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Short communication

# Prostaglandin $E_1$ suppresses tumor necrosis factor-$\alpha$ and interleukin-10 production by lipopolysaccharides-stimulated mononuclear cells

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**Abstract**

Prostaglandin $E_1$ has been clinically used in a variety of vascular occlusive diseases. We investigated the in vitro effect of prostaglandin $E_1$ on the synthesis of tumor necrosis factor-$\alpha$, interleukin-1, interleukin-6, interleukin-10 and transforming growth factor $\beta$ by human peripheral blood mononuclear cells stimulated with lipopolysaccharides. Prostaglandin $E_1$ significantly suppressed both the mRNA expression and the production of tumor necrosis factor-$\alpha$ and interleukin-10 by lipopolysaccharides-stimulated peripheral blood mononuclear cells in a dose-dependent manner ($1 \times 10^{-6}$–$1 \times 10^{-8}$ M). Since tumor necrosis factor-$\alpha$ is a potent proinflammatory cytokine involved in certain inflammatory diseases, our findings suggest the possibility to expand the clinical application of prostaglandin $E_1$. © 1998 Elsevier Science B.V.

*Keywords:* Interleukin-10; Lipopolysaccharide; Mononuclear cell; Prostaglandin $E_1$; TNF-$\alpha$ (tumor necrosis factor-$\alpha$)

## 1. Introduction

Prostaglandin $E_1$ is one of the prostanoids synthesized from linoleic acid in vivo and differs from the products of the arachidonate cascade. Two major functions of prostaglandin $E_1$, vasodilatation and anti-platelet aggregation, have been extensively investigated (Schrror, 1991) and its clinical efficacy has been proven in arterial occlusive ischemia (Carlson and Eriksson, 1973; Carlson and Olsson, 1976), systemic sclerosis (Shimizu et al., 1994; Yamanaka et al., 1997) and other conditions. In these days, prostaglandin $E_1$ is commonly used in patients with congenital or acquired pulmonary hypertension. In contrast, the pharmacological actions of prostaglandin $E_1$ on cytokine production by immune competent cells have been poorly investigated (Haynes et al., 1992). Cells of monocyte/macrophage lineage produce interleukin-1$\beta$, interleukin-6, tumor necrosis factor $\alpha$ and prostaglandins (normally prostaglandin $E_2$) after various endogenous or exogenous stimuli. We carried out an in vitro study to clarify the effects of prostaglandin $E_1$ on several cytokines secreted by human peripheral blood mononuclear cells stimulated with lipopolysaccharides.

## 2. Materials and methods

### 2.1. Cell culture

Peripheral blood was obtained from 4 healthy men aged 26, 27, 28 or 43 years who took no medication. Peripheral blood mononuclear cells were isolated by using the Ficoll-Hypaque technique. After being washed with RPMI 1640 medium three times, peripheral blood mononuclear cells were suspended in RPMI 1640 supplemented with 10% fetal calf serum at a concentration of $1 \times 10^6$ cells/ml. For RNA extraction, 10 ml of cell suspension was dispensed into 10-cm culture dishes. Peripheral blood mononuclear cells were stimulated with lipopolysaccharides (Cpaesel, Germany) at a concentration of 10 $\mu$g/ml. Prostaglandin $E_1$, a kind gift from Ohtsuka Pharmaceutical, Japan, was simultaneously added at 0, $1 \times 10^{-6}$, $1 \times 10^{-7}$ or $1 \times 10^{-8}$ M. Peripheral blood mononuclear cells were incubated for 3 and 6 h at 37°C, 5% $CO_2$. Cells were collected at the indicated times for RNA extraction. For measurement of tumor necrosis factor-$\alpha$ and interleukin-10 in the supernatant, 2 ml of cell suspension was incubated for 18 h in the same way as described above. The experiment was done in triplicate and the cell viability in each peripheral blood mononuclear cells was more than 95% after an 18-h incubation.

