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Prostaglandins E_1 and E_2 inhibit lipopolysaccharide-induced interleukin-18 production in monocytes

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Abstract

The purpose of this present study was to explore the therapeutic potential of prostaglandins E_1 and E_2 on the systemic inflammatory response evoked by endotoxin. Since interleukin-18, a monocyte-derived cytokine, is increased during sepsis, decreasing the production of interleukin-18 is important in treating this condition. Prostaglandin E_1 and E_2 inhibited interleukin-18 production in human monocytes treated with lipopolysaccharide and prostanoid IP-, EP_2- and EP_4-receptor agonists mimicked the effects of prostaglandins E_1 and E_2. Therefore, prostanoid IP, EP2- and EP4-receptors might be involved in the decrease in interleukin-18 production during sepsis.

Keywords: Prostaglandin; Monocyte; Adhesion molecule

1. Introduction

Lipopolysaccharide, a major component of the outer membrane of Gram-negative bacteria, interacts with host immune cells by binding to surface CD14 molecules or by forming a complex with soluble CD14 and binding to the toll-like receptor-4 (TLR-4) on monocytes, macrophages and dendritic cells (Cauwels et al., 1999; Verbon et al., 2001). In murine models of endotoxemia, interleukin (IL)-18 in blood stimulated with lipopolysaccharide induces the secretion of proinflammatory cytokines (Schumann et al., 1998). IL-18 is an inducer of Th1 cytokines, such as interferon (IFN)-γ and tumor necrosis factor (TNF)-α (Yoshimoto et al., 1998; Takahashi et al., 2002). The administration of IFN-γ to trauma and high-risk surgical patients improves their immune function by increasing their resistance to infection and enhancing their chances of survival (Mock et al., 1996; Mordue et al., 2001). However, IL-18 exerts its toxic activity through IFN-γ-dependent and -independent mechanisms (Nakamura et al., 2000). The treatment using anti-IL-18 antibody prolongs survival but does not protect mice against sepsis (Netea et al., 2000), suggesting that a decrease in IL-18 production might be beneficial in the clinical treatment for sepsis.

The therapeutic value of prostaglandin E_1 in sepsis in animal and human models was previously reported (Zhang et al., 1994; Eierman et al., 1995). Prostaglandin E_1 has high affinity for prostanoid IP-, EP1-, EP2-, EP3- and EP4-receptors (Coleman et al., 1994), which are expressed on monocytes or dendritic cells (Takahashi et al., 2003; Kubo et al., 2004), while prostaglandin E_2 has high affinity for prostanoid EP_1-, EP_2-, EP_3- and EP_4-receptors (Narumiya et al., 1999). The prostanoid IP-, EP_2- and EP_4-receptors are coupled to Gs and mediate increases in cyclic adenosine monophosphate (cAMP) and the activation of protein kinase A (PKA) (Coleman et al., 1994; Narumiya et al., 1999). In a previous study, we reported that prostaglandin E_1 and E_2 inhibits TNF-α production induced by IL-18 at 100 ng/ml

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and lipopolysaccharide at 1 ng/ml via prostanoid IP-, EP_2- and EP_4-receptors in human peripheral blood mononuclear cells (Takahashi et al., 2002, 2003, in press). However, little is known about the effects of prostaglandin E_1 and E_2 on the production of IL-18. We also found that lipopolysaccharide at 1 μg/ml induces the production of IL-18 in human monocytes (Takahashi et al., 2004). In the present study, we examined the effects of prostaglandin E_1 and E_2 on IL-18 production induced by lipopolysaccharide at 1 μg/ml in monocytes isolated from human peripheral blood mononuclear cells.

2. Materials and methods

2.1. Reagents and drugs

Lipopolysaccharide from *Escherichia coli* (L8274) was purchased from Sigma Chemical (St. Louis, MO) and pure water produced by MILLIPORE (MILLIPORE JAPAN, Tokyo, Japan) was used as its solvent solution. Prostaglandin E_1 and E_2 along with ONO-1301 (7,8-dihydro-5-[(E)-[[α-(3-pyridyl)benzylidene]aminooxy]ethyl]-1-naphthyloxy)acetic acid), ONO-DI-004 (17S-2,5-ethano-6-oxo-17,20-dimethyl prostaglandin E_1), ONO-AE1-259-01 (11,15-O-dimethyl prostaglandin E_2), ONO-AE-248 (16S-9-deoxy-9beta-chloro-15-deoxy-16-hyfroxy-17,17-trimethylene-19,20-didehydro prostaglandin F_2) and ONO-AE1-329 (16-(3-methoxymethyl)phenyl-omega-tetranor-3,7-dithia prostaglandin E_1) were kindly provided by Ono Pharmaceutical Co. Ltd. (Tokyo, Japan).

2.2. Isolation of monocytes

Samples of 50 ml of peripheral blood were withdrawn from the forearm vein of ten human volunteers with their oral informed consent, after which human peripheral blood mononuclear cells were isolated as previously described (Takahashi et al., 2003, 2004). Monocytes isolated from human peripheral blood mononuclear cells were enriched by counterflow centrifugal elutriation using a SRR6Y elutriation system (Hitachi Koki Co. Ltd, Tokyo, Japan) as previously described (Takahashi et al., 2003, 2004). The cell populations, which were determined by flow cytometry with PE-conjugated anti-CD14 antibody (monocytes) (DAKO, Glostrup, Denmark), contained 85% CD14+ monocytes.

2.3. Cytokine assay

Monocytes at 1×10^6 cells/ml were incubated for 24 h at 37 °C in a humidified atmosphere of 5% CO_2 in air under different conditions. After culturing, the cell-free supernatant fractions were assayed for IL-18 protein by enzyme-linked immunoadsorbent assay (ELISA) employing the multiple Abs sandwich principle

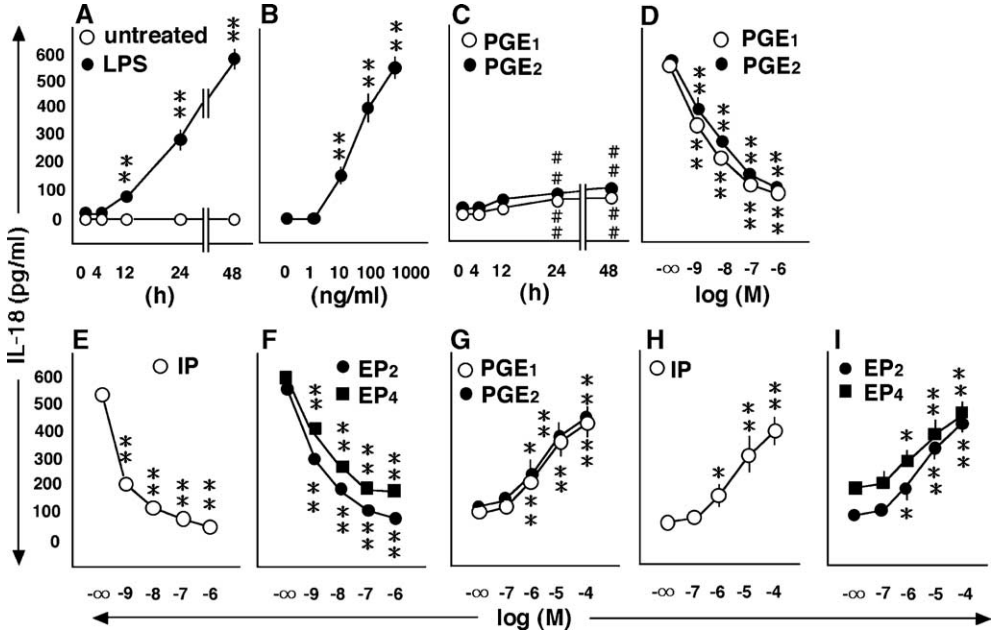

Fig. 1. The effects of prostaglandins E_1 and E_2 on lipopolysaccharide-induced IL-18 production in monocytes. (A) The kinetic effect of lipopolysaccharide on IL-18 production was determined. Monocytes at 1×10^6 cells/ml were incubated with lipopolysaccharide at 1 μg/ml for 0–48 h. After treatment, the level of IL-18 in the conditioned media was determined by ELISA. $^{**}P<0.01$ compared with the corresponding value for 0 h. (B) The dose-dependent effect of lipopolysaccharide on IL-18 production was determined. Monocytes at 1×10^6 cells/ml were incubated with different concentration of lipopolysaccharide for 24 h. $^{**}P<0.01$ compared with the corresponding value in the absence of LPS. (C) The kinetic effect of prostaglandin E_1 and E_2 on IL-18 production was determined. $^{\#\#}P<0.01$ as compared with the corresponding value for 1 μg/ml lipopolysaccharide at each time point. (D–F) The dose-dependent effects of prostaglandin E_1, E_2, ONO-1301, a prostanoid IP-receptor agonist, ONO-AE1-259-01, a prostanoid EP_2-receptor agonist, and ONO-AE1-329, a prostanoid EP_4-receptor agonist, in the presence of lipopolysaccharide at 1 μg/ml were determined at 24 h. $^{**}P<0.01$ compared with the corresponding value for lipopolysaccharide. (G–I) The effect of H89, a PKA inhibitor, in the presence of prostaglandin E_1, E_2, prostanoid IP-, EP_2- and EP_4-receptor agonists at 1 μM and lipopolysaccharide at 1 μg/ml was determined. $^{*}P<0.05$, $^{**}P<0.01$ compared with the corresponding value in the absence of H89. The results are the means ± SEM of five donors. When an error bar was within a symbol, the bar was omitted.

