator in this study. The amount of cytokines except for IL-5 and chemokines detected was higher that the detection limits in the Lot (#500-2617) of assay kit used in this study.

2.6. Immunoassays

To examine the response of PGD_2 or BW245C or db-cAMP on the production of MCP-1 and IL-8, and the effect of pinagladin or H-89 on their production, the amounts of MCP-1 and IL-8 in the cell supernatants were measured using ELISA kits (R&D Systems, Minneapolis, MN, USA).

2.7. Statistical analysis

The data were expressed as means±S.E.M. Statistical significance of the data was assessed by means of Dunnett's test for multiple comparison, or Student's or Welch's *t* test for comparison between two groups.

3. Results

3.1. Expression of mRNA and functional second signal for prostanoid DP receptor in THP-1 cells

At first, RT-PCR was performed to observe the expression of prostanoid DP receptor transcripts in THP-1 cells. As shown in Fig. 1A, the expression of prostanoid DP receptor mRNA was observed in THP-1 cells. We then performed the cAMP assay by stimulation of prostanoid DP receptor agonists in THP-1 cells to examine whether or not they have functionally active receptors. As shown in Fig. 1B, THP-1 cells significantly generated cAMP by stimulation with 10 μM PGD_2 or BW245C (data not shown), a selective prostanoid DP receptor agonist (Town et al., 1983; Abramovitz et al., 2000), and 10 μM pinagladin completely suppressed prostanoid DP receptor-dependent cAMP generation. In our previous study, we revealed that pinagladin was a potent, orally active and highly selective antagonist for prostanoid DP receptor (Mitsumori et al., 2003). Indeed, more than 100-times selectivity to prostanoid DP receptor in comparison to the other prostanoid receptors was observed (data not shown). These results suggest that THP-1 cells express transcripts and functional protein for prostanoid DP receptor.

3.2. Profile of cytokines and chemokines produced by THP-1 cells

To examine the profile of the production of cytokines and chemokines from THP-1 cells, the supernatants were assessed 24 h after stimulation with a protein array system. As shown in Fig. 2A, TNF-α (10 ng/ml) significantly induced production of 24 out of 26 cytokines and chemokines, except for IL-5 and G-CSF, in THP-1 cells. By contrast, 10 μM of PGD_2 or BW245C in the absence of TNF-α did not produce significantly any cytokines and chemokines with protein array system (data not shown). On the other hand, the synergic effects of PGD_2 or BW245C and TNF-α were observed only for the production of IL-8 and MCP-1 (Fig. 2B).

