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Effects of prostaglandins and cyclic AMP on cytokine production in rat leukocytes

Sachiko Oh-ishi [a,*], Iku Utsunomiya [a], Takaaki Yamamoto [b], Yoshihiro Komuro [b], Youichi Hara [b]

[a] *Department of Pharmacology, School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108, Japan*
[b] *Research Center, Sumitomo Pharmaceutical Co., Ltd., Konohana-ku, Osaka 554, Japan*

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Abstract

Prostaglandins E_1, prostaglandin E_2, 3-oxa-methano-prostaglandin I_1 (SM-10906), a stable prostaglandin I_2 analog, and dibutyryl cyclic AMP suppressed the production of tumor necrosis factor and interleukin-1 in lipopolysaccharide-stimulated rat pleural resident monocytic cells, whereas they enhanced the production of interleukin-6 and cytokine-induced neutrophil chemoattractant (CINC), a rat interleukin-8-like chemokine, in these cells. SM-10906 also inhibited the in vivo production of tumor necrosis factor and interleukin-1 in pleural exudates, when injected into the rat pleural cavity concomitantly with carrageenin. The cyclic AMP (cAMP) level in the lipopolysaccharide-stimulated resident cells was increased when the cells were incubated in the presence of prostaglandin E_1, prostaglandin E_2 or SM-10906. Prostaglandin I_2 showed only slight effects. The addition of pentoxifylline, a phosphodiesterase inhibitor, to the incubation mixture increased the cAMP level and also enhanced the effect of prostaglandins, indicating that these regulating actions of prostaglandins may be exerted partly through a mechanism involving an increased intracellular cAMP level.

Keywords: CINC (cytokine-induced neutrophil chemoattractant); cAMP; Interleukin-1; Interleukin-6; Prostaglandin E_2; Prostaglandin I_2; TNF (tumor necrosis factor)

1. Introduction

It is known that in inflammatory exudates, such as mouse pleural exudates and those from rats with pleurisy, proinflammatory cytokines are sequentially involved in the local inflammatory reactions (Goto et al., 1984; Utsunomiya et al., 1991). We found earlier that pretreatment of rats with anti-inflammatory agents, such as indomethacin, changed the cytokine levels in the inflammatory exudates, i.e., indomethacin enhanced the production of tumor necrosis factor and interleukin-1, but suppressed the interleukin-6 level in the exudates of carrageenin-induced rat pleurisy (Utsunomiya et al., 1994). Furthermore, when rat pleural resident cells were cultured with bacterial lipopolysaccharide in vitro, the production of tumor necrosis factor and interleukin-1 was suppressed in the presence of prostaglandin E_2 (Utsunomiya et al., 1994). Recently many reports have appeared describing the regulation of cytokine production by prostaglandins or cyclic AMP (cAMP), i.e., suppression of tumor necrosis factor and interleukin-1 production in human monocytic cells by prostacyclin analogs (Crutchley et al., 1994), inhibition of lipopolysaccharide-induced interleukin-1 transcription by cAMP in human astrocytic cells (Willis and Nisen, 1995), and suppression of tumor necrosis factor production by xanthine derivatives (Semmler et al., 1993). Therefore in this study we examined the effects of several prostaglandins on cytokine production and also conducted experiments to determine if cAMP, a second messenger of prostaglandin E-type receptor activation, is involved in the regulation of cytokine production in inflammatory cells.

2. Materials and methods

2.1. Animals

Six to seven-week-old male Sprague-Dawley rats (Japan SLC, Hamamatsu, Japan) and 5-week-old female C3H/HeJ mice (CLEA Japan, Tokyo, Japan) were used.

* Corresponding author. Tel.: +81-3-3444-6161; fax: +81-3-3442-3875.

