ELSEVIER

Biochemical Pharmacology 61 (2001) 1153–1160

Biochemical Pharmacology

Regulation of TNFα and interleukin-10 production by prostaglandins I_2 and E_2: studies with prostaglandin receptor-deficient mice and prostaglandin E-receptor subtype-selective synthetic agonists

Shiho Shinomiya[a], Hiroaki Naraba[a], Akinori Ueno[a], Iku Utsunomiya[a], Takayuki Maruyama[a], Shuichi Ohuchida[b], Fumitaka Ushikubi[c], Koichi Yuki[c], Shuh Narumiya[d], Yukihiko Sugimoto[e], Atsushi Ichikawa[e], Sachiko Oh-ishi[f,*]

[a]*Department of Pharmacology, School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan*
[b]*Minase Research Institute, Ono Pharmaceutical Co. Ltd., Osaka 618-8585, Japan*
[c]*Department of Pharmacology, Asahikawa Medical College, Asahikawa, 078-8510, Japan*
[d]*Department of Physiological Chemistry, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto 606-8501, Japan*
[e]*Department of Pharmacology, Faculty of Medicine, Kyoto University, Kyoto 606-8501, Japan*
[f]*Kitasato Institute, 5-9-1 Shirokane, Minatoku, Tokyo, 108-8642, Japan*

Received 28 June 2000; accepted 27 October 2000

Abstract

To know which receptors of prostaglandins are involved in the regulation of TNFα and interleukin 10 (IL-10) production, we examined the production of these cytokines in murine peritoneal macrophages stimulated with zymosan. The presence of PGE_2 or the PGI_2 analog carbacyclin in the medium reduced the TNFα production to one-half, whereas IL-10 production increased several fold; and indomethacin caused the reverse effects, suggesting that endogenous prostaglandins may have a regulatory effect on the cytokine production. Among prostaglandin E (EP) receptor-selective synthetic agonists, EP2 and EP4 agonists caused down-regulation of the zymosan-induced TNFα production, but up-regulation on the IL-10 production; while EP1 and EP3 agonists showed no effect. Macrophages harvested from prostaglandin I (IP) receptor-deficient mice showed the up- and down-regulatory effects on the cytokine production by the EP2 and EP4 agonists or PGE_2, but no effect was obtained by carbacyclin. On the contrary, macrophages from EP2-deficient mice showed the effect by PGE_2, carbacyclin, and the EP4 agonist, but not by the EP2 agonist; and the cells from EP4-deficient mice showed the effect by PGE_2, carbacyclin, and EP2 agonist, but not by the EP4 agonist. These functional effects of prostaglandins well accorded with the mRNA expression of TNFα and IL-10 when such expression was examined by the RT-PCR method. The peritoneal macrophages from normal mice expressed IP, EP2, and EP4 receptors, but not EP1 and EP3, when examined by RT-PCR. Thus the results suggest that PGI_2 and PGE_2 generated simultaneously with cytokines by macrophages treated with zymosan may influence the cytokine production through IP, EP2, and EP4 receptors.

Keywords: IP receptor; EP2 receptor; EP4 receptor; TNFα; IL-10; Zymosan

1. Introduction

Prostaglandins are well-known modulators of biological functions, such as body-defense mechanisms against infections or trauma [1,2]. It has been reported that cytokine production at an inflammatory site may lead to the induction of particular enzymes and receptors for inflammatory mediators including those for eicosanoids [3] and that eicosanoids in turn act to co-operate with other mediators to cause an enhanced inflammatory reaction [4]. Prostaglandins modulate further cytokine production by increasing the intracellular cAMP level [5]. We reported earlier that TNFα, IL-1, IL-6, and CINC (cytokine-induced neutrophil chemoattractant) were sequentially produced in the exudates of rats with carrageenin-induced and zymosan-induced pleurisy during the early stage of pleurisy [6–7].

* Corresponding author. Tel.: +81-3-3444-6161 ext 2120; fax: +81-3-5791-6120.
E-mail address: oh-ishi@kitasato.or.jp (S. Oh-ishi).

Abbreviations: cAMP, 3′,5′-cyclic adenosine monophosphate; EP, prostaglandin E receptor; IL-10, Interleukin-10; IP, prostacyclin receptor; PG, prostaglandin; RT-PCR, reverse transcriptase-polymerase chain reaction; TNFα, tumor necrosis factor alpha.

0006-2952/01/$ – see front matter
PII: S0006-2952(01)00586-X

These chemokines in the inflammatory site cause further chemotaxis to attract granulocytes and monocytes, and then migrating leukocytes in turn produce further cytokines and other mediators [7–8]. As therapeutic agents, non-steroidal anti-inflammatory drugs have been used as remedies for these inflammatory diseases, but several reports including ours have warned that indomethacin increased the production of pro-inflammatory cytokines, such as TNFα and IL-1, but suppressed that of IL-6 and IL-8 in the inflammatory exudates, and that the addition of PGE_2 or PGI_2 reversed these effects [9].

Furthermore, the production of TNFα and IL-1 by rat leukocytes was found to be related inversely to the content of cAMP in the cells; and treatment with phosphodiesterase inhibitors significantly suppressed the production of these cytokines [10]. However, the receptors on the cells that may have caused these effects have not yet been precisely identified. In a previous paper we demonstrated that PGE_1, PGE_2, and PGI_2 almost equally regulated the cytokine production in rat resident pleural cells [10].

Because IP, EP2 and EP4 receptor stimulation is known to increase cAMP level, while EP1 receptor relates to phosphoinisitide turnover, and EP3 stimulation may decrease cAMP level [11], in this present study, therefore, we intend to clarify which prostaglandin receptors would contribute to the essential regulating action on cytokine production by using some selective synthetic agonists for EP receptors [12] and prostaglandin receptor-deficient mice, i.e. IP, EP2, and EP4 receptor-deficient mice [13–14].