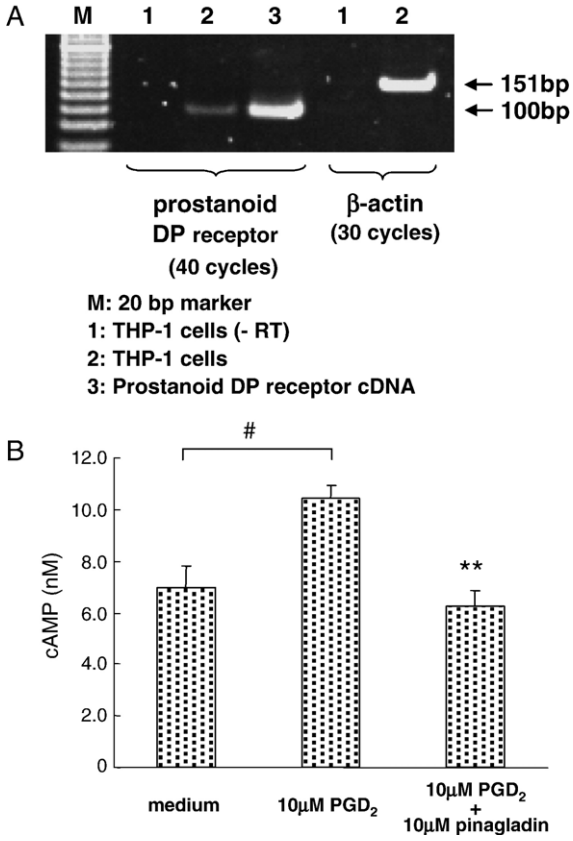

Fig. 1. Expression of mRNA and functional second signal for prostanoid DP receptor in THP-1 cells. Expression of prostanoid DP receptor mRNA in THP-1 cells was determined by RT-PCR (A) in the presence (lane 1) or absence (lane 2) of RT, using total RNA isolated from THP-1 cells. As a positive control, prostanoid DP receptor cDNA inserted into vectors was amplified by PCR (lane 3). Elevation of cAMP level after stimulation with PGD_2, and inhibition of it by pinagladin were determined (B). THP-1 cells were stimulated by 10 μM PGD_2 for 10 min, with or without pre-treatment with 10 μM pinagladin. Data represent means±S.E.M. of three independent experiments. Significant differences were analyzed by Student's *t* test. #$P<0.05$ between vehicle treatment and PGD_2 treatment; **$P<0.01$ between with and without pre-treatment with pinagladin.

3.3. Involvement of prostanoid DP receptor in the enhancement of TNF-α-induced MCP-1 production by PGD_2

Although stimulation with PGD_2 alone hardly produced MCP-1 (Fig. 3A, small column), PGD_2 (1.0–10 μM) concentration dependently upregulated TNF-α-induced MCP-1 production with an EC_{50} value of 2.69 μM, and significant production was observed at 3 and 10 μM (Fig. 3A). We next examined the effects of pinagladin on TNF-α-induced MCP-1 release, enhanced by PGD_2 at a concentration approximately equal to the EC_{50} value. MCP-1 production with drug free stimulation (positive control in this study) significantly increased in comparison to that with 10 ng/ml TNF-α alone (negative control in this study) ($P<0.05$ for 3 μM PGD_2). As

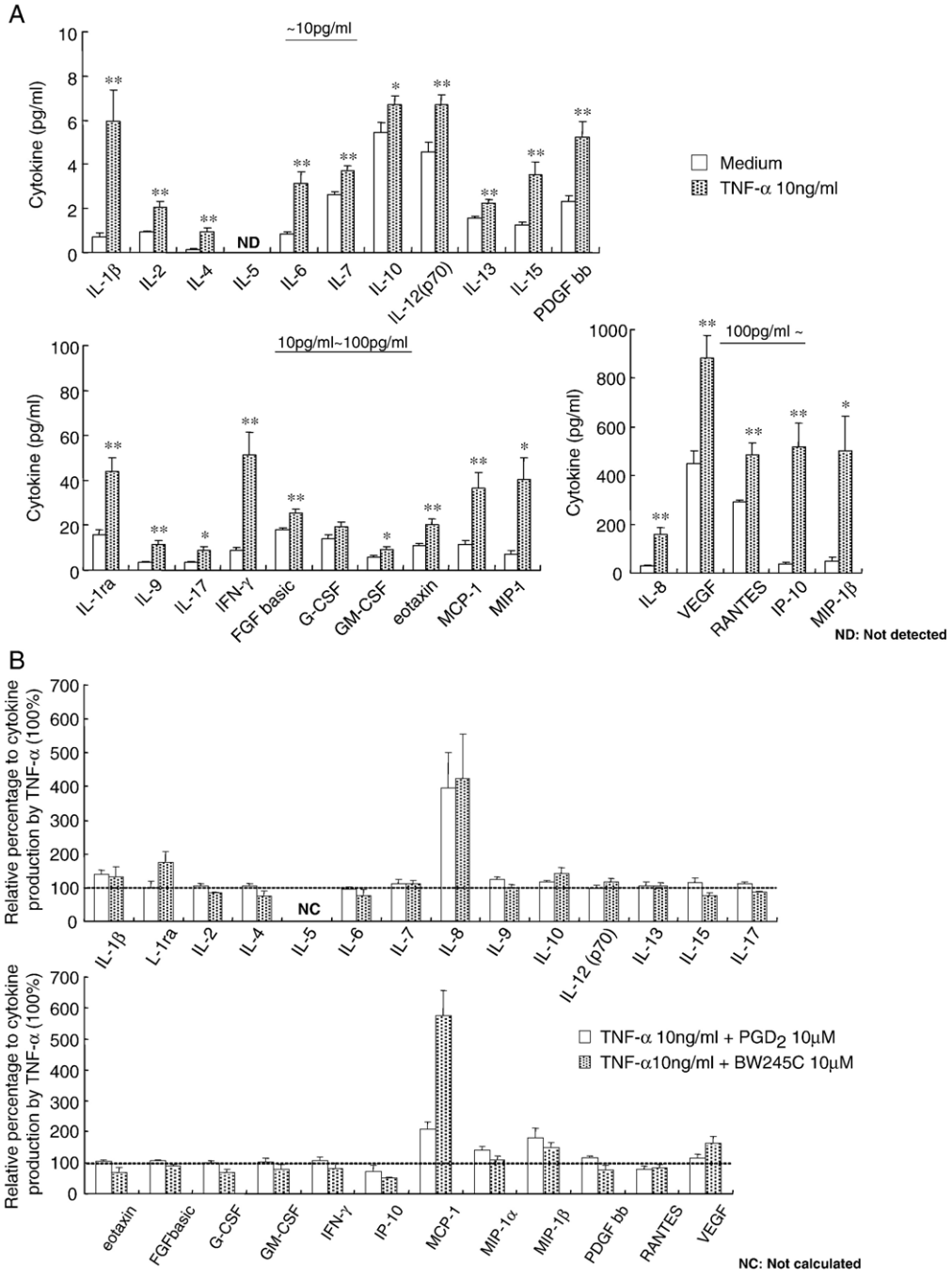

Fig. 2. Production profiles of cytokines and chemokines in THP-1 cells induced by TNF-α alone (A) and by costimulation with prostanoid DP activator and TNF-α (B). THP-1 cells were stimulated by 10 μM PGD_2 or 10 μM BW245C in the presence of 10 ng/ml TNF-α. Cytokine and chemokine levels in the supernatants collected 24 h after each stimulation were measured using a Bioplex assay kit. Data represent the mean ± S.E.M. of two to four independent experiments. Significant differences were analyzed by Student's *t* test. $*P < 0.05$, $**P < 0.01$ between vehicle and TNF-α treatment (A). The relative percentage of cytokine and chemokine production following control stimulation with TNF-α alone (expressed as 100% production) ($n = 2–4$) was represented (B).

shown in Fig. 3B, pinagladin showed concentration dependence and complete inhibition of the TNF-α-induced MCP-1 production enhanced by PGD_2. The IC_{50} value was 1.23 μM. Pinagladin also inhibited MCP-1 production induced by TNF-α and BW245C, with an IC_{50} value of 5.79 μM (data not shown). These results suggest that the concentration-dependent and synergistic upregulation of TNF-α-induced MCP-1 production was caused by the activation of prostanoid DP receptor.

3.4. Involvement of cAMP and PKA signaling pathway on prostanoid DP receptor-dependent enhancement of MCP-1 production

We investigated the involvement of the cAMP and PKA signaling pathway in TNF-α-induced MCP-1 production enhanced by PGD_2 in THP-1 cells. In the first set of experiments, to verify the role of cAMP, dibutyryl cAMP (db-