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2.2. Agents

Carrageenin, dibutyryl cyclic AMP, and pentoxifylline were purchased from Sigma Chemical Co., St. Louis, MO, USA. Prostaglandins E_1, E_2, and I_2 (Funakoshi Chemical Co., Tokyo, Japan), phytohemagglutinin (Wellcome, Dartford, UK), and lipopolysaccharide (*Escherichia coli*; 055:B5, Difco, MI, USA) were obtained from the sources indicated. 3-Oxa-methano-prostaglandin I_1 (SM-10906, i.e., (+)-[2-[(2*R*,3*aS*,4*R*,5*R*,6*aS*)-octahydro-5-hydroxy-4-[(*E*)-(3*S*,5*S*)-3-hydroxy-5-methyl-1-nonenyl]-2-pentalenyl]ethoxy]acetic acid) was synthesized as reported earlier (Kawakami et al., 1993). Recombinant human tumor necrosis factor (3×10^6 U/mg) and recombinant human interleukin-1α (2.26×10^7 U/mg) were generously provided by Dainippon Pharmaceutical Co. (Osaka, Japan). Recombinant human interleukin-6 (5.18×10^6 U/mg) and the MH60.BSF2 cell line were kind gifts of Dr. Toshio Hirano (Biomedical Research Center, Osaka University Medical School, Osaka, Japan). The L929 cell line was generously donated by Dr. Motohiro Matsuura (Jichi Medical School, Tochigi, Japan).

2.3. Rat pleurisy

Rat pleurisy was produced by intrapleural injection of 2% carrageenin, 0.1 ml, as described previously (Oh-ishi et al., 1989). SM-10906, 10 μg/0.1 ml alone or SM-10906 mixed with carrageenin, was also injected into the pleural cavity, and exudates at 1, 3 and 5 h after the injection were collected. Exudates or a saline wash (1 ml) of the pleural cavity were obtained at the indicated times and measured for cytokines.

2.4. Cytokine assay

Tumor necrosis factor levels in samples were assessed in terms of cytotoxicity toward murine fibroblast cell line L929, and interleukin-1 was assayed by thymocyte proliferation as previously reported (Utsunomiya et al., 1991). Interleukin-6 was measured by the use of interleukin-6-dependent murine hybridoma as previously reported (Utsunomiya et al., 1994). Cytokine-induced neutrophil chemoattractant (CINC) was assayed with an enzyme-linked immunosorbent assay (ELISA) kit (Iida et al., 1992; Nakagawa et al., 1992) from Immuno-Biological Laboratories, Co., kindly donated by Drs. Tsurufuji and Watanabe, Inst. Cytosignal Res., Tokyo, Japan.

2.5. Cytokine production by resident cells

Resident pleural cells were collected by washing of the pleural cavity with sterile saline solution as described previously (Utsunomiya et al., 1994) The cells (1×10^6) were cultured with lipopolysaccharide (10 μg/ml) and prostaglandins (1 μM) or dibutyryl cAMP (0.1 mM) with or without pentoxifylline (0.5 mM) in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin, and 50 μl 2-mercaptoethanol in 24-well culture plates for 24 h. Indomethacin (10 μM) was added to the medium to inhibit endogenous prostaglandin production so that only the effect of exogenous prostaglandins would be examined. The supernatant was assayed for cytokine levels.

2.6. cAMP assay

Pleural resident cells were incubated in the same way as above and cells were harvested at 10 and 60 min after incubation. Cyclic AMP in the stimulated cells was measured by a radioimmunoassay kit (Yamasa Shoyu Co., Tokyo, Japan) after extraction as previously reported (Yamamoto et al., 1994).

2.7. Statistics

The values are expressed as means $\pm$ S.E., and the statistical significance of differences between groups was analyzed by two-way analysis of variance with Student's *t*-test (Dunnett's multiple comparison). *P* values of less than 0.05 were considered as significant. Curve fitting to estimate correlation coefficients for cytokine levels versus cAMP level was done by use of a computer software program (CA Cricket Graph III, Computer Assoc., NY, USA).

3. Results

3.1. Effect of 3-oxa-methano-prostaglandin I_1 (SM-10906) on the cytokine level in the exudate of rats with pleurisy

Tumor necrosis factor and interleukin-1 levels in the exudate of rats with carrageenin-induced pleurisy increased to a peak at 3 h and then decreased at 5 h and the co-administration of 3-oxa-methano-prostaglandin I_1 (SM-10906) with carrageenin significantly decreased tumor necrosis factor and interleukin-1 levels at 3 h and the tumor necrosis factor level at 5 h also (Fig. 1). However, the levels of interleukin-6 and CINC that were elevated by carrageenin at 3 h were unaffected when the prostaglandin I_1 analog was also present (Fig. 1). No significant difference was seen in the exudate volume and leukocyte migration between the exudates of carrageenin alone and carrageenin with SM-10906-treated animals (Fig. 2).

3.2. Effect of prostaglandins, dibutyryl cAMP, and pentoxifylline on the cytokine levels in rat resident cells in vitro in response to LPS

Fig. 3 shows that tumor necrosis factor (A), interleukin-1 (B), interleukin-6 (C), and CINC (D) were produced in rat

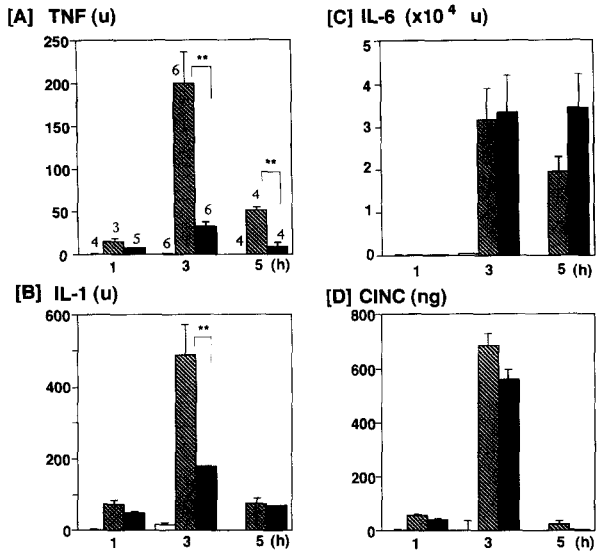

Fig. 1. Effect of 3-oxa-methano-prostaglandin I_1 (SM-10906) on the production of cytokines in the exudates of rats with carrageenin-induced pleurisy. Rats received an intrapleural injection of SM-10906 (10 μg/0.1 ml) alone (open columns), carrageenin (2%, 0.1 ml) alone (hatched columns) or a carrageenin mixed with SM-10906 (black columns). Tumor necrosis factor (A), interleukin-1 (B), interleukin-6 (C), and CINC (D) levels in the exudates at 1, 3, and 5 h after the injection were assayed as described in Materials and methods. * and ** indicate statistically significant difference ($P < 0.05$ and $P < 0.01$, respectively) between the groups of carrageenin alone versus carrageenin with SM-10906. Numbers above columns indicate numbers of rats used.

resident pleural cells treated with lipopolysaccharide for 24 h. This increased tumor necrosis factor production (Fig. 3A) was significantly suppressed by the presence of prostaglandin E_1, E_2, SM-10906, and dibutyryl cAMP, but prostaglandin I_2 showed no effect. In the presence of

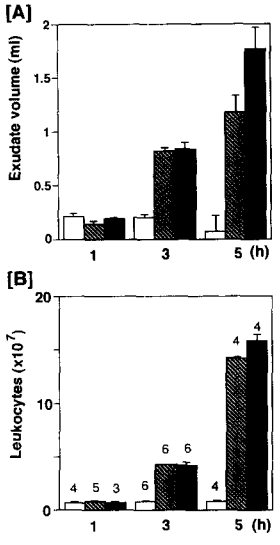

Fig. 2. Effect of SM-10906 on the exudate and total leukocyte number in rats with carrageenin-induced pleurisy. Pleural exudate volume (A) and total leukocyte number (B) in the exudate were assessed at 1, 3, and 5 h after the carrageenin injection as described in Materials and methods. Column marks are the same as those in Fig. 1.

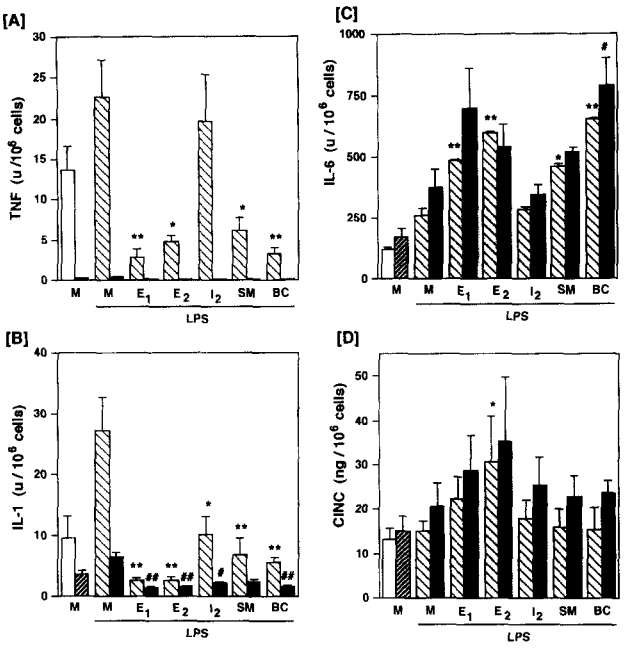

Fig. 3. Effects of prostaglandins on the cytokine production of rat resident cells in response to lipopolysaccharide and enhancing effect of pentoxifylline. Pleural resident cells were cultured for 24 h with lipopolysaccharide (10 μM) plus 1 μM prostaglandin E_1 (E_1), prostaglandin E_2 (E_2), prostaglandin I_2 (I_2), SM-10906 (SM) or 100 μM dibutyryl cAMP (BC) in the presence or absence of pentoxifylline (0.5 mM). Tumor necrosis factor (A), interleukin-1 (B), interleukin-6 (C), and CINC (D) in the supernatant were then assayed, and expressed as the means with S.E.M. of three samples. Column types are defined as follows: open columns, medium only; dark hatched columns, medium plus pentoxifylline (0.5 mM); hatched columns, lipopolysaccharide; black columns, lipopolysaccharide plus pentoxifylline. * and ** indicate significant differences from the value of the group incubated with lipopolysaccharide alone at $P < 0.05$ and $P < 0.01$, respectively. # and ## indicate significant difference at $P < 0.05$ and $P < 0.01$, respectively, from the value of the group incubated with lipopolysaccharide and pentoxifylline alone.

pentoxifylline the tumor necrosis factor level of most cultures was below the detection limit. As shown in Fig. 3B, the lipopolysaccharide-stimulated interleukin-1 level was also significantly suppressed in the presence of prostaglandin E_1, E_2, I_2, SM-10906, and dibutyryl cAMP. In the presence of pentoxifylline all agents caused a further suppression, and this suppression by prostaglandins was significant when compared with the level of interleukin-1 in the cultures treated with lipopolysaccharide and pentoxifylline alone. In contrast to tumor necrosis factor and interleukin-1, the lipopolysaccharide-stimulated interleukin-6 level was increased significantly by prostaglandin E_1, E_2, SM-10906 and dibutyryl cAMP, but not by prostaglandin I_2. Pentoxifylline showed a tendency to further increase the interleukin-6 level induced by the prostaglandins, but the difference was not significant when these values were compared with the value for lipopolysaccharide and pentoxifylline alone. In Fig. 3D, the lipopolysaccharide-induced CINC level tended to be increased by these prostaglandins, and prostaglandin E_2 had a significant effect. Pentoxifylline also showed a tendency to in-

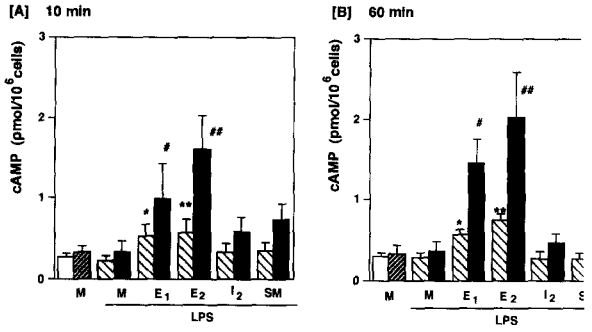

Fig. 4. Effects of prostaglandins and pentoxifylline on the cAMP level in rat resident cells in response to lipopolysaccharide. Pleural resident cells collected as described in the legend of Fig. 3 were cultured with lipopolysaccharide (10 μM) and prostaglandins (1 μM) in the presence or absence of pentoxifylline (0.5 mM). Cyclic AMP in the cells after 10 min (A) and 60 min (B) of culture was measured by the method described in Materials and methods. Column types and marks are the same as in Fig. 3.

crease further the level elicited by these prostaglandins, but the differences were not statistically significant.

3.3. Effect of prostaglandins on cAMP levels in the resident cells in response to lipopolysaccharide

As shown in Fig. 4, prostaglandin E_1 and E_2 significantly increased the cAMP level already at 10 min, and the effect was still seen at 60 min. Also, pentoxifylline further increased the cAMP level increased by either prostaglandin E_1 or E_2. This effect was statistically significant when compared with the response to cultures of lipopolysaccharide plus pentoxifylline alone. Prostaglandin I_2 and SM 10906 tended to increase the cAMP level in the presence of pentoxifylline.

3.4. Correlation coefficient for cytokine levels versus cAMP level

As shown in Fig. 5, the exponential relationships of the four cytokines versus cAMP were plotted. Negative exponential relationships of tumor necrosis factor and interleukin-1 to cAMP were observed, i.e., $r = 0.742$ and 0.904, respectively, whereas positive ones of interleukin-6 and CINC were found: $r = 0.746$ and 0.901, respectively.

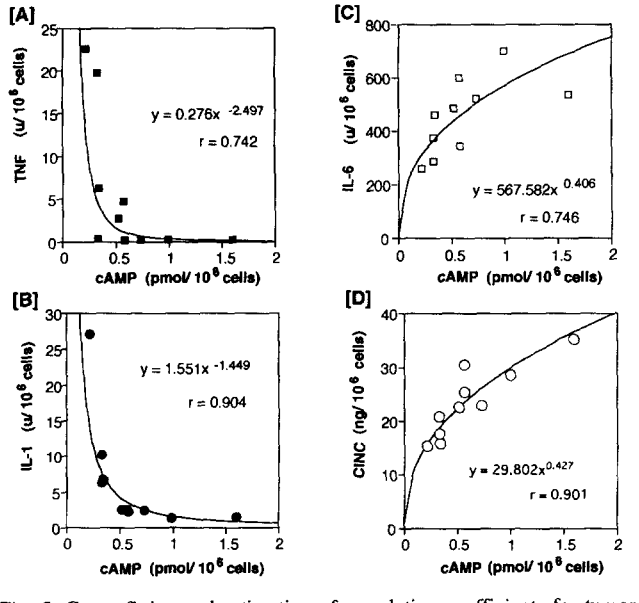

Fig. 5. Curve fitting and estimation of correlation coefficients for tumor necrosis factor (A), interleukin-1 (B), interleukin-6 (C), and CINC (D) versus cAMP. Rat pleural resident cells were stimulated with lipopolysaccharide (10 μM) plus prostaglandins (1 μM) in the presence or absence of pentoxifylline (0.5 mM). Mean values of intracellular cAMP level in 10-min cultures and means of tumor necrosis factor (A), interleukin-1 (B), interleukin-6 (C) and CINC (D) levels in the supernatants of 24-h cultures were assessed from 3 samples prepared on the same day. The mean values of cAMP were plotted on the abscissa, and cytokine levels on the ordinate; and a curve fitting as an exponential equation was done for each group.

