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Prostaglandin E\textsubscript{2} Up-Regulates Macrophage-Derived Chemokine Production but Suppresses IFN-Inducible Protein-10 Production by APC\textsuperscript{1}

Etsushi Kuroda, Tsutomu Sugiura, Kazumasu Okada, Kazuya Zeki, and Uki Yamashita\textsuperscript{2}

PGE\textsubscript{2} has been known to suppress Th1 responses. We studied the role of PGE\textsubscript{2} in two representative chemokines, macrophage-derived chemokine (MDC) and IFN-inducible protein-10, production by LPS- or CD40-stimulated spleen cells. The production of MDC, one of the ligands for CCR4 preferentially expressed on Th2, was enhanced in nonstimulated, LPS-, CD40-, or CD3-stimulated spleen cells by the pretreatment with PGE\textsubscript{2}, while the production of IFN-inducible protein-10, a representative ligand for CXC chemokine receptor 3 expressed on Th1, was suppressed. MDC production was also enhanced by IL-4, IL-5, and intracellular CAMP-elevating agents such as dibutylryl CAMP and 3-isobutyl-1-methylxanthine, and the effect of IL-4, IL-5, and PGE\textsubscript{2} was additive. However, the pretreatment with IL-6, IL-10, or TGF-\(\beta\), or the neutralization of IFN-\(\gamma\) or IL-12 had no effect on MDC production. B cells, macrophages, and dendritic cells were main producers of MDC, while T cells produced only a small amount of MDC. Production by B cells was equally stimulated by LPS and anti-CD40 Ab, while that by macrophages and dendritic cells was more markedly stimulated by anti-CD40 Ab, and PGE\textsubscript{2} further enhanced MDC production by these stimulated cells. These results indicate that PGE\textsubscript{2} regulates Th1/Th2-related chemokine production by B cells, macrophages, and dendritic cells, and that this is a new function of PGE\textsubscript{2} for the regulation of Th2 immune responses at the induction and activation stages.


The activation of CD4\textsuperscript{+} T cell subsets, Th1 and Th2, is one of the most important factors to determine the characters of several immunological diseases. In general, the activation of Th1 promotes cellular immune responses via IFN-\(\gamma\) production and is related with the elimination of intracellular pathogens, while the activation of Th2 enhances humoral immune responses via IL-4 and IL-5 and is related with allergic diseases. Th1 and Th2 are differentiated from naive T cells after the Ag recognition, which are influenced by surface molecules of APCs, Ag doses, and cytokines (1–6). The selective recruitment of Th subset to an inflammatory site is also important to determine the character of immune responses. Although the mechanism of this selective recruitment still remained unclear, recent studies suggest that the patterns of chemokines and their receptors determine the selective recruitment of Th1 and Th2 (7–10).

Th1 and Th2 express different chemokine receptors and respond to distinct types of chemokines. CXC chemokine receptor 3 (CXCR3)\textsuperscript{3} and CCR5 are highly expressed on Th1, and IFN-inducible protein-10 (IP-10, new nomenclature: CXCL10 (7)), which binds to CXCR3, induces chemotaxis of Th1, while CCR3 and CCR4 are preferentially expressed on Th2, and ligands for CCR4, macrophage-derived chemokine (MDC, CCL21 (7)), and thymus and activation-regulated chemokine (TARC, CCL17 (7)) promote the migration of Th2 (7–13). The relationship between chemokines and Th1/Th2 responses in vivo has been also investigated in several human diseases and mouse models. In multiple sclerosis and rheumatoid arthritis, which are Th1-related diseases, CCR5\textsuperscript{+} and CXCR3\textsuperscript{+} T cells are accumulated, and their ligands, macrophage-inflammatory protein (MIP)-1\(\alpha\) (CCL3 (7)) and IP-10, are detected at inflammatory sites (14, 15). On the other hand, a higher expression of TARC is detected on Reed-Sternberg cells of Hodgkin’s lymphoma that have the characteristic feature of infiltration of Th2 (16). In murine models of allergic airway inflammation and atopic dermatitis, NC/Nga mouse, and endotoxin-induced liver injury, the accumulation of CCR4\textsuperscript{+} Th2 and the production of MDC and TARC play an important role in the induction of tissue injuries (17–20).