2. Materials and methods

2.1. Agents

Carbacyclin (Biomol Res.), prostaglandin E_2 (Cayman Chem. Co.,) dibutyryl cyclic AMP (Sigma), pentoxyfilline (Sigma), indomethacin (Sigma), H89 dihydrochloride (Calbiochem.), recombinant murine IL-10 (Biosource International), RPMI medium (Life Technologies), and thioglycollate (Difco Labs.) were purchased. Anti mouse IL-10 (rat IgG_1, Endogen) was also purchased, and anti mouse TNFα was a kind gift from Professor Screiber [15]. EP subtype agonists, Ono-DI-004 (EP1 agonist), OnoAE1-257 (EP2 agonist), Ono-AE1-248 (EP3 agonist), and Ono-AE1-329 (EP4 agonist), were prepared by Ono Pharmaceutical Co. [12].

2.2. Animals and peritoneal cell collection

Male and female C57BL/6 mice were purchased from Japan SLC, IP receptor-deficient mice [14] and EP2 receptor-deficient ones [16] were prepared as described previously, and back crossed over at least six generations to C57BL/6. The EP4 receptor knock out mice used in this experiment had a mixed genetic background of 129sv/ola and C57BL/6 mice, because all the EP4 knock-out mice born from the back-crossed parents died within 2 days after birth from patent ductus arteriosus [17].

2.3. Macrophage collection

Adherent macrophages were prepared from the peritoneal exudate cells of mice that had received thioglycollate medium (1 mL for 20 g body weight) 4 days before, as follows: the peritoneal cells obtained from 3–4 mice were mixed and seeded into 24-well plates (Falcon, Becton Dickinson and Co.) at the density of 10^6 cells in 1 mL RPMI medium per well. After a 2-hr incubation in a CO_2-incubator, the supernatants and non-adherent cells were discarded; and the adherent cells, over 90% macrophages, were used for the experiments. The cells were preincubated in RPMI for 20 min and then opsonized zymosan (prepared as described previously [18] was added, after which the cells were incubated for 2, 5, 16, and 24 hr. In some wells prostaglandins and agents were added during the preincubation. After the desired incubation time had passed, the medium of each well was collected for measurement of cytokines and prostaglandins, and stored at $-70°$ until analyzed.

2.4. Detection of prostaglandin receptors

The adherent cells were used for extraction of RNA to detect mRNA of prostaglandin receptors by the reverse transcriptase-polymerase chain reaction (RT-PCR) using the following probes [14,16,17]: IP receptor primers used were 5′-CCTGCAGTGTTTGTGGCCTATGCTCGAAAC-3′ and 5′-CTGCTGTCTGGGGCGATGGCCTGAGTGAAG3′; EP1 primers were 5′-ACCCTGCATCCTGAGCAGCACTGGCCCTCT-3′ and 5′-CGATGGCCAACACCACCAACACCAGCAGGG-3′; EP2 primers were 5′-TTCATATTCAAGAAACCAGACCCTGGC3′ and 5′-AGGGAAGAGGTTTCATCCATGTAGGCAAAG-3′; EP3 primers were 5′-ATCCTCGTGTACCTGTCACAGCGACGCTGG-3′ and 5′-TGCTCAACCGACATCTGATTGAAGATCATT-3′, and EP4 primers were 5′-CGTAGTATTGTGCAAGTCGC-3′ and 5′-GGCGATGAGTAAGATGACCA-3′. The RT-PCR was performed using a SuperScript preamplification system (Life Technologies), according to the manufacturer's instructions, with 1 μg of total RNA from macrophages used as a template. Equal amounts of each RT product were amplified by PCR with Taq polymerase (Life Technologies) for 30 cycles consisting of 30 sec each at 94°, 56°, and 72°. The amplified cDNA fragments were resolved electrophoretically on 2% (w/v) agarose gels and visualized by staining with ethidium bromide.

2.5. Assay for TNFα and IL-10

Assays were performed with commercial ELISA kits for TNFα and IL-10 (Biosource).

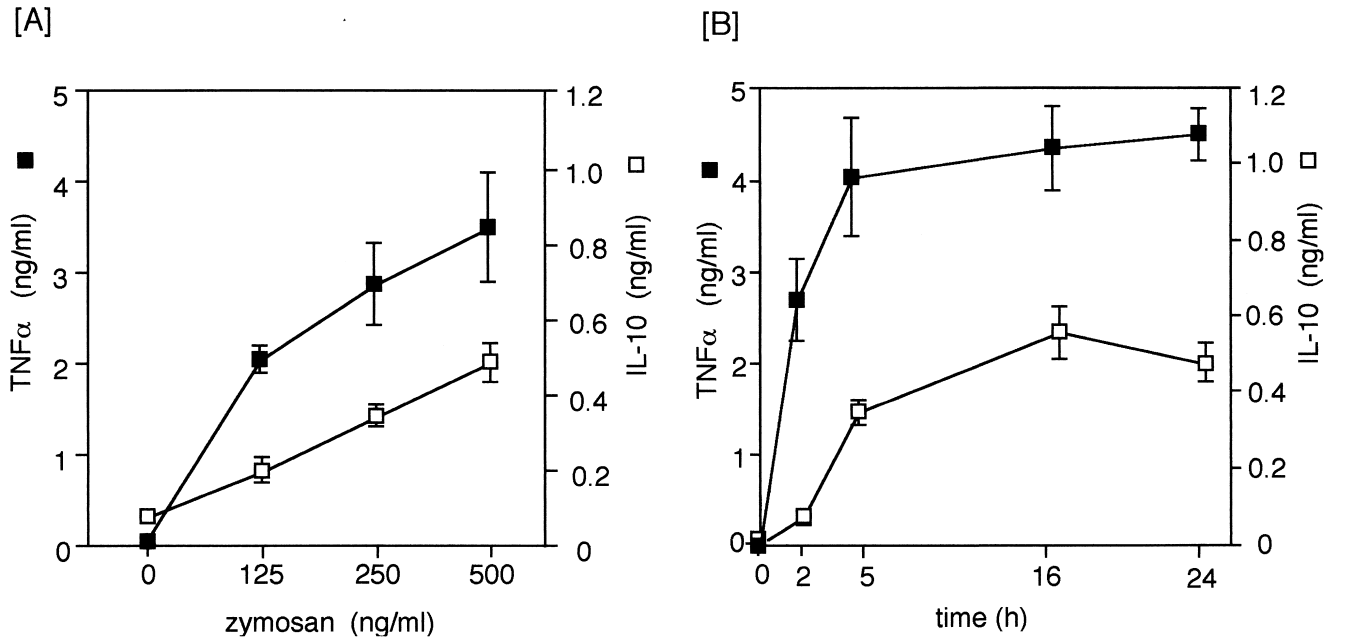

Fig. 1. Concentration-response relationships of zymosan in the production of TNFα (closed squares) and interleukin-10 (open squares) over a 5-hr incubation (A), and time course of the cytokine production (B) of murine peritoneal macrophages treated with zymosan. (A) Thioglycollate medium-elicited peritoneal murine macrophages pooled from 3–4 C57 BL/6 mice were stimulated with a zymosan suspension of 0, 125, 250, or 500 μg/mL/10^6 cells for 5 hr. TNFα and IL-10 in the medium were then assayed. Data are the means with SEM of 3 independent experiments. In B, macrophages were incubated with zymosan at the concentration of 500 μg/mL/10^6 cells, and the supernatants of the incubation medium were collected at the indicated times to measure TNFα (closed symbols) and IL-10 (open symbols). Data are the means with SEM of 3 to 5 independent experiments.

2.6. Detection of mRNA for TNFα and IL-10

RNA was extracted as the same way as that for the receptor detection (section 1–4) from the macrophage cultures after a 5-hr incubation. The RT-PCR was carried out in the same way as described in the above section, and the primers used were 5′-TCCGCTTCTCCGCTGCCA-3′ and 5′-CACCTTTGTGTCTGGGACCT-3′ for TNFα and 5′-CTTCAGCCAGGTGAAGAC-3′ and 5′-TGGAGTCCAGCAGACTCAAT-3′ for IL-10; and they were purchased from Amersham Pharmacia Biotech. The annealing temperature for TNFα was 56°; and that for IL-10, 59°.

2.7. Assay for prostaglandins

PGE_2 and 6-keto-$PGF_{1\alpha}$ in the incubation mixture were assayed by the use of ELISA kits (Cayman), after purification by SEP-pak isolation, as described previously [20].

3. Results

3.1. Production of TNFα and IL-10 in murine peritoneal macrophages in response to zymosan, and effects of prostaglandins and cAMP

Thioglycollate-elicited peritoneal macrophages obtained from C57BL/6 mice produced significant amounts of TNFα and IL-10 in response to zymosan dose- and incubation time-dependently. The amounts of both cytokines produced by macrophages (10^6/mL) linearly increased with the dose of zymosan (Fig. 1A). The time course of TNFα production showed a plateau after 5 hr, whereas IL-10 increased gradually up to 16 hr and then decreased slightly by 24 hr (Fig. 1B).

The prostacyclin analog carbacyclin and PGE_2 significantly decreased TNFα and increased IL-10; and pentoxyfilline, which is an inhibitor of phosphodiesterases, and dibutyryl cAMP dose-dependently decreased TNFα and increased IL-10 (Fig. 2). On the contrary, indomethacin increased TNFα and decreased IL-10 significantly. The presence of exogenous PGE_2 or carbacyclin with zymosan stimulation reduced the amount of TNFα approximately to one-half, while either of them increased the amount of interleukin-10 several fold. These features are similar to those reported by us for rat macrophages [9], and by others for murine or human leukocytes [5,15]. The involvement of A-kinase in cytokine regulation was proved by an experiment with the A-kinase inhibitor H89. As shown in Table 1, H89 (30 μM) mostly reversed the effects of carbacyclin and PGE_2 on the down-regulation of TNFα production and up-regulation of IL-10 production.

3.2. Prostaglandins produced by macrophages in response to zymosan

According to the results of a previous study [21], 6-keto-$PGF_{1\alpha}$ and PGE_2 were the major prostaglandins produced

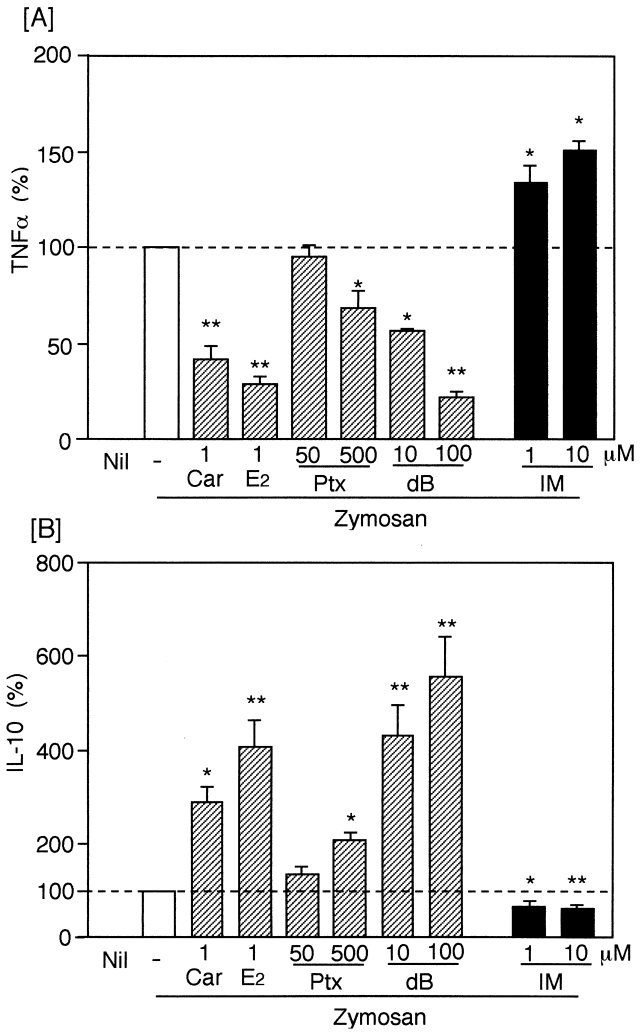

Fig. 2. Effect of prostaglandins and agents that increase intracellular cAMP. Macrophages were stimulated with zymosan (500 μg/mL) in the presence of the indicated concentrations of carbacyclin, PGE_2, pentoxiphylline (Ptx), dibutyryl cyclic AMP (dB), or indomethacin (IM) for 5 hr. Data are shown as the means with SEM of 3–7 independent experiments, expressed as a percent of those obtained with zymosan stimulation alone. * and ** indicate that the measured values are statistically significantly different at $P < 0.05$ and $P < 0.01$, respectively, from the value obtained for zymosan alone, when calculated by 2-way analysis of variance and evaluated with Dunett's t-test.