4. Discussion

In in vitro experiments using lipopolysaccharide-stimulated resident cells, we demonstrated that prostaglandins exerted a negative effect on tumor necrosis factor and interleukin-1 production, but a positive one on interleukin-6 and CINC production, in the stimulated cells. These effects were more prominent with E-type analogs than with I-type ones, even though one of the latter is a stable synthetic analog. These effects seem to be closely related to the increased level of cAMP in the stimulated cells, since a phosphodiesterase inhibitor, pentoxifylline, enhanced the effects of the prostaglandins. This close relationship may be explained as follows. (1) When we measured the cAMP level in the cells stimulated with lipopolysaccharide in the presence of prostaglandins, we found that the increase in the cAMP level in the lipopolysaccharide-stimulated cells in the presence of prostaglandin E_1 or E_2 was significant and that prostaglandin I_2 and SM-10906 tended to increase it only slightly (Fig. 4). (2) Pentoxifylline further enhanced the increase in cAMP elicited by these two E-type prostaglandins. (3) These potencies of prostaglandins to increase cAMP mostly agree with their activities on cytokine production.

In the in vivo experiment SM-10906 suppressed tumor necrosis factor and interleukin-1 levels in the exudates, indicating that this stable analog could affect cytokine production also in vivo. However, it showed a slight effect on interleukin-6 and no effect on CINC production. These features of SM-10906 were in line with the results of the in vitro experiment presented in Fig. 3, where SM-10906 showed a slight enhancement of interleukin-6 production and only tended to increase CINC production. The lack of effect of SM-10906 on the carrageenin-induced leukocyte migration may be because of the lack of increase in CINC which is a representative of chemokines in this inflammation (Ito et al., 1993; Utsunomiya et al., in press).

As shown in Fig. 5, plots of cytokine levels versus cAMP level clearly demonstrated down-regulation of tumor necrosis factor and interleukin-1, and up-regulation of interleukin-6 and CINC by cAMP levels. Correlation coefficients calculated by exponential curve fitting demonstrated fairly good relationships for each cytokine to the cAMP level.

The effects of prostaglandins and pentoxifylline were most prominent on tumor necrosis factor among the cytokines in these rat monocytic cells. This suppression well agrees with data for murine macrophages (Kunkel et al., 1988), human mononuclear cells (Semmler et al., 1993) and human monocytic THP-1 (American Type Culture Collection) cells (Crutchley et al., 1994). However, there is some discrepancy in the sensitivity of cytokine production; for example, in human monocytic THP-1 cells, interleukin-1 production was not affected by pentoxifylline (Crutchley et al., 1994), whereas in our rat monocytes pentoxifylline suppressed interleukin-1 production. This difference might be attributable to the species difference, because our results are consistent with the report by Haynes et al. (1992), who found that prostaglandin E_1 analogs suppressed interleukin-1 production and enhanced interleukin-6 production in rat and mouse macrophages.

The mechanism of the effects of cAMP elevation on cytokine production may operate at the level of cytokine transcription, as a paper has appeared describing the cAMP-responsive transcription factors CREB (cAMP responsive element binding protein) and C/EBP (CCAAT enhancer-binding protein) involved in interleukin-1β transcription (Willis and Nisen, 1995). Up-regulation or down-regulation of these cAMP-dependent transcription factors may be a consequence of which type of complex interaction with other transcription factors occurs (Sasson-Corsi, 1994; Hills and Treisman, 1995). The elucidation of this complex regulatory process is a topic for future study.

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