MBL (Nagoya, Japan). The detection limit of the ELISA for IL-18 was 10 pg/ml.

2.4. Flow cytometric analysis

Monocytes were cultured as described in cytokine assay. Cultured cells at 5×10^5 cells/ml were incubated with 1 μg of PE-conjugated anti-CD14 and FITC-conjugated anti-TLR-4 antibodies (MBL) for 20 min at 4 °C and then analyzed using a FACSCalibur (Becton Dickinson, Biosciences, San Jose, CA), after which the data was processed using the CELL QUEST program (Becton Dickinson Biosciences).

2.5. Statistical analysis

The statistical significance of differences was evaluated by analysis of variance (ANOVA) followed by Dunnet's test. A probability value of less than 0.05 was considered to indicate statistical significance.

3. Results

3.1. The effects of PGE_1 and E_2 on lipopolysaccharide-induced IL-18 production in monocytes mediated through the IP-, EP_2- and EP_4-receptors

Lipopolysaccharide at 1 μg/ml time-dependently induced IL-18 production. The production of IL-18 was significant at 12 h and reached a maximum at 48 h (Fig. 1A). Moreover, the effect of different concentrations from 0 to 1 μg/ml of LPS on changes in the production of IL-18 in the supernatant at 24 h was examined. LPS concentration-dependently induced IL-18 production (Fig. 1B).

Prostaglandins E_1 and E_2 abolished lipopolysaccharide-enhanced IL-18 production at 24 and 48 h (Fig. 1C) and in a concentration-dependent manner (Fig. 1D). The IC50 values for the effects of prostaglandins E_1 and E_2 were 500 nM. As shown in Fig. 1E and F, ONO-1301, a prostanoid IP-receptor agonist, ONO-AE1-259-01, a prostanoid EP_2-receptor agonist, and ONO-AE1-329, a prostanoid EP_4-receptor agonist, inhibited lipopolysaccharide-induced IL-18 production in a concentration-dependent manner. However, ONO-DI-004, a prostanoid EP_1-receptor agonist, and ONO-AE-248, a prostanoid EP_3-receptor agonist, had no effect (data not shown). The IC50 values for the inhibitory effects of IP, EP_2- and EP_4-receptor agonists were 500 nM.

3.2. Involvement of PKA in the effects of PGE_1, E_2, IP-, EP_2- and EP_4-receptor agonists

H89, a PKA inhibitor, abolished the inhibitory effects of lipopolysaccharide-induced prostaglandin E_1, E_2, ONO-1301, ONO-AE1-259-01 and ONO-AE1-329 on IL-18 production in monocytes in a concentration-dependent manner (Fig. 1G–I).

3.3. The effects of PGE1 and E2 on the expression of CD14 and TLR-4 in monocytes

Prostaglandins E_1 and E_2 inhibited the expression of CD14 and TLR-4 in the absence of lipopolysaccharide (Fig. 2). In the presence of lipopolysaccharide, the expression of CD14 and TLR-4

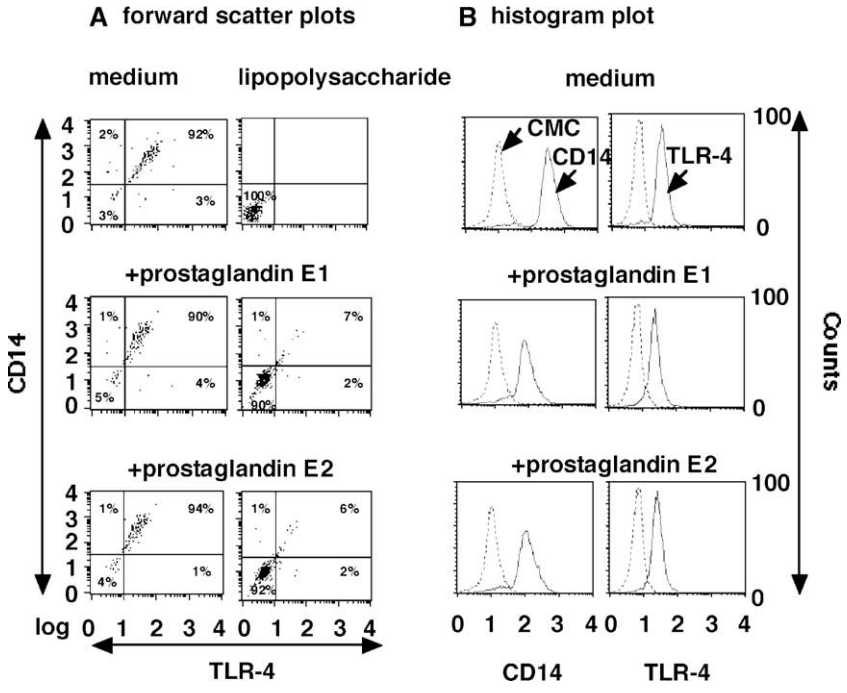

Fig. 2. The effects of prostaglandin E_1 and E_2 on the expression of CD14 and TLR-4 in monocytes. (A) Forward scatter plots are shown. The expression of CD14 and TLR-4 on monocytes in the presence of lipopolysaccharide at 1 μg/ml and prostaglandins E_1 and E_2 at 1 μM was examined by double-labeling flow cytometry using a combination of anti-CD14 and anti-TLR-4 antibodies. (B) The mean fluorescence intensity is shown by the histogram plot. Typical results of the expression of CD14 and TLR-4 on monocytes in the presence of prostaglandins E_1 and E_2 at 1 μM are shown. The experiments were repeated 10 times using different donors. The dotted lines represent the mean fluorescence intensity using mouse IgG_1 as a class-matched control (CMC).

was not detected and prostaglandins E_1 and E_2 had no effect on CD14 and TLR-4 expression.

4. Discussion

Prostaglandins E_1 and E_2 suppressed lipopolysaccharide-elicited IL-18 production (Fig. 1A). Recent studies reported that the release of prostaglandin E_2 is induced by healthy donor monocytes incubated in serum and separated plasma from septic patients (Astiz et al., 1996) and that lipopolysaccharide at 10 mg/ml concentration- and time-dependently causes the release of prostaglandin E_2 at 60 nM in human peripheral blood mononuclear cells (Yaqub et al., 2003). However, we found that the level of prostaglandin E_1 and E_2 production in the presence of lipopolysaccharide at 1 μg/ml was less than 10 pg/ml (data not shown). Prostanoid IP-, EP_2- and EP_4-receptor agonists mimicked the effects of prostaglandins E_1 and E_2 (Fig. 1B) and the IC50 values for the inhibitory effects of prostaglandin E_1, E_2, prostanoid IP, EP_2- and EP_4-receptor agonists on IL-18 production were 500 nM. These findings indicate that the stimulation of prostanoid IP-, EP_2- and EP_4-receptors might be involved in the effects of prostaglandins E_1 and E_2. As shown in Fig. 1C, the effects of prostaglandin E_1, E_2, prostanoid IP-, EP_2- and EP_4-receptor agonists on IL-18 production in monocytes were abolished by the PKA inhibitor. Recently, we reported that dibutylyl cAMP, an analog of cAMP, inhibits IL-18 production induced by lipopolysaccharide at 1 μg/ml (Takahashi et al., 2004). These results suggested that cAMP/PKA activation might be involved in the modulation of IL-18 production.

The effect of lipopolysaccharide on cytokine production depends on stimulation of the CD14/TLR-4 complex. In the absence (Fig. 2) or presence (data not shown) of lipopolysaccharide at 1 ng/ml, prostaglandins E_1, E_2, prostanoid IP-, EP_2- and EP_4-receptor agonists inhibited the expression of CD14 and TLR-4. However, CD14+ or TLR-4+ monocytes were not detected by double-stained flow cytometric analysis in the presence of lipopolysaccharide at 1 μg/ml (Fig. 2). Therefore, we could not find any effects of prostaglandins E_1, E_2, prostanoid IP-, EP_2- and EP_4-receptor agonists on the expression of CD14 and TLR-4 in the presence of lipopolysaccharide at 1 μg/ml. The cell viabilities of monocytes in the presence of lipopolysaccharide at 1 ng/ml and 1 μg/ml were 88% and 80% after 24 h of incubation, suggesting that lipopolysaccharide at 1 μg/ml might not induce cell death. Further study on the mechanism responsible for the prostaglandin-initiated inhibitory effect on IL-18 production should be carried out.

In conclusion, we found that the stimulation of prostanoid IP-, EP2- and EP4-receptors inhibits lipopolysaccharide-induced IL-18 production in monocytes. This might indicate therapeutic potential for prostanoid IP-, EP2- and EP4-receptor stimulation during sepsis.

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