by macrophages; therefore we measured their levels and time course in zymosan-stimulated macrophages, as shown in Fig. 3. We also measured prostaglandins in the samples from IP receptor-deficient mice (Fig. 3) and found no difference with those from the wild-type mice, confirming our previous study, in which we had found no difference in the prostaglandin production by resident peritoneal macrophages from IP receptor-deficient and its wild-type mice during the acetic acid-induced writhing reaction [14]. As seen in Fig. 3, 6-keto-$PGF_{1\alpha}$ almost reached its plateau at 5 hr, and PGE_2 was still increasing at 16 hr; and both levels reached the level (approximately 0.1 μM) that could affect cytokine production by 16 hr.

Table 1
Effect of H89 on the regulation of zymosan-induced cytokine production by prostaglandins

	carbacyclin (1 μM)		PGE_2 (1 μM)	
H89 (30 μM)	−	+	−	+
TNFα (%)	64.4	96.7	26.0	85.5
IL-10 (%)	122.9	84.5	230.6	110.9

The macrophages were stimulated for 5 hr with 500 μg/mL zymosan in the presence of carbacyclin or PGE_2 with or without H89. Cytokines in the medium were measured and expressed as a percent of each cytokine value obtained with zymosan alone. Data represent those of a typical experiment.

3.3. Effect of synthetic agonists for EP receptor on the zymosan-induced cytokine production

As shown in Fig. 4, EP2 agonist and EP4 agonist as well as PGE_2 and carbacyclin showed dose-related and significant up-regulation of IL-10, and down-regulation of TNFα production. However, EP1 and EP3 agonists had no significant effect, suggesting that PGE_2 may exert its regulatory effect on cytokine production through its EP-receptor subtypes EP2 and EP4.

3.4. Expression of PG receptors on the macrophages

The RT-PCR method clearly demonstrated the presence of mRNA for IP, EP2, and EP4 receptors on the murine peritoneal macrophages, but no significant band was found for EP1 or EP3 receptors (Fig. 5).

3.5. Cytokine production and effect of IP and EP agonists on macrophages obtained from PG receptor-deficient mice

The results for the cytokine production in IP receptor-, EP2 receptor-, and EP4 receptor-deficient mice are shown in

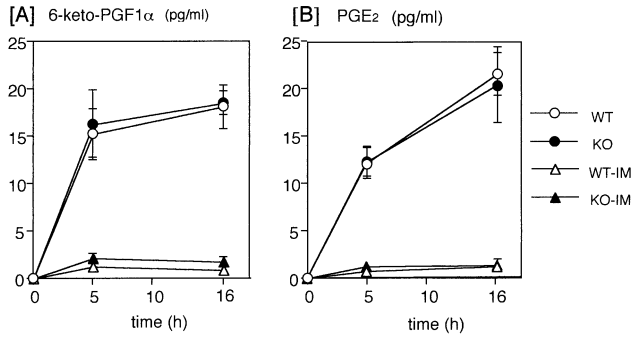

Fig. 3. Prostaglandin production of macrophages during stimulation with zymosan. Macrophages from IP receptor-deficient (KO) or wild-type mice (WT) were incubated with zymosan in the absence or presence of 10 μM indomethaci (IM), in the same way as described in previous figures for 5 and 16 hr. PGE_2 and 6-keto-PGF1α in the incubation medium were measured with ELISA kits as described in the Methods section. Data are the means with SEM of 4 independent experiments.