IP-10 and MDC are mainly produced by APCs such as dendritic cells, monocytes, macrophages, and B cells, and highly regulated by Th1- and Th2-derived cytokines. In general, IFN-\(\gamma\)-up-regulates IP-10, but suppresses MDC production, while IL-4 and IL-13 have opposite effects (9, 12, 13, 21–23). Thus, Th1 and Th2 cytokines regulate Th1- and Th2-related chemokine (IP-10 and MDC) productions, and reversely these chemokines also regulate Th1/Th2 responses. However, there is no report about the role of PGE\textsubscript{2} in Th1/Th2-related chemokine productions.

PGE\textsubscript{2}, an arachidonic acid metabolite, produced in various types of cells, regulates broad range of physiological functions (24). In the immune system, PGE\textsubscript{2} is also mainly produced by APCs, and the effects are almost suppressive on Th1-related immune responses and augment Th2-related immune responses. PGE\textsubscript{2} preferentially down-regulates IL-12R expression and inhibits the differentiation of Th1 (25, 26). PGE\textsubscript{2} also suppresses LPS-induced

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\textsuperscript{3} Abbreviations used in this paper: CXCR, CXC chemokine receptor; CD40L, CD40 ligand; dbcAMP, dibutylryl cAMP; IBMX, 3-isobutyl-1-methylxanthine; IP-10, IFN-inducible protein-10; LT\(\beta\), leukotriene \(\beta\); MDC: macrophage-derived chemokine; MIP, macrophage-inflammatory protein; TARC, thymus and activation-regulated chemokine.

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IL-12 production by macrophages, and IL-12 and PGE₂ derived from APCs down-regulate IFN-γ production by T cells (27, 28). In B cell function, PGE₂ enhances IgE production of IL-4- and LPS-stimulated B cells in vitro (29). In these reports, the effect of PGE₂ is similar to IL-4 in immune responses.

In this study, we examined the effect of PGE₂ on IP-10 and MDC production in vitro, and demonstrate that PGE₂ up-regulates MDC production, but down-regulates IP-10 production by APCs induced with LPS and CD40 stimulation. These results suggest that PGE₂ plays an important role not only for the differentiation of Th₂, but also for the recruitment of Th₂-related chemokines.

**Materials and Methods**

**Mice**

BALB/c male mice, 7–8 wk old, were purchased from Seac (Oita, Japan), and were maintained in our laboratory under specific pathogen-free condition.

**Cytokines, chemokines, Abs, and other reagents**

PGE₂, indomethacin, dibutyryl cAMP (dbcAMP), 3-isobutyl-1-methylxanthine (IBMX), and anti-phenylephedrine (Sigma, St. Louis, MO). Recombinant mouse IL-12, IFN-γ, and MDC were purchased from R&D Systems (Minneapolis, MN). Recombinant mouse IL-4, IL-5, IL-6, and IL-10 were purchased from PeproTech (London, U.K.). Human TGF-β, anti-mouse MDC, and anti-mouse CRG-2 (IP-10) Abs were purchased from Genzyme/Technie (Cambridge, MA). Anti-mouse IFN-γ and anti-mouse IL-12 Abs were purchased from PharMingen (San Diego, CA). Recombinant mouse IP-10/CRG-2 was purchased from Dako Japan (Kyoto, Japan). Peroxidase-conjugated rabbit anti-antibody IgG Ab was purchased from MBL (Nagoya, Japan).

**Preparation of cell**

Spleen cell suspension was prepared and maintained in RPMI 1640 medium (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10 nM HEPES, pH 7.2, and 10% FBS (BioWhittaker, Walkersville, MD). B cells (B220⁺ cells), macrophages (CD11b⁻ cells), dendritic cells (CD11c⁺ cells), and Th cells (CD4⁺ cells) were purified from spleen cells by magnetic cell sorting according to the manufacturer’s procedures (MACS; Miltenyi Biotech, Bergish-Gladbach, Germany). Purity of sorted cells was >95%, as determined by flow cytometry. In some experiments, B220⁺, CD11b⁻, and CD11c⁻negative cells were used as T cells (≤5% Ig⁻, ≤5% Mac-1⁻, and >95% CD³⁺). The number of whole spleen cells and sorted cells were adjusted in 5 × 10⁶/ml (whole spleen cells, macrophages, and T cells) or 2 × 10⁶/ml (macrophages, dendritic cells, and macrophages/dendritic cells fraction), and used for experiments.

**In vitro culture and stimulation of cells**

Whole spleen cells or sorted cells were cultured in 24-well culture plates (Falcon 3047; Becton Dickinson, Franklin Lakes, NJ) with or without PGE₂ (1–100 nM), IL-4 (10 ng/ml), IFN-γ (50 ng/ml), IL-12 (50 ng/ml), IL-5 (10 ng/ml), IL-6 (10 ng/ml), IL-10 (10 ng/ml), TGF-β (4 ng/ml), dbcAMP (50 μM), IBMX (50 μM), anti-IFN-γ Ab (1 μg/ml), or anti-IL-12 Ab (1 μg/ml) in the presence of indomethacin (1 μM) for 12 h, and then they were stimulated with LPS (10 μg/ml), anti-CD40 Ab (10 μg/ml), or anti-CD3 Ab (1 μg/ml) for an additional 24, 48, or 72 h. Purified CD4⁺ T cells were stimulated with plate-coated anti-CD3 Ab in the presence or absence of PGE₂ or IL-4 for 48 h. In some experiments, cells were cultured without indomethacin for 12 h, and then stimulated. The culture supernatants were collected and used for the assay of chemokine or PGE₂ as described below.

**Chemokine mRNA analysis**

mRNA expression was detected by RT-PCR. Total cellular RNA was extracted by TRIZol (Life Technologies, Rockville, MD), according to the manufacturer’s protocol. First strand cDNA was synthesized from 5 μg of total RNA by Superscript II RT Kase H-reverse transcriptase (Life Technologies), following the instruction of manufacturer using 0.25 μg of random primer (Life Technologies). One-tenth of synthesized cDNA was amplified by PCR using 50 pmol sense and antisense primers with 1 U of Taq polymerase (Boehringer Mannheim, Mannheim, Germany) in a total volume of 50 μl. PCR cycles were performed for 1 min at 94°C for denaturation, 1 min at 55°C for annealing, and 2 min at 72°C for extension, and at the first cycle, denaturation was run for 2 min at 94°C. For the amplification of sense and antisense primers, products size, and PCR cycles are as follows: β-actin, 5'-CTCAACTCGGAGCAGCATGAG-3' and 5'-GTGTTGCTTGAAGCTGTAGCC-3'; 380 bp, 30 cycles; MDC, 5'-CTGGTGGAAGAAGCTACTCCAT-3' and 5'-TAAAGACCTGGGTGATCTTG-3'; 493 bp, 35 cycles; IP-10, 5'-GGGCGAGTGATTAGGAGGC-3' and 5'-TGAGTTGGAGGACGAGG-3'; 544 bp, 35 cycles. A 10 μl of PCR products was electrophoresed using a 1.5% agarose gel. After ethidium bromide staining (Sigma), PCR products were visualized by UV illumination.

**Assay of chemokine**

The concentrations of MDC in the supernatants were measured by competitive ELISA inhibition assay (30). In brief, ELISA plates (Nunc-Immuno Plate MaxiSorp; Nunc, Roskilde, Denmark) were coated with 100 μl of 250 ng/ml MDC in PBS at 4°C for 18 h. The wells were then washed and blocked with 300 μl of RPMI 1640 containing 10% FBS at 37°C for 30 min. Standard solutions (40,000–625 pg/ml in culture medium) or culture supernatants and equal volume of 400 ng/ml anti-MDC Ab were mixed well in microtubes and incubated at 37°C for 1 h. After incubation, 200 μl of prepared standard or sample solutions was transferred to each well of MDC-coated plates, and the plates were kept at 4°C for 18 h. The wells were washed and filled with 100 μl of peroxidase-conjugated rabbit anti-goat IgG Ab (diluted 1/2000 in PBS containing 0.05% Tween 20 and 1% BSA). The plates were incubated at 37°C for 2 h, washed, and then substrate (0.04% o-phenylenediamine, 0.01% H₂O₂, in 50 mM disodium hydrogen phosphate, and 150 mM citric acid, pH 5) was added to the wells. Thirty minutes later, the enzyme reaction was stopped by the addition of 25 μl of 8 N H₂SO₄, and the absorbance at 490 nm was measured. The results are expressed as mean ± SD of chemokine produced (pg/ml) in triplicate cultures. Competitive ELISA inhibition assay for IP-10 and TARC was also performed by a similar method except for Ab concentration. The concentration of anti-IP-10 Ab and anti-TARC Ab was used at 100 ng/ml. Detection limit of these assays is 1000 pg/ml.