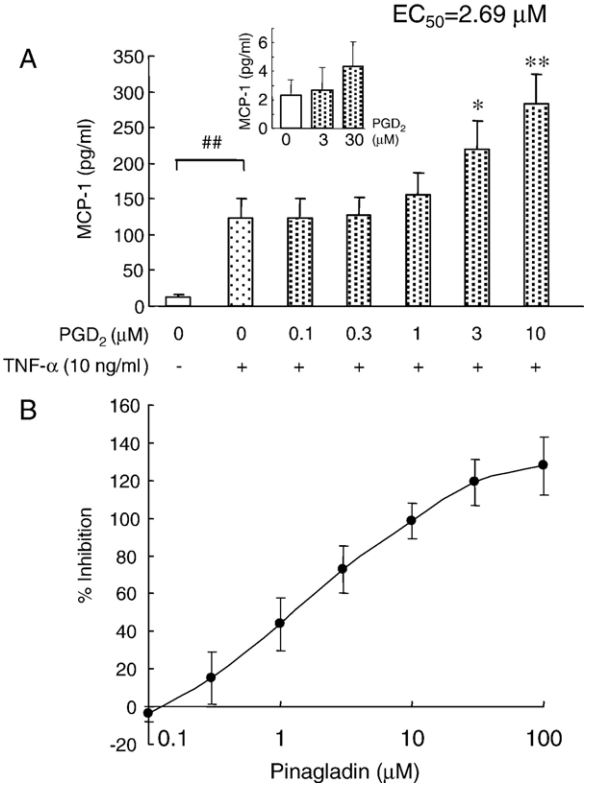

Fig. 3. Concentration response of PGD_2 (A) on TNF-α-induced MCP-1 production and inhibitory effects of pinagladin on MCP-1 enhancement by PGD_2 (B). THP-1 cells were stimulated by various concentration of PGD_2, in the presence or absence of 10 ng/ml TNF-α (A). THP-1 cells pre-treated with various concentrations of pinagladin were stimulated by 10 ng/ml TNF-α and 3 μM PGD_2 (B). MCP-1 levels in the supernatant collected 24 h after each stimulation were measured using ELISA. Data represent the mean±S.E.M. of five to eight independent experiments (A, $n=7–8$; B, $n=5$). Significant differences between vehicle and TNF-α stimulation were analyzed by Student's t test ($\#\#P<0.01$), and between TNF-α stimulation and combined stimulation with TNF-α and PGD_2 were analyzed by Dunnett's test ($*P<0.05$, $**P<0.01$).

cAMP; cAMP analogue) was costimulated with TNF-α. As shown in Fig. 4A, db-cAMP concentration dependently induced MCP-1 production in the presence of TNF-α, and a significant enhancement was observed at 100 μM. Next, to further estimate the role of the cAMP/PKA transduction pathway, we examined the effects of PKA inhibitor H89 on MCP-1 production. H89 at higher concentrations significantly inhibited MCP-1 production induced by combined stimulation with TNF-α and PGD_2 (Fig. 4B).

3.5. Enhancement of IL-8 production by prostanoid DP activators

To investigate whether or not the enhancement of IL-8 production was also regulated by prostanoid DP receptor via the cAMP/PKA pathway, we examined the effects of pinagladin, db-cAMP and H89 on IL-8 production. Synergistic production of IL-8 was observed by combined stimulation with TNF-α and PGD_2, and pinagladin and H89 significantly inhibited PGD_2-induced IL-8 production (Fig. 5A and C). Moreover, db-cAMP significantly increased TNF-α-induced IL-8 production (Fig. 5C). These results indicate that IL-8 production is also regulated by prostanoid DP receptor in the same way as MCP-1. Complete inhibition of IL-8 production with pinagladin was observed in the case of stimulation by BW245C (Fig. 5D), but not by PGD_2 (62.2% inhibition, Fig. 5A), suggesting that the production of IL-8 by PGD_2 might involve, at least partially, a prostanoid DP-independent pathway.

4. Discussion

In the present study, we showed that the THP-1 human monocytic cell line expressed the functional prostanoid DP receptor, using RT-PCR and a cAMP generation assay. The involvement of the prostanoid DP receptor in the production of cytokines and chemokines from THP-1 cells was estimated using a protein array system. Stimulation with 10 μM PGD_2 or BW245C, in the absence of TNF-α, did not produce any cytokines and chemokines in THP-1 cells, although activation