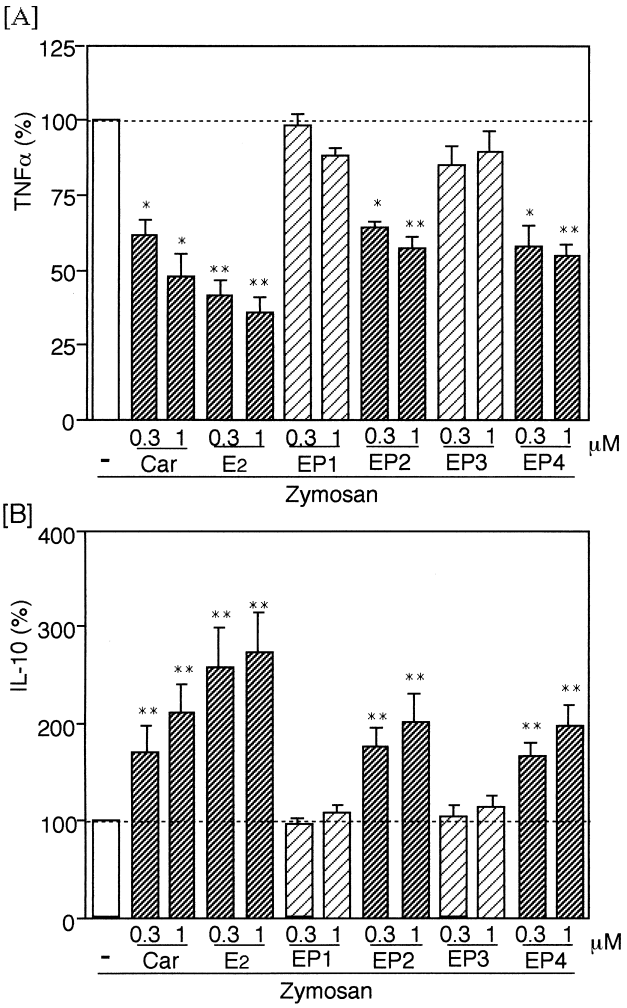

Fig. 4. Effects of PGE_2, carbacyclin, and EP receptor agonists on the zymosan-stimulated production of TNFα and IL-10 by macrophages. Mouse peritoneal macrophages were stimulated with zymosan for 5 hr in the presence of the indicated concentrations of carbacyclin (Car), PGE2 (E2), or EP1, EP2, EP3, or EP4 receptor agonist. Data are the means with SEM of 4 independent experiments, and are expressed as a percent of those obtained with zymosan stimulation alone. * and ** indicate that the measured values are statistically significantly different at $P < 0.05$ and $P < 0.01$, respectively, from the value obtained for zymosan alone, when calculated by 2-way analysis of variance and evaluated with Dunett's t-test.

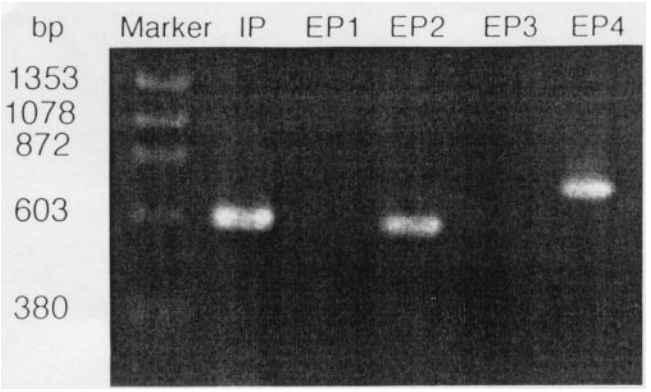

Fig. 5. Expression of prostaglandin receptors on the murine peritoneal macrophages. Total RNA was extracted from murine peritoneal macrophages as described in the section 2 and 1 μg was subjected to RT-PCR and electrophoresis.

Fig. 6. Macrophages collected from IP receptor-deficient mice expressed the up- or down-regulatory effect by PGE_2 and EP2 and EP4 agonists on their cytokine production, but the production was not affected by carbacyclin. Macrophages harvested from EP2 receptor-deficient mice showed the regulatory effect on the cytokines in the presence of EP4 agonist, PGE_2, and carbacyclin, but no effect with the EP2 agonist. On the contrary, those cells from EP4 receptor-deficient mice showed the effect with the EP2 agonist but not with the EP4 agonist. However, they showed the effects by PGE_2 and carbacyclin. Thus, the above results all together indicate that PGI_2 and PGE_2 may cause down-regulation of the TNFα production and up-regulation of IL-10 production via their receptors IP, EP2, and EP4, when murine macrophages are stimulated with zymosan. The effects of indomethacin were not significant in these receptor-deficient mice except for a slight effect in the EP4 deficient ones, suggesting that the presence of any one of these receptors may enough to cause the intrinsic regulation of the cytokine production.

3.6. Effects of prostaglandin agonists on the expression of TNFα and IL-10 mRNA

Fig. 7 shows RT-PCR results for TNFα and IL-10 expression in the macrophages incubated for 5 hr with zymosan in the presence of prostaglandin agonists. Zymosan stimulation of macrophages caused clear expression of RNA for TNFα (Fig. 7A), and slight expression for that of IL-10 (Fig. 7B). Effects of indomethacin and the reverse effect of dibutyryl cAMP were observed with all macrophage samples (i.e. WT, IP receptor-deficient, EP2 and EP4 receptor-deficient mice). The macrophages from IP receptor-deficient mice lacked an effect of carbacyclin on TNFα and IL-10 messages. In the case of EP2 receptor-deficient macrophages, the effects of PGE_2 and EP4 agonist (down-regulation for TNFα and up-regulation of IL-10) were observed, but no effect of EP2 agonist was found. The macrophages obtained from EP4 receptor-deficient mice manifested effects of PGE_2 and EP2 agonist, but no effect of the EP4 agonist. EP1 and EP3 agonists showed no effect on any macrophages. These results well agree with the data for the cytokine protein production assessed by the immunoassay (Fig. 6).

3.7. Interaction of TNFα and IL-10

As shown in Table 2, simultaneous addition of IL-10 (1 ng) with zymosan significantly suppressed the production of TNFα. Furthermore, the presence of antibody to IL-10 increased TNFα production dose-dependently, but antibody