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### 2.2. Reverse transcriptase polymerase chain reaction (RT-PCR)

The sets of specific primers for tumor necrosis factor-$\alpha$, interleukin-1$\beta$, interleukin-6, interleukin-10, and transforming growth factor-$\beta$ were purchased from Clontech (Palo Alto, CA) and glyceraldehyde-3-phosphate dehydrogenase from Stratagene (La Jolla, CA). Total RNA was extracted from peripheral blood mononuclear cells by using a RNA extraction kit (Isogen, Nippon gene, Japan). One $\mu$g of total RNA was reverse-transcribed by the GeneAmp RNA PCR kit (Perkin-Elmer Cetus, Norwalk, CT), and cDNA was amplified by PCR according to the manufacturer's protocol with some modifications. Denaturation of cDNA was done for 1 min at 95°C, annealing for 1 min at 60–65°C (depending upon the target being amplified), and polymerization for 2 min at 72°C. Cycle numbers were determined by titration to establish a linear standard curve: 33 cycles for interleukin-6 and tumor necrosis factor-$\alpha$, 30 cycles for interleukin-10, 28 cycles for glyceraldehyde-3-phosphate dehydrogenase and 23 cycles for transforming growth factor-$\beta$ and interleukin-1$\beta$. Each PCR product was size-fractionated on a 1% agarose gel and stained with ethidium bromide. Stained gel was photographed under ultraviolet A light. Primer sets used are as follows;

interleukin-1$\beta$:
sense primer, ATGGCAGAAGTACCTAAGCTCGC
antisense primer, ACACAAATTGCATGGTGAAGTCAGTT
interleukin-6:
sense primer, ATGAACTCCTTCTCCACAAGCGC
antisense primer, GAAGAGCCCTCAGGCTGGACTG
interleukin-10:
sense primer, AAGCTGAGAACCAAGACCCAGACATCAAGGCG
antisense primer, AGCTATCCCAGAGCCCCAGATCCGATTTTGG
tumor necrosis factor-$\alpha$:
sense primer, GAGTGACAAGCCTGTAGCCCATGTTGTAGCA
antisense primer, GCAATGATCCCAAAGTAGACCTGCCCAGACT
transforming growth factor-$\beta$:
sense primer, GCCCTGGACACCAACTATTGCT
antisense primer, AGGCTCCAAATGTAGGGGCAGG
glyceraldehyde-3-phosphate dehydrogenase:
sense primer, CCACCCATGGCAAATTCCATGGCA
antisense primer, TCTAGACGGCAGGTCAGGTCCACC.

### 2.3. Enzyme-linked immunosorbent assay

The concentration of tumor necrosis factor-$\alpha$ and interleukin-10 in the culture supernatant was measured in duplicate by using an enzyme-linked immunosorbent assay kit (Biosource, Belgium) according to the manufacturer's protocol. Before quantification, the culture supernatant collected was diluted 1:4 for tumor necrosis factor-$\alpha$ or 1:5 for interleukin-10 with dilution buffer supplied by the manufacturer. The measurement was done in duplicate. The detection range was from 15 to 1500 pg/ml for tumor necrosis factor-$\alpha$ and 11 to 1335 pg/ml for interleukin-10. Statistical analyses were done by analysis of variance (ANOVA) followed by Dunnett's two-tailed test.

## 3. Results

Stimulation with lipopolysaccharides increased mRNA messages for interleukin-1$\beta$, interleukin-6, interleukin-10 and tumor necrosis factor-$\alpha$ in peripheral blood mononuclear cells as compared with those in unstimulated peripheral blood mononuclear cells at 3 h or 6 h. Simultaneous addition of lipopolysaccharides and prostaglandin $E_1$ suppressed the mRNA expression of tumor necrosis factor-$\alpha$ and interleukin-10 in a dose-dependent manner at 3 h (Fig. 1) and 6 h (data not shown). The mRNA expression of