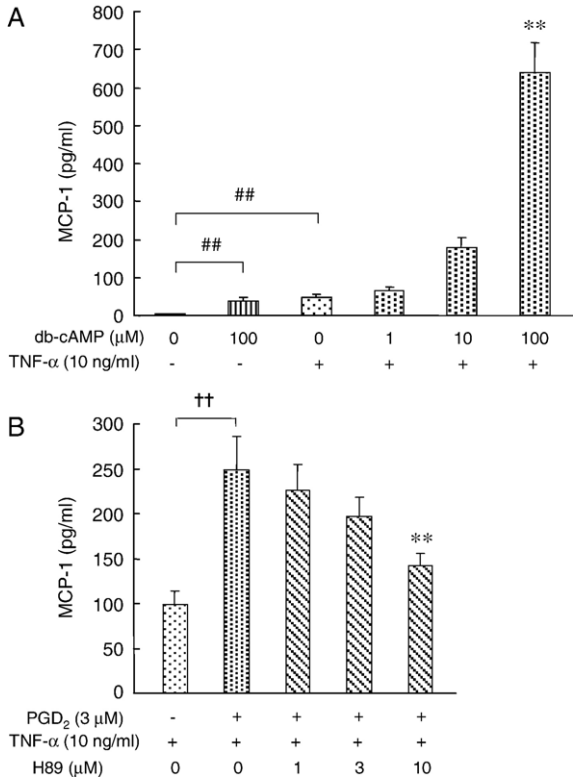

Fig. 4. Involvement of cAMP/PKA signaling in prostanoid DP receptor-dependent enhancement of MCP-1 production. THP-1 cells were stimulated by several concentrations of db-cAMP in the presence of 10 ng/ml TNF-α (A). The effect of H89 on TNF-α-induced MCP-1 production enhanced by PGD_2 (B) in THP-1 cells was examined. THP-1 cells pre-treated with various concentrations of H89 were stimulated with 10 ng/ml TNF-α and 3 μM PGD_2 (B). MCP-1 levels in the supernatants collected 24 h after each stimulation were measured using ELISA. Data represent the mean±S.E.M. of eight independent experiments (A, B). Significant differences between vehicle stimulation and TNF-α or db-cAMP stimulation were analyzed by Student's t test ($\#\#P<0.01$). Significant differences between TNF-α stimulation and combined stimulation with TNF-α and db-cAMP (A), and between combined stimulation with TNF-α and PGD_2 and H89 treatment (B) were analyzed by Dunnett's test ($**P<0.01$). Significant differences between TNF-α stimulation and combined stimulation with TNF-α and PGD_2 were analyzed by Student's t test ($††P<0.01$) (B).