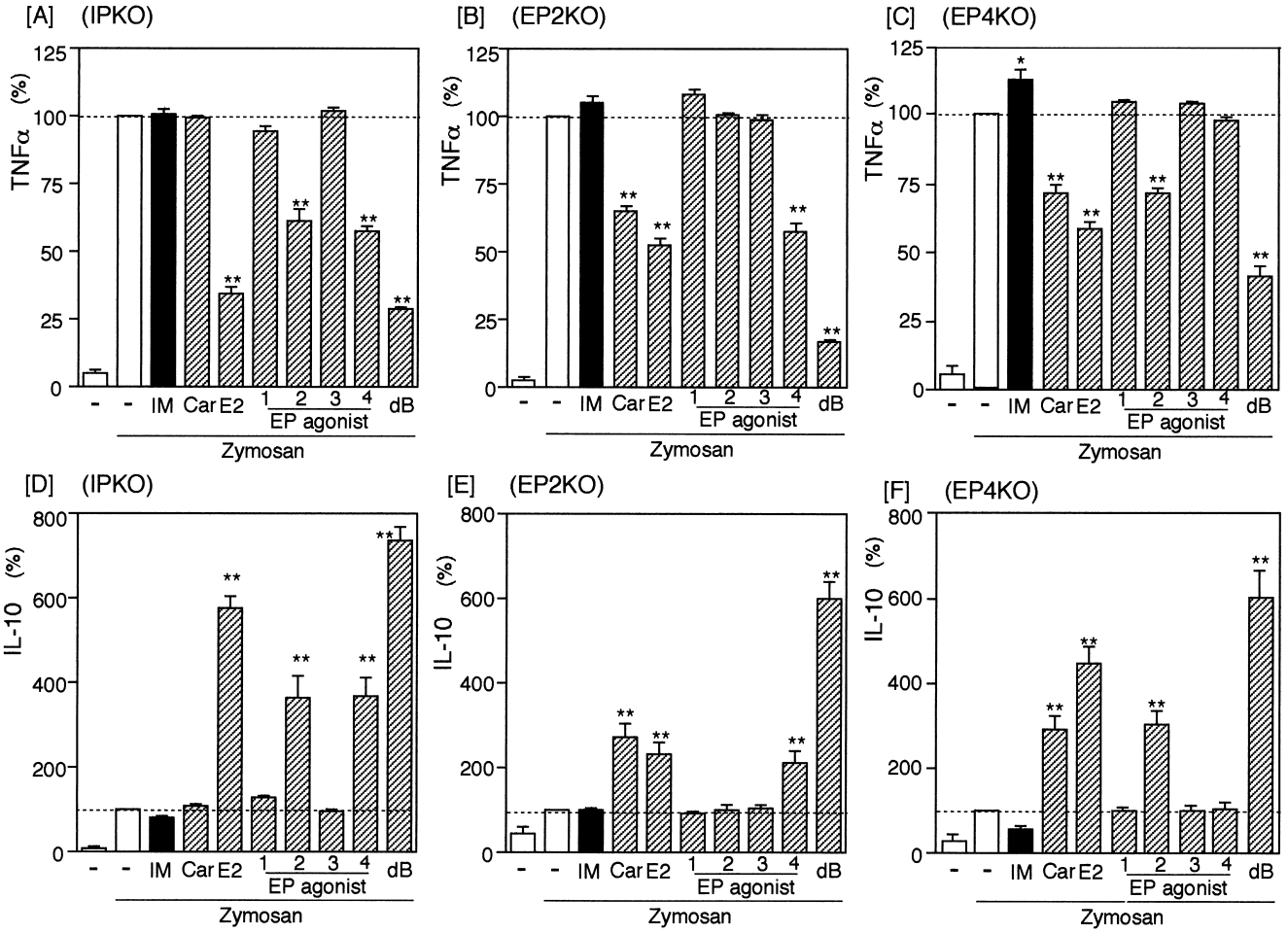

Fig. 6. Effect of prostaglandin agonists on the TNFα and IL-10 production in prostaglandin receptor-deficient mice. Macrophages obtained from IP receptor- (IPKO: A, D), EP2 receptor- (EP2KO: B, E), and EP4 receptor- (EP4KO: C, F) deficient mice were subjected to zymosan stimulation in the presence of indomethacin (IM, 10 μM), carbacyclin (Car, 1 μM), PGE2 (E2, 1 μM), EP1, EP2, EP3, EP4 agonists (each 1 μM), or dibutyryl cAMP (dB, 100 μM) for 5 hr. TNFα and IL-10 were measured and expressed as a percent of the level obtained with zymosan alone. * and ** show that measurements are statistically significantly different at $P < 0.05$ and $P < 0.01$, respectively, from each value for zymosan alone, when calculated by 2-way analysis of variance and evaluated with Dunett's *t*-test. Data express the means with SEM of 3–4 independent experiments.

to TNFα did not show significant effect on IL-10 production (data not shown). These results suggest that endogenously produced IL-10 may probably partly down-regulate TNFα production during the incubation with zymosan.

4. Discussion

In this experiment we found that opsonized zymosan could stimulate the mouse peritoneal macrophages to produce TNFα and IL-10, and this result is consistent with previous work showing that cytokines were produced in the pleural cavity of rats during zymosan-induced pleurisy [7]. As for the time courses of production of TNFα and IL-10, the onset of IL-10 production was slower than that of TNFα. In considering the data of Table 2, 0.5 ng/mL of endogenous IL-10 could inhibit TNFα production; however, at the early phase, i.e. before 5 hr, the concentration of IL-10 was not high enough to suppress TNFα production. At the later phase, however, its concentration became high enough to suppress TNFα. After 5 hr the level of TNFα was almost constant; therefore a partial inhibitory effect of endogenous IL-10 could explain this plateau.

The effects of PGE_2, carbacyclin, pentoxyfilline, and dibutyryl cAMP on the cytokine production (Fig. 2) are consistent with previous findings [10], i.e., down-regulation of TNFα and up-regulation of IL-10. The reverse effect of indomethacin was also expected [9]. All of these effects on the cytokine production could be explained as the regulatory effects on the cytokine mRNA expression shown in Fig. 7.

The experiment with EP receptor agonists demonstrated that these synthetic agonists showed fairly excellent selectivity over the range of concentrations used [12]. Because mRNA for EP1 and EP3 receptors were not detected in the macrophages, the findings of no effect of either EP1 and EP3 agonists is reasonable. Important well-known signal

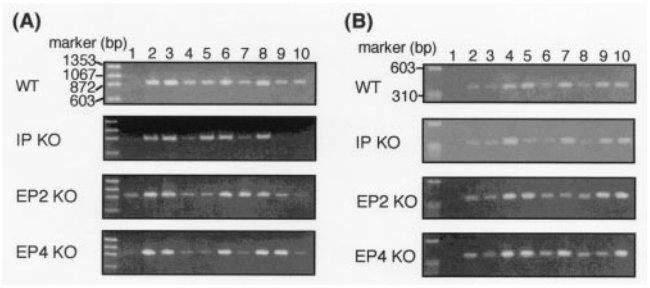

Fig. 7. Effects of IP or EP agonists on the expression of mRNAs for TNFα (A) and IL-10 (B) in the zymosan-stimulated macrophages. Typical results of RT-PCR for TNFα and IL-10 mRNA are shown. Total RNA was extracted from the cell lysates of the macrophage samples incubated for 5 hr from wild-type mice (WT), IP receptor-deficient mice (IPKO), EP2 receptor deficient mice (EP2KO), and EP4 receptor-deficient mice (EP4KO), and 1 μg of each was subjected to RT-PCR and electrophoresis. Lane 1 expresses macrophages incubated with vehicle alone; lane 2 shows macrophages incubated with zymosan alone (500 μg/mL); lane 3, those incubated with zymosan plus indomethacin; 10 μM, lane 4, zymosan plus PGE_2 1 μM; lane 5, zymosan plus carbacyclin 1 μM; lane 6, zymosan plus EP1 agonist 1 μM; lane 7, zymosan plus EP2 agonist 1 μM; lane 8, zymosan plus EP3 agonist 1 μM; lane 9, zymosan plus EP4 agonist; and lane 10, zymosan plus dibutyrylcAMP 100 μM.