**PGE₂ enzyme immunoassay**

PGE₂ in the culture supernatant was measured using STAT-PGE₂ Enzyme Immunoassay Kit (Cayman Chemicals, Ann Arbor, MI), according to the manufacturer’s protocol.

**Statistics**

All experiments were repeated at least three times, and one representative result of each experiment is shown in figures. Statistical analyses were performed using the Student’s t test. A confidence level of <0.05 was considered significant (31).

**Results**

**PGE₂ enhances MDC production, but suppresses IP-10 production in murine spleen cells**

At first, we investigated the effect of PGE₂ on Th₁- and Th₂-related chemokine (IP-10 and MDC, respectively) mRNA expression in murine spleen cells. Spleen cells were pretreated with PGE₂ for 12 h, then stimulated with LPS, anti-CD3 Ab, or anti-CD40 Ab for an additional 24 h, and mRNA expression was detected by RT-PCR. As shown in Fig. 1, both IP-10 and MDC mRNA expressions were up-regulated following stimulation. The pretreatment with PGE₂ suppressed the expression of IP-10 mRNA. On the contrary, the expression of MDC mRNA was enhanced by the pretreatment with PGE₂.

Next, we assessed IP-10 and MDC protein productions in murine spleen cells. Spleen cells were pretreated with PGE₂ for 12 h, then stimulated with LPS, anti-CD3 Ab, or anti-CD40 Ab for an additional 24 h. The supernatants were assessed by competitive ELISA inhibition assay. As shown in Fig. 2, IP-10 production was not influenced. On the other hand, IFN-γ suppressed MDC production, but enhanced IP-10 production. These findings were more clearly observed in stimulated spleen cells. In spleen cells stimulated with LPS (a model of bacterial...
stimulation), anti-CD40 Ab (a model of T-APC interaction), or anti-CD3 Ab (a model of T-APC interaction), PGE$_2$ enhanced MDC production (about 3-fold higher), and this capacity was stronger than that of IL-4. On the other hand, IP-10 production was suppressed by the pretreatment with PGE$_2$ or IL-4. IFN-$\gamma$ had quite opposite effects of IL-4 and PGE$_2$, which was the same as previously reported (23). We also assessed MDC and IP-10 productions in spleen cells stimulated with soluble anti-CD3 Ab that is known to activate APC via CD40-CD40 ligand (CD40L) interaction (32). The pretreatment with PGE$_2$ enhanced MDC production, and suppressed IP-10 production in anti-CD3 Ab-stimulated spleen cells the same as anti-CD40 stimulation. The amount of MDC was much higher in CD40-stimulated spleen cells than in LPS-stimulated ones, and was moderate in CD3-stimulated ones.

We also investigated the kinetics of PBS-, LPS-, or anti-CD40 Ab-induced MDC production. Spleen cells were pretreated with PGE$_2$, and then stimulated. Supernatants were harvested at 24, 48, or 72 h after stimulation, and MDC in the supernatants were determined. As shown in Fig. 3, the amounts of MDC were increased in a time-dependent manner, and pretreatment with PGE$_2$ clearly enhanced MDC production at 48 and 72 h.

Enhancement of MDC production by PGE$_2$ is mediated by the accumulation of intracellular cAMP

A representative activity of PGE$_2$ is the elevation of intracellular cAMP (24). We examined whether the enhanced production of MDC is mediated by intracellular accumulation of cAMP in stimulated spleen cells. Spleen cells were pretreated with 1–100 nM PGE$_2$, dbcAMP (membrane-permeable cAMP analogue), or IBMX (cAMP phosphodiesterase inhibitor), and then stimulated with LPS or anti-CD40 Ab for 48 h. As shown in Fig. 4, A and B, in both stimuli, the pretreatment with dbcAMP or IBMX also enhanced MDC production the same as PGE$_2$ treatment, but the degree of increase in IBMX treatment was lower than PGE$_2$ or dbcAMP. This difference seems to be caused by a different mechanism of cAMP accumulation between PGE$_2$ and IBMX.