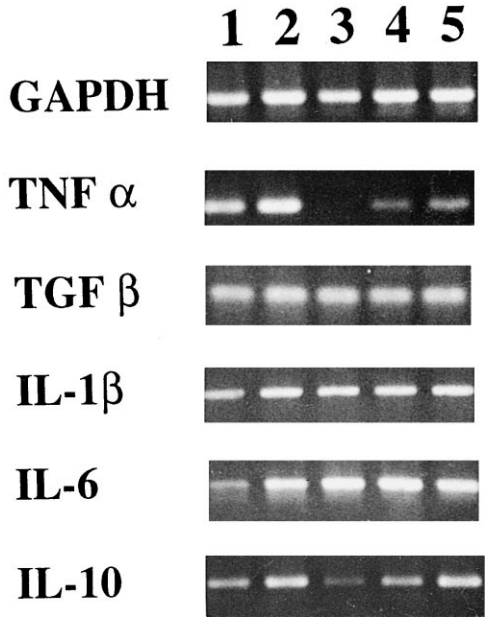


Fig. 1. Expression of glyceraldehyde-3-phosphate dehydrogenase, transforming growth factor-$\beta$, tumor necrosis factor-$\alpha$, interleukin-1, interleukin-6 and interleukin-10 mRNAs by lipopolysaccharides-stimulated peripheral blood mononuclear cells incubated with prostaglandin $E_1$. Isolated peripheral blood mononuclear cells ($1\times10^6$/ml) were incubated with lipopolysaccharides (10 $\mu$g/ml) and $1\times10^{-6}$, $1\times10^{-7}$ or $1\times10^{-8}$ M of prostaglandin $E_1$ for 3 h. The expression of mRNA for tumor necrosis factor-$\alpha$ and interleukin-10 was suppressed in a dose-dependent manner. The figure shows the representative results of one sample (43 year-old man). Lane 1: unstimulated peripheral blood mononuclear cells, lane 2: lipopolysaccharides-stimulated peripheral blood mononuclear cells, lane 3: lipopolysaccharides-stimulated peripheral blood mononuclear cells with $1\times10^{-6}$ M prostaglandin $E_1$, lane 4: lipopolysaccharides-stimulated peripheral blood mononuclear cells with $1\times10^{-7}$ M prostaglandin $E_1$, lane 5: lipopolysaccharides-stimulated peripheral blood mononuclear cells with $1\times10^{-8}$ M prostaglandin $E_1$.

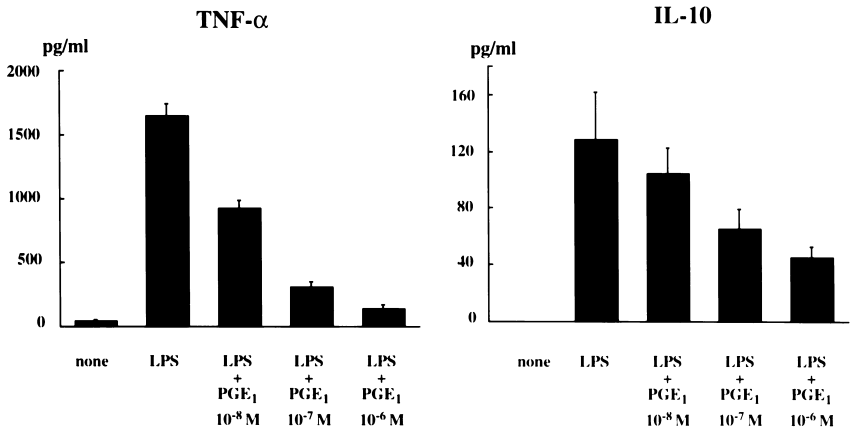


Fig. 2. Amounts of tumor necrosis factor-$\alpha$ and interleukin-10 in the culture supernatant. The amounts of tumor necrosis factor-$\alpha$ and interleukin-10 in the supernatant of peripheral blood mononuclear cells incubated for 18 h were measured by enzyme-linked immunosorbent assay. The peripheral blood mononuclear cells were from 3 healthy subjects (26, 27, and 28 year-old men). The amount of both tumor necrosis factor-$\alpha$ and interleukin-10 was significantly suppressed in a dose-dependent manner ($P < 0.01$).

other cytokines, however, was not affected by prostaglandin $E_1$ at the concentrations examined. We obtained constant results with peripheral blood mononuclear cells from 4 normal individuals.

The amount of tumor necrosis factor-$\alpha$ and interleukin-10 in the culture supernatant decreased in a dose-dependent manner ($P < 0.01$). The result for peripheral blood mononuclear cells from 3 normal individuals is shown in Fig. 2. The amounts of tumor necrosis factor-$\alpha$ were $1647 \pm 98$ pg/ml (lipopolysaccharides alone), $925 \pm 59$ pg/ml (lipopolysaccharides + prostaglandin $E_1$ $1 \times 10^{-8}$ M), $305 \pm 44$ pg/ml (lipopolysaccharides + prostaglandin $E_1$ $1 \times 10^{-7}$ M), $143 \pm 27$ pg/ml (lipopolysaccharides + prostaglandin $E_1$ $1 \times 10^{-6}$ M) and $46.2 \pm 9.5$ pg/ml (none). The amounts of interleukin-10 were $129 \pm 34$ pg/ml (lipopolysaccharides alone), $105 \pm 18$ pg/ml (lipopolysaccharides + prostaglandin $E_1$ $1 \times 10^{-8}$ M), $66 \pm 14$ pg/ml (lipopolysaccharides + prostaglandin $E_1$ $1 \times 10^{-7}$ M), $45 \pm 7$ pg/ml (lipopolysaccharides + prostaglandin $E_1$ $1 \times 10^{-6}$ M) and $0.1 \pm 0.1$ pg/ml (none).