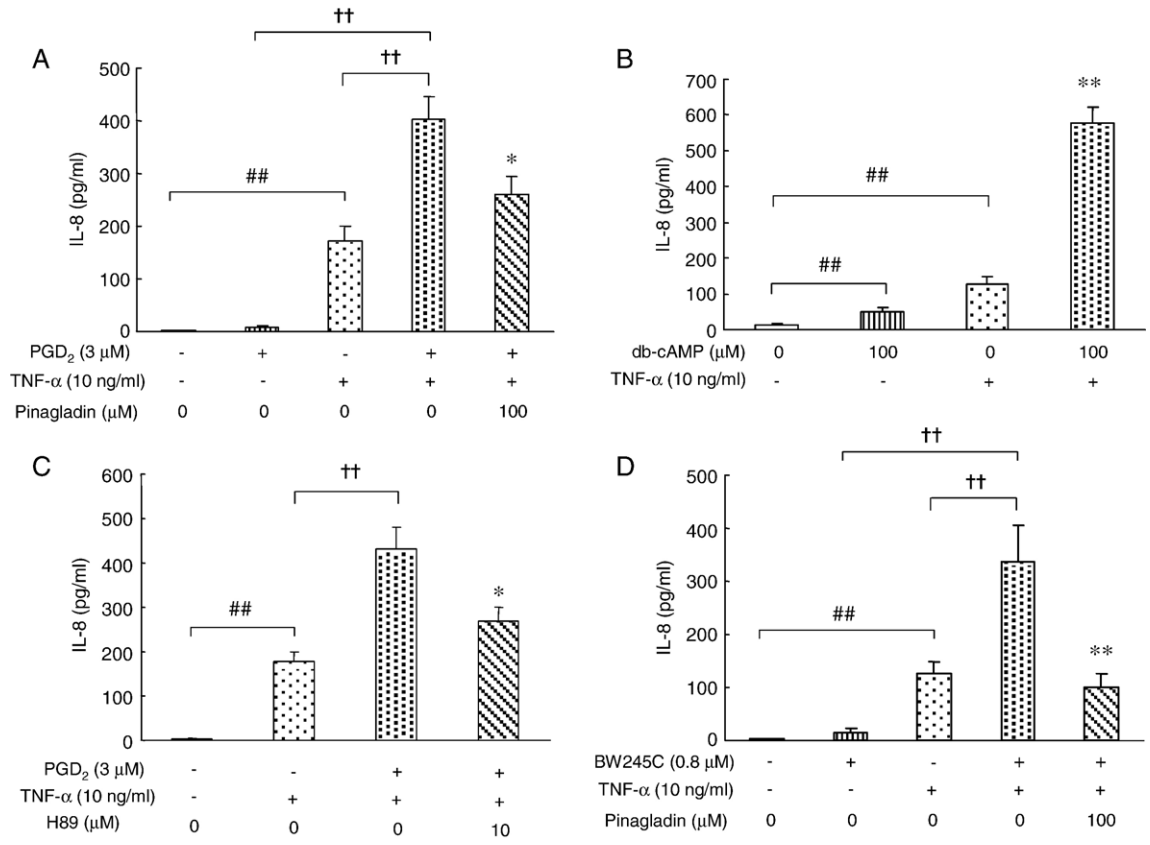

Fig. 5. Enhancement of IL-8 production by prostanoid DP receptor activation. The effect of pinagladin on TNF-α-induced IL-8 production enhanced by PGD_2 (A) or BW245C (D) was examined. THP-1 cells pre-treated by 100 μM pinagladin were costimulated with 10 ng/ml TNF-α and 3 μM PGD_2 (A) or 0.8 μM BW245C (D). Involvement of cAMP and PKA with IL-8 production was also determined using db-cAMP (B) and H89 (C). THP-1 cells were stimulated by 100 μM db-cAMP in the presence of 10 ng/ml TNF-α (B). H89 at 10 μM was pre-treated following stimulation with 10 ng/ml TNF-α and 3 μM PGD_2 (C). IL-8 levels in the supernatants collected 24 h after each stimulation were measured using ELISA. Data represent the mean ± S.E.M. of four to eight independent experiments (A, $n=4$; B, $n=8$; C, $n=7$; D, $n=8$). Significant differences were analyzed by Student's t test. ##$P<0.01$ between vehicle stimulation and TNF-α (A, B, C, D) or db-cAMP (B) stimulation; ††$P<0.01$ between single stimulation with TNF-α (A, C, D), PGD_2 (A) or BW245C (D) and combined stimulation with TNF-α and PGD_2 (A, C) or BW245C (D); *$P<0.05$, **$P<0.01$ between combined stimulation with TNF-α and PGD_2 (A, C) or BW245C (D), and pinagladin (A, D) or H89 treatment (C). **$P<0.01$ between TNF-α alone and combind stimulation with TNF-α and db-cAMP (B).

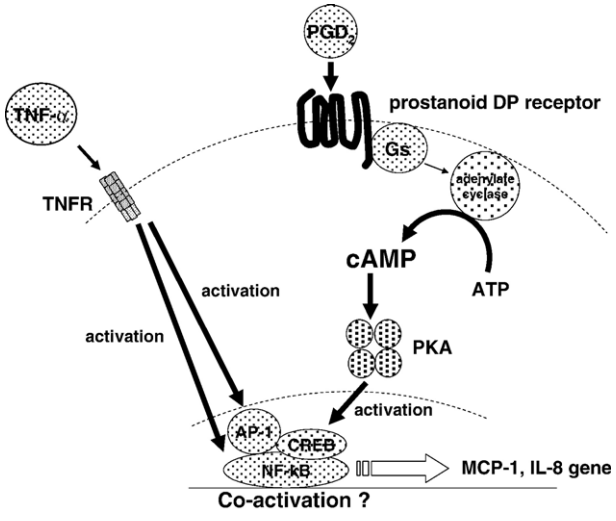

Fig. 6. Hypothetical model of MCP-1 and IL-8 produced by combined stimulation with PGD_2 and TNF-α in THP-1 cells. Following stimulation of prostanoid DP receptor, the cAMP/PKA pathway was activated. TNF-α might activate NF-κB and AP-1, and PGD_2 might activate CREB via cAMP/PKA pathway. The formation of complex of these three transcription factors might induce synergistic production of MCP-1 and IL-8.

of the prostanoid DP receptor alone generated intracellular cAMP (Fig. 1B). These results suggest that there is a gap between cAMP generation and protein synthesis, and that costimulation with TNF-α is needed to activate protein synthesis.