PGE$_2$ is a potent inhibitor of IFN-$\gamma$ and IL-12, and an inducer of IL-10 (25–28). Next, we examined whether the enhanced production of MDC is due to a secondary effect of the suppression of IFN-$\gamma$ and IL-12 or the induction of IL-10. As shown in Fig. 4, C and D, neutralization of IFN-$\gamma$ or IL-12 was not sufficient to enhance MDC production in LPS- or anti-CD40 Ab-stimulated spleen cells. Moreover, the pretreatment with IL-10 suppressed MDC production. Another representative inhibitory cytokine, TGF-$\beta$, had no effect on MDC production. Interestingly, IL-5 also enhanced MDC production the same as IL-4, but IL-4 and IL-5 were not detected in PGE$_2$-treated spleen cells stimulated with LPS or anti-CD40 Ab (data not shown). However, IL-6, another Th2 cytokine, had no effect on MDC production. IFN-$\gamma$ suppressed MDC production the same as previously reported (23), but IL-12 had no effect.

Enhanced production of MDC by PGE$_2$ is mainly mediated by B cells, macrophages, and dendritic cells

It has been reported that MDC is produced by various types of cells, such as B cells, T cells, and macrophages (33–38). We investigated the producer of MDC by LPS- or anti-CD40 Ab-stimulated spleen cells in the presence of PGE$_2$. Spleen cells were separated into B cells (B220$^+$ cells), macrophages/dendritic cells (CD11b$^+$ or CD11c$^+$ cells), and T cells (B220$^-$, CD11b$^-$, and CD11c$^-$ spleen cells). Purified cell fractions were pretreated with PGE$_2$ or IL-4 for 12 h, and then stimulated with LPS or anti-CD40 Ab for 48 h. PGE$_2$-pretreated B cells produced a higher amount of MDC as compared with nontreated cells in both LPS and anti-CD40 Ab stimulation (Fig. 5, A and B). The amount of MDC is much higher in CD40-stimulated macrophages/dendritic cells than other types of cells, and PGE$_2$ pretreatment further enhanced MDC production in macrophages/dendritic cells treated with anti-CD40 Ab (Fig. 5D). Contrary to our expectation, however, PGE$_2$ pretreatment slightly reduced MDC production in LPS-stimulated macrophages/dendritic cells (Fig. 5C). Macrophages and dendritic cells were further purified, and MDC production was assessed. Dendritic cells produced higher amount of MDC than macrophages did after LPS stimulation, but PGE$_2$ pretreatment had no effect on MDC production in both cells (Fig. 5E). Macrophages and dendritic cells produced the same amount of MDC after anti-CD40 Ab stimulation, and PGE$_2$ pretreatment similarly enhanced MDC production in both cells (Fig. 5F). It has been reported that CD4$^+$ T cells, especially Th2, are MDC-producing cells. Although we could not detect MDC production in LPS-, anti-CD40 Ab-, or plate-coated anti-CD3 Ab-stimulated T cells (Fig. 5G, and data not shown), purified CD4$^+$ T cells produced only a small amount of MDC following the stimulation with immobilized anti-CD3 Ab in the presence of PGE$_2$ (Fig. 5H). IP-10 production was also assessed. IP-10 production was not detectable in B cells, T cells, and CD4$^+$ T cells stimulated with anti-CD40 Ab, LPS, or anti-CD3 Ab (data not shown). Macrophages/dendritic cells produced high amount of IP-10 after stimulation with LPS or anti-CD40 Ab, and PGE$_2$ pretreatment suppressed, but IFN-$\gamma$ pretreatment enhanced IP-10 production in these cells the same as spleen cells (Fig. 5, I).
and J). These results indicate that PGE2 mainly affects APCs (B cells, macrophages, and dendritic cells) to enhance MDC production and to suppress IP-10 production.

Modulation of enhanced production of MDC in PGE2-treated APCs by cytokines

Next we investigated the effect of cytokines on PGE2-enhanced MDC production in B cells, macrophages, and dendritic cells. As shown in Fig. 6, PGE2, IL-4, and IL-5 alone once again enhanced MDC production. The combination of PGE2 and IL-4 or IL-5 had additive effect on MDC production in B cells and macrophages/dendritic cells. The combination of PGE2 and IL-5 was the strongest in MDC production in both cell types, especially in macrophages/dendritic cells. IFN-γ, IL-10, and TGF-β suppressed MDC production, as shown in Fig. 4. These cytokines also suppressed the enhanced production of MDC by PGE2 in B cells and macrophages/dendritic cells. IL-6 had no effect on the PGE2-induced enhanced production of MDC. Leukotriene B4 (LTB4), another arachidonic acid derivative, alone or in combination with PGE2, had only a little effect on MDC production in macrophages/dendritic cells.