## 4. Discussion

It has been reported that prostaglandin $E_1$ inhibits tumor necrosis factor-$\alpha$ and -$\beta$ production and enhances interleukin-6 production by rat and mouse peritoneal macrophages or human peripheral blood mononuclear cells stimulated with lipopolysaccharides (5 $\mu$g/ml) (Haynes et al., 1992). They, however, observed these effects at concentrations of prostaglandin $E_1$ higher than $1 \times 10^{-6}$ M. Since the clinical dose of prostaglandin $E_1$ is 20–60 $\mu$g for intravenous perfusion, the maximum concentration of prostaglandin $E_1$ in peripheral blood is assumed to be approximately $1 \times 10^{-7}$ M. Therefore, we examined lower concentrations, i.e., $1 \times 10^{-6}$, $1 \times 10^{-7}$ and $1 \times 10^{-8}$ M. In addition, they used a biological cytotoxicity assay to measure the production of cytokines: the L929 cell line for tumor necrosis factor assay and the 7TD1 cell line for interleukin-6 assay. We measured tumor necrosis factor-$\alpha$ and interleukin-10 directly in the culture supernatant by using more sensitive enzyme-linked immunosorbent assays. Thus, we first demonstrated that prostaglandin $E_1$ suppressed interleukin-10 production as well as tumor necrosis factor-$\alpha$ production by lipopolysaccharides-stimulated peripheral blood mononuclear cells at lower concentrations relevant to those used clinically.

It was suggested that prostaglandin $E_1$ suppressed tumor necrosis factor-$\alpha$ production through a decreased mRNA expression of tumor necrosis factor-$\alpha$. It has been recognized that prostaglandin $E_2$ increases interleukin-10 production in lipopolysaccharides-stimulated whole blood cell cultures (Van der Pouw Kraan et al., 1995). Since interleukin-10 is a potent inhibitor of the production of several monocyte-derived cytokines, including interleukin-12 and tumor necrosis factor-$\alpha$, we expected an increased interleukin-10 production by prostaglandin $E_1$ treatment. On the contrary, both mRNA expression and synthesis of interleukin-10 were suppressed by prostaglandin $E_1$. These results strongly suggest that the decreased tumor necrosis factor-$\alpha$ production was not caused by an indirect effect of interleukin-10. Further studies are required to elucidate how prostaglandin $E_1$ regulates tumor necrosis factor-$\alpha$ or interleukin-10 synthesis, at the transcriptional level or at the post-transcriptional level.

Tumor necrosis factor-$\alpha$ is a potent cytokine with diverse biological activities in the immune system (Solis-Herruzo et al., 1988), extracellular matrix metabolism (Piguet et al., 1990), or cell proliferation and differentiation (Vilcek et al., 1987). Tumor necrosis factor-$\alpha$ is reported to have an important role in various diseases such as rheumatoid arthritis (DiGiovine et al., 1988; Leirisalo-Repo et al., 1995), systemic sclerosis (Hasegawa et al., 1997), Kawasaki disease (Maury et al., 1989), cachexia in neoplastic diseases (Tracey et al., 1988) and fulminant hepatitis (Muto et al., 1988). Tumor necrosis factor-$\alpha$ also

has an important role in certain skin diseases such as psoriasis, contact dermatitis (Piguet et al., 1991; Groves et al., 1995) or graft versus host disease (Piguet et al., 1987).

The clinical efficacy of prostaglandin $E_1$ in fulminant hepatitis has been reported (Abecassis, 1987). Our results indicate that we can anticipate anti-inflammatory effects as well as vasodilator and anti-platelet aggregation actions of prostaglandin $E_1$.

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