transduction pathways mediated via IP, EP2, and EP4 and triggered by an increase in intracellular cAMP were also demonstrated in this experiment by use of these agonists; and at the same time, their receptors were found to be expressed on the macrophages.

Salient facts demonstrated by the results of this study on prostaglandin receptor-deficient mice (Fig. 6) are the following: 1) Support for selectivity of each agonist was obtained, because macrophages from each receptor-deficient mouse showed only the lack of effect of each corresponding agonist. 2) Both EP2 and EP4 receptors mediate the action of PGE_2, because both of each type of receptor-deficient mice expressed the appropriate effect of PGE_2, mediated by each counterpart, and the effect of PGE_2 in IP receptor-deficient mice, mediated by both subtypes, was twice as high as that in EP2 or EP4 receptor-deficient mice. However, the EP2 receptor-mediated effect of PGE_2 may be more pronounced than the EP4 receptor-mediated one, since the effect of indomethacin was more apparent in the EP4 receptor-deficient mice. 3) This experiment also suggests some redundant effects of these prostaglandin receptors, IP, EP2, and EP4. These functional effects well accord with the expression of mRNAs for TNFα and IL-10 in the above samples (Fig. 7), and the data suggests that prostaglandins may regulate the cytokine production via the mRNA expression.

Regulation of cytokine production by an increase in intracellular cAMP or by PGE_2 has been reported in LPS-stimulated macrophages and many other cells [21–23]. The mechanism of regulation of cytokine production by increased intracellular cAMP could be hypothesized as its action on nuclear factor cAMP-responsive element modulators that participate in transcription factor assembly, such as CREB, CREM (cAMP responsive element modulator) or C/EBP (ACAAT enhancer-binding protein) [24–26]. Recently CREM was found to be a cAMP-inducible component in the neuroendocrine system as one of the early response gene [27]. Therefore, these transcription factors could be new targets for development for modulating agents on prostaglandin receptors. Some studies suggest that PGE2 regulates the cytokine production via increasing the cAMP level leading to production of IL-10, which cytokine then suppresses TNFα production [28], while another study argues there is no involvement of IL-10 in the cAMP-induced inhibition of TNFα production [29]. However, our data suggest that the inhibitory effect of IL-10 on TNFα production may be partial, and can not explain the whole potent suppressive effect of prostaglandins or cAMP.

Taken together the present results demonstrate that PGI_2 and PGE_2 regulate production of proinflammatory and anti-inflammatory cytokines through their receptors, IP, EP2 and EP4, in inflammatory macrophages. Therefore, prostaglandins, which are produced at a local inflammatory site, could have an anti-inflammatory effect on the cytokine production by causing an increase in cAMP, in addition to the classical pro-inflammatory effect, such as an increase in vascular permeability or sensitization of pain receptors.

Table 2
Effect of IL-10 and antibody to IL-10 on the zymosan-induced TNFα production

IL-10 (ng/mL)		anti-IL-10 antibody (μg/mL)		
0.5	1.0	10	20	40
57.3 ± 2.4	45.7 ± 1.2	111.5 ± 2.1	130.0 ± 1.0	144.9 ± 4.1

TNFα amount is calculated as a percent of the level with zymosan alone after a 5-hr incubation and shown as the mean with SEM of 4 experiments. IL-10 or its antibody was added 15 min before the start of incubation.

Acknowledgment

We are grateful to Ms Miwako Tarui for her assistance with some of the experiments. This work was supported in part by a grant-in-aid (No. 09557213) for scientific-research from the Ministry of Education, Science and Culture of Japan.

References

[1] Samuelsson B, Goldyne M, Granstrom E, Hamberg M, Hammastrom S, Malmstein C. Prostaglandins and thromboxane. Ann Rev Biochem 1978;47:997–1029.
[2] McGiff JC. Prostaglandins, prostacyclin, and thromboxanes. Ann Rev Pharmacol Toxicol 1981;21:479–509.
[3] Movat HZ, Cybulsky MI, Colditz IG, Chan MK, Dinarello CA. Acute inflammation in gram-negative infection: endotoxin, interleukin 1, tumor necrosis factor, and neutrophils. Fed Proc 1987;46:97–104.