Endogenous PGE2 also serves as an enhancing factor for MDC production

In all experiments to date, cells were pretreated with indomethacin to inhibit endogenous production of PGE2 after stimulation. PGE2 was mainly produced by APCs such as macrophages and dendritic cells. Then we investigated the effect of endogenous PGE2 from macrophages/dendritic cells on MDC production. Splenic macrophages/dendritic cells were pretreated with PBS, PGE2, indomethacin, or PGE2 plus indomethacin for 12 h, and then stimulated with anti-CD40 Ab for an additional 48 h. As shown in Fig. 7, indomethacin treatment suppressed MDC production by anti-CD40 Ab stimulation. However, the addition of PGE2 reversed the suppressed production of MDC by indomethacin. PGE2 only slightly enhanced MDC production by anti-CD40 Ab stimulation in the absence of indomethacin. We also assessed the amount of PGE2 in the supernatants after stimulation. Macrophages/dendritic cells

FIGURE 2. PGE2 regulates IP-10 and MDC production by spleen cells. Spleen cells were pretreated with PBS, PGE2, IL-4, or IFN-γ in the presence of indomethacin for 12 h, and then stimulated with indicated stimulators for additional 48 h. After stimulation, cell culture supernatants were collected, and the amounts of IP-10 and MDC were detected by competitive ELISA inhibition assay. The results are expressed as mean ± SD of chemokines (pg/ml) produced by 5 × 10^6 spleen cells in triplicate cultures of a representative experiment. *, Significantly increased from PBS-pretreated group. #, Significantly decreased from PBS-pretreated group.

FIGURE 3. Time course of MDC production by spleen cells. Spleen cells were pretreated with or without 100 nM PGE2 in the presence of indomethacin for 12 h, and then stimulated with indicated stimulators for additional 24, 48, and 72 h. Culture supernatants were collected, and the amount of MDC was detected by competitive ELISA inhibition assay. *, Significantly increased from PBS-pretreated group.
produced PGE₂ about 4000 pg/2 × 10⁶ (about 1 nM), and indomethacin treatment completely inhibited PGE₂ production (Fig. 7). These results indicate that endogenously produced PGE₂ plays a role to enhance MDC production by macrophages/dendritic cells.

**Discussion**

There are many reports showing that PGE₂ plays an important role for Th2 activation and that PGE₂ primarily affects the induction phase of Th differentiation, such as suppression of IL-12 production and Th1 development, and polarization to Th2 (25–28). PGE₂ also suppresses several chemokine production by various types of cells (39–43). To our knowledge, there has been no report on the up-regulation of chemokine production by various types of cells (39–43). To our knowledge, there has been no report on the up-regulation of chemokine production by various types of cells (39–43). To our knowledge, there has been no report on the up-regulation of chemokine production by various types of cells (39–43).

MDC and TARC are known as specific Th2 chemoattractants (11–13). Several reports have shown that the preferential chemotactic properties of these chemokines are found not only in vitro but also in vivo using model mice with several Th2-related diseases. In the mouse model of airway inflammation, macrophages in lungs (resident and infiltrating macrophages) produced MDC, and the neutralization of MDC by the administration of specific Ab in vivo reduced Th2 recruitment to the inflammatory sites and inhibited airway hyperreactivity (17, 18). In NC/Nga mice, model mice of atopic dermatitis, MDC was highly produced by dermal dendritic cells in the lesional skin (19). In the mouse model of endotoxin liver injury, MDC and TARC were produced by cells in the granuloma, which was mainly composed of macrophages/dendritic cells, and neutralization of TARC protected the mice from acute lethal liver damage caused by Th2 (20). These reports suggest that not only Th2 and eosinophils, but also APCs recruited to the inflamed sites, and Th2 attractant chemokines, MDC or TARC, were mainly produced by APCs. Many reports have indicated that Th2 cytokines, IL-4 and IL-13, are strong inducers of MDC and TARC from APCs (12, 13, 23). However, we found that cell to cell interaction between T cells and APCs was more important for the chemokine production. Furthermore, MDC productions are regulated not only by cytokines, but also by PGE₂. PGE₂ up-regulated MDC production by CD40-stimulated macrophages/dendritic cells, but the enhancing effect of PGE₂ on LPS-stimulated ones was weak (Fig. 5). On the other hand, IL-4 up-regulated MDC production by both LPS- and CD40-stimulated macrophages/dendritic cells. These differential effects probably depend on different signal transduction pathways. LPS is a representative APC stimulator. However, CD40 stimulation is a more appropriate model for an activation of APCs in vivo than LPS stimulation, because chemokine productions in vivo are not limited to endotoxin stimulation, and higher amounts of MDC were produced by CD40 stimulation than by LPS stimulation. We consider that, in the induction of chemokines by APCs, T-APC interaction is more important than the stimulation with LPS or cytokines alone, and