TNF-α is a potent multifunctional cytokine that plays a central role in the pathogenesis of many inflammatory diseases such as asthma (Gosset et al., 1991) and allergic rhinitis (Bradding et al., 1995). This proinflammatory cytokine is produced by a variety of cells, such as mast cells and macrophages at the inflammation site. TNF-α is also known to induce the production of various cytokines and chemokines in inflammatory cells (Aloisi et al., 1992; Cromwell et al., 1992; Zhao et al., 2003; Schwamborn et al., 2003). In the present study, stimulation with TNF-α alone significantly produced 24 out of 26 cytokines and chemokines. Of particular interest from our results is that the synergistic effects with prostanoid DP activator and TNF-α were observed only for the production of MCP-1 and IL-8. A specific common pathway might be involved in the production of these two chemokines after stimulation with PGD_2 and TNF-α. The relationship of cAMP/PKA in this signaling pathway was clearly demonstrated in the

present study. Moreover, we observed that the synergistic increase in the expression of MCP-1 transcripts was induced by combined stimulation with PGD_2 and TNF-α (data not shown). Therefore, a common pathway does indeed seem to exist between cAMP/PKA and transcription for the production of MCP-1 and IL-8. To further understand the signaling pathway, we examined the translocation of NF-κB to the nucleus after stimulation of THP-1 cells (data not shown). No upregulation was observed in the translocation of NF-κB to the nucleus after combined stimulation with PGD_2 and TNF-α, although TNF-α by itself induced significant translocation (data not shown). Jaramillo and Olivier (2002) have suggested that MCP-1 gene transcription by H_2O_2 is mediated by NF-κB, activator protein (AP)-1 and cAMP-dependent cAMP response element binding protein (CREB), and requires the formation of an essential transcriptional complex of these three factors for maximal and fine tuning of the MCP-1 gene in a murine macrophage cell line. TNF-α induces activation of NF-κB and AP-1 (Barnes and Karin, 1997; Karin et al., 1997), whereas PGD_2 activates CREB via cAMP signaling (Gosset et al., 2003). Therefore, formation of a transcriptional complex may be needed for the production of IL-8, as well as MCP-1 by THP-1 cells after stimulation with prostanoid DP activator and TNF-α (Fig. 6).

Interestingly, the production of IL-8, but not MCP-1, induced by PGD_2 and TNF-α was not inhibited completely by a high concentration of pinagladin (Fig. 5A), suggesting the existence of a prostanoid DP receptor-independent pathway. Chiba et al. (2006) have shown that PGD_2 induces IL-8 production in bronchial epithelial cells, via an unknown Gi-coupled receptor, in a CRTH2- and prostanoid DP receptor-independent manner. On the other hand, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, which is a metabolite of PGD_2, also induces IL-8 gene via peroxisome proliferator-activated receptor gamma (PPAR-γ) in THP-1 cells (Zhang et al., 2001). In that study, PGD_2 or its metabolite, without TNF-α, induced IL-8, whereas we only observed IL-8 production after combined stimulation with PGD_2 and TNF-α. The relationship between TNF-α and the unknown receptor and/or PPAR-γ should be investigated further.

MCP-1, which is produced in patients with asthma, provokes aggregation of mast cells (Conti et al., 1995), histamine release from mast cells (Suzuki et al., 1996), and the formation of leukotriene C_4 in bronchial alveolar lavage fluids and in culture supernatants of pulmonary mast cells (Weber et al., 1996; Campbell et al., 1999). Moreover, MCP-1 is reported to induce chemotaxis of eosinophils (Dunzendorfer et al., 2001). On the other hand, IL-8, which is a potent neutrophil recruiting and activating factor, is produced in patients with asthma, and is thought to be involved in severe acute asthma (Pease and Saboroe, 2002; Tillie-Leblond et al., 2005). In addition, MCP-1 and IL-8 have also been detected in patients with atopic dermatitis and allergic rhinitis (Kimata and Lindley, 1994; Ohkubo et al., 1998; Vestergaard et al., 2004; Bruno et al., 2002). At such inflammation sites, PGD_2 and TNF-α may be produced at the same time and may work cooperatively, resulting in exacerbation of allergic inflammation by induction of IL-8 and MCP-1 production by monocytes.

We have demonstrated, possibly for the first time, that PGD_2/prostanoid DP receptor interaction synergistically upregulates MCP-1 and IL-8 production by TNF-α, via the cAMP/PKA signaling pathway in human monocytic THP-1 cells. Further studies with primary cells are needed to clarify the importance of these phenomena in human pathogenesis, and molecular biological techniques should be used to elucidate the signaling pathway for the production of MCP-1 and IL-8.

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