[4] Oh-ishi S, Hayashi M, Yamaki K. Inflammatory effects of acetylglycerylether phosphorylcholine; Vascular permeability increase and induction of pleurisy in rats. Prost Leukotr Med 1986;22:21–33.
[5] Kunkel SL, Spengler M, May MA, Spengler R, Larrick J, Remick D. Prostaglandin E2 regulates macrophage-derived tumor necrosis factor gene expression. J Biol Chem 1988;263:5380–4.
[6] Utsunomiya I, Nagai S, Oh-ishi S. Sequential appearance of IL-1 and IL-6 activities in rat carrageenin-induced pleurisy. J Immunol 1991; 147:1803–18.
[7] Utsunomiya I, Ito M, Oh-ishi S. Generation of inflammatory cytokines production in zymosan-induced pleurisy in rats: TNF induces IL-6 and cytokine-induced neutrophil chemoattractant (CINC) in vivo. Cytokine 1998;10:956–63.
[8] Utsunomiya I, Ito M, Watanabe K, Tsurufuji S, Matsushima K, Oh-ishi S. Infiltration of neutrophils by intrapleural injection of tumor necrosis factor, interleukin-1, and interleukin-8 in rats and its modification by actinomysin D. Br J Pharmacol 1996;70:285–90.
[9] Utsunomiya I, Nagai S, Oh-ishi S. Differential effects of indomethacin and dexamethasone on cytokine production in carrageenin-induced rat pleurisy. Eur J Pharmacol 1994;252:213–8.
[10] Oh-ishi S, Utsunomiya I, Yamamoto T, Komuro Y, Hara Y. Effects of prostaglandins and cyclicAMP on cytokine production in rat leukocytes. Eur J Pharmacol 1996;300:255–9.
[11] Ushikubi F, Hirata M, Narumiya S. Molecular biology of prostanoid receptors; an overview. J Lipid Med Cell Sign 1995;12:343–59.
[12] Suzawa T, Miyaura C, Inada M, Maruyama T, Sugimoto Y, Ushikubi F, Ichikawa A, Narumiya S, Suda T. The role of prostaglandin E receptor subtypes (EP1, EP2, EP3 and EP4) in bone resorption: An analysis using specific agonists for the respective EPs. Endocrinology 2000;141:1554–9.
[13] Narumiya S, Sugimoto Y, Ushikubi F. Prostanoid receptors: structures, properties, and function. Physiol Rev 1999;79:1193–226.
[14] Murata T, Ushikubi F, Matsuoka T, Hirata M, Yamasaki M, Sugimoto Y, Ichikawa A, Aze Y, Tanaka T, Yoshida N, Ueno A, Oh-ishi S, Narumiya S. Altered pain perception and inflammatory response in mice lacking prostacyclin receptor. Nature 1997;388:678–82.
[15] Sheehan KC, Ruddle NH, Schreiber RD. Generation and characterization of hamster monoclonal antibodies that neutralize murine tumor necrosis factors. J Immunol 1989;142:3884–90.
[16] Hiraki H, Segi E, Sugimoto Y, Hirose M, Saji T, Ushikubi F, Matsuoka T, Noda Y, Tanaka T, Yoshida N, Narumiya S, Ichikawa A. Abortive expansion of the cumulus and impaired fertility in mice lacking the prostaglandin E receptor subtype EP2. Proc Natl Acad Sci USA 1999;96:10501–6.
[17] Segi E, Sugimoto Y, Yamasaki A, Aze Y, Oida H, Nishimura T, Murata T, Matsuoka T, Ushikubi F, Hirose M, Tanaka T, Yoshida N, Narumiya S, Ichikawa A. A patent ductus arteriosus and neonatal death in prostaglandin receptor EP4-deficient mice. Biochem Biophys Res Commun 1998;246:7–12.
[18] Naraba H, Imai Y, Hayashi M, Oh-ishi S. Enhanced production of platelet-activating factor in rat stimulated leukocytes by blockades of acylation of lysophospholipids. Jpn J Pharmacol 1993;61:109–13.
[19] Katsuyama M, Ikegami R, Karahashi H, Amano F, Sugimoto Y, Ichikawa A. Characterization of the LPS-stimulated expression of EP2 and EP4 prostaglandin E receptors in mouse macrophage-like cell line, J774.1. Biochem Biophys Res Commun 1998;251:727–31.
[20] Matsumoto H, Naraba H, Murakami M, Kudo I, Yamaki K, Ueno A, Oh-ishi S. Concordant induction of prostaglandin E_2 synthase with cyclooxygenase-2 leads to preferred production of prostaglandin E_2 over thromboxane and prostaglandin D_2 in lipopolysaccharide-stimulated rat peritoneal macrophages. Biochem Biophys Res Commun 1997;230:110–4.
[21] Renz H, Gong J, Schmidt A, Nain M, Gemsa D. Release of tumor necrosis factor-alpha from macrophages. Enhancement and suppression are dose-dependently regulated by prostaglandin E_2 and cyclic nucleotides. J Immunol 1988;141:2388–93.
[22] Semmler J, Gebert U, Eisenhut T, Moeller J, Schonearting M, Allera A, Endres S. Xanthine derivatives: comparison between suppression of tumor necrosis factor-α production and inhibition of cAMP phosphodiesterase activity. Immunology 1993;78:520–5.
[23] Kambayashi T, Alexander HR, Fong M, Strassmann G. Potential involvement of IL-10 in suppressing tumor-associated macrophage. J Immunol 1995;154:3383–90.
[24] Hill CS, Treisman R. Transcription regulation by extracellular signals: mechanisms and specificity. Cell 1995;80:199–211.
[25] Willis SA, Nisen PD. Inhibition of lipopolysaccharide-induced IL-1b transcription by cyclic adenosine monophosphate in human atrocytic cells. J Immunol 1995;154:1399–406.
[26] De Cesare D, Fimia GM, Sassone-Corsi P. Signaling routes to CREM and CREB: plasticity in transcriptional activities. Trends Biochem Sci 1999;24:281–5.
[27] Monaco L, Lamas M, Tamai K, Lalli E, Zazopoulos E, Penna L, Nantel F, Foulkes NS, Mazzucchelli C, Sassone-Corsi P. Coupling transcription to signaling pathways: cAMP and nuclear factor cAMP responsive element modulator. Adv Second Mess Phos Res 1997;31: 63–74.
[28] Kambayashi T, Jacob CO, Zhou D, Mazurek N, Fong M, Strassmann G. Cyclic nucleotide phosphodiesterase type VI participates in the regulation of IL-10 and in the subsequent inhibition of TNFα and IL-6 release by endotoxin-stimulated macrophages. J Immunol 1995; 155:4909–16.
[29] Seldon PM, Barnes PJ, Giembycz MA. Interleukin-10 does not mediate the inhibitory effect of PDE-4 inhibitors and other cAMP-elevating drugs on lipopolysaccharide-induced tumor necrosis factor-alpha generation from human peripheral blood monocytes. Cell Biochem Biophys 1998;29:179–201.