![FIGURE 4](http://www.jimmunol.org/) Enhanced production of MDC by PGE₂ depends on the elevation of intracellular cAMP, but not the activation or suppression of other cytokine production. A and B, Spleen cells were pretreated with PBS, PGE₂, dbcAMP, or IBMX in the presence of indomethacin for 12 h, and then stimulated with LPS (A) or anti-CD40 Ab (B) for additional 48 h. C and D, Spleen cells were pretreated with PBS, PGE₂, IL-4, IL-5, IL-6, IFN-γ, IL-12, IL-10, TGF-β, anti-IFN-γ Ab, or anti-IL-12 Ab for 12 h, and then stimulated with LPS (C) or anti-CD40 Ab (D) for additional 48 h. Culture supernatants were collected, and the amount of MDC was detected by competitive ELISA inhibition assay. * Significant increased from PBS-pretreated group.
propose that T-APC interaction, especially CD40-CD40L interaction, is a more effective signal for the induction of chemokine production by APCs. Kornbluth et al. also proposed the importance of CD40-CD40L interaction in chemokine production in an HIV infection model (44).

There are several reports indicating that PGE2 suppresses chemokine mRNA expression and chemokine production in various types of cells, and this suppressive effect depends on the accumulation of intracellular cAMP. For example, PGE2 or intracellular accumulation of cAMP suppresses RANTES production by murine mesangial cells (39), MIP-1α production by RAW264.7 macrophage cell line (40), IL-8 release from human neutrophils (41), MIP-1α production by PBMC (42), and IP-10 mRNA expression by cultured keratinocytes (43). Our results also indicate that the IP-10 production of spleen cells is reduced by PGE2 (Fig. 1), which is consistent with the report on IP-10 production by keratinocytes (43), and that the enhanced production of MDC by PGE2 also depends on the accumulation of intracellular cAMP (Fig. 2). It has also been reported that PGE2 alters the cytokine profile in several cells, for example, PGE2 suppresses IL-12, but enhances IL-10 production via accumulation of intracellular cAMP (27). However, neutralization of IFN-γ or IL-12 and the addition of IL-10 had only a little effect on MDC production (Fig. 2). Furthermore, cAMP-enhancing agents such as dbcAMP and IBMX also enhanced MDC production (Fig. 4). These results suggest that PGE2-induced elevation of cAMP is an important signal for MDC production. The accumulation of intracellular cAMP is not restricted to the regulation of cytokine and chemokine productions.

FIGURE 5. IP-10 and MDC were mainly produced by APCs in spleen cells. B cells (A and B) and macrophages (Mφ)/dendritic cells (DC) (C, D, I, and J) were pretreated with PGE2, IL-4, or IFN-γ in the presence of indomethacin, and then they were stimulated with LPS (A, C, and I) or anti-CD40 Ab (B, D, and J) for additional 48 h. Macrophages and dendritic cells (E and F) were purified from spleen cells, respectively. Each cell was pretreated for 12 h, and then stimulated for 48 h, as described above. T cells (G) and CD4⁺ T cells (H) were stimulated with plate-coated anti-CD3 Ab in the presence or absence of PGE2 or IL-4 for 48 h. Culture supernatants were collected, and the amount of MDC and IP-10 was assessed by competitive ELISA inhibition assay. *, Significantly increased from PBS-pretreated group. #, Significantly decreased from PBS-pretreated group.
Several reports have indicated that the elevation of cAMP enhanced the expression of costimulatory molecules, B7-2, on macrophages, and these macrophages preferentially activated Th2 (45, 46). Another report showed that the cAMP accumulation in naive T cells inhibited Th1 development in vitro (25). These reports and our findings indicate that cAMP regulates Th1/Th2 balance at a number of different stages. Interestingly, a representative Th2 activator, IL-4, does not participate in the elevation of cAMP (47). This finding indicates that PGE2 engages Th2 activation in a quite different way from IL-4.

We determined cell types of MDC production and found that all cell types, such as T cells, B cells, macrophages, and dendritic cells, produced MDC following appropriate stimuli and were up-regulated by PGE2 (Fig. 5). Several reports have indicated that MDC is produced by T cells and is regulated by Th1/Th2 cytokines such as IL-4 and IFN-γ, and that T cell-derived MDC is very important in several diseases (37, 38). We also found that T cells, especially CD4+ T cells, produced MDC after CD3 ligation in the presence of PGE2 the same as the MDC production by APCs (Fig. 5). However, the amount of MDC produced by CD4+ T cells was much lower than that of APCs (B cells, macrophages, and dendritic cells). Therefore, we consider that the main cell types of MDC production are APCs. MDC production by B cells is also an interesting finding, and ABCD-1, a mouse homologue of human MDC, was found to be expressed in B cells and dendritic cells (33, 34). It is well known that B cells participate in the development of Th2 subset, and it has been reported that CD40 stimulation is necessary for the induction of Th2 by B cells (6, 48). However, we suggest that T-B interaction also induces T cell recruitment via B cell-derived chemokines, and PGE2 up-regulate chemokine production by B cells.

We also found an interesting phenomenon regarding IL-5. IL-5 is a well-known cytokine generally detected in allergic diseases and produced by Th2, mast cells, and eosinophils (1). Our results indicate that IL-5 is also an enhancing factor for MDC production by APCs (Figs. 4 and 6). Surprisingly, IL-5 in cooperation with PGE2 induced a much higher amount of MDC from APCs, which was stronger than the combination of IL-4 and PGE2 (Fig. 6).

**FIGURE 6.** Effect of Th1 or Th2 cytokines on enhanced production of MDC by PGE2-treated cells. B cells (5 × 10⁶/ml, A and B) or macrophages/dendritic cells (DC) (2 × 10⁶/ml, C) were pretreated with IL-4, IL-5, IL-6, IL-10, IFN-γ, TGF-β, or LTB4 in the presence of PGE2 and indomethacin for 12 h, and then stimulated with LPS (A) or anti-CD40 Ab (B and C) for 48 h. Culture supernatants were collected, and the amount of MDC was assessed by competitive ELISA inhibition assay or enzyme immunoassay kit, respectively. *, Significantly different.

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These results suggest that IL-5, PGE₂, or the combination of both factors are more important than IL-4 in the recruitment of Th2. LTβR, another arachidonic acid derivative that is one of the chemical mediators detected in allergic response, alone and in combination with PGE₂, had only a little effect on MDC production (Fig. 6).

In conclusion, we found a novel function of PGE₂ in Th2 activation. PGE₂ preferentially enhanced MDC, but suppressed IP-10 production. These effects depended on the accumulation of intracellular cAMP, but not on the suppression of Th1 cytokines, IFN-γ and IL-12, and the induction of suppressive cytokines, IL-10 and TGF-β. APCs produced especially higher amounts of MDC via CD40 stimulation and PGE₂, suggesting that T-APC interaction is very important signal for the induction of MDC. PGE₂ has been known as one of the immunosuppressive factors. PGE₂ mainly inhibited Th1 development at the induction phase of Th subset differentiation. There are also several reports that PGE₂ production is enhanced in Th2-related diseases (49–51). From our findings, we propose that PGE₂ also affects Th2 activation at an effector phase, that is, PGE₂ up-regulates Th2-related chemokine production and enhances Th2 recruitment to the inflammatory sites. Large amounts of PGE₂ are produced by suppressor macrophages and endothelial cells in chronic inflammation (52). Therefore, we consider that PGE₂ plays an important role for the progression of Th2-type inflammation and Th2-related diseases. Further clarification of the regulation mechanism of not only cytokine, but also chemokine and PGE₂ productions will be required to understand the precise mechanism of immunological diseases such as autoimmune or allergic diseases.

References


