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Differential effect of prostaglandins E_1 and E_2 on lipopolysaccharide-induced adhesion molecule expression on human monocytes

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Abstract

The effect of prostaglandins E_1 and E_2 on the 1 ng/ml lipopolysaccharide-induced expression of intercellular adhesion molecule (ICAM)-1, B7.1, B7.2, CD40 and CD40 ligand (CD40L) on monocytes was examined. Prostaglandin E_1 suppressed B7.1 and CD40 expression, but prostaglandin E2 did not effect on any type of adhesion molecule expression. Both prostaglandins inhibited tumor necrosis factor (TNF)-α production and T-cell proliferation of lipopolysaccharide-treated human peripheral blood mononuclear cells (PBMC). Among prostaglandin E_1 receptors (IP/EP_1/EP_2/EP_3/EP_4) agonists, ONO-1301, a prostanoid IP-receptor agonist, prevented B7.1 and CD40 expression. ONO-AE1-259-01 a prostanoid EP_2-receptor agonist, ONO-AE1-329, a prostanoid EP_4-receptor agonist, and ONO-1301 inhibited TNF-α production and T-cell proliferation. Moreover, anti-B7.1 and anti-CD40 Abs prevented lipopolysaccharide-induced TNF-α production and T-cell proliferation. Therefore, the effect of prostaglandin E_1 on TNF-α production and T-cell proliferation might depend on the inhibition of B7.1 and CD40 expression, but that of prostaglandin E_2 might be independent of adhesion molecules expression. In conclusion, the mechanism responsible for the effect of prostaglandin E_1 on lipopolysaccharide-induced responses is distinct from that of prostaglandin E_2.

Keywords: Prostaglandin; Monocyte; Adhesion molecule

1. Introduction

Serious infections involving Gram-negative bacteria often result in the development of sepsis, which is largely induced by lipopolysaccharide-induced secretion of tumor necrosis factor (TNF)-α (van Deuren et al., 1995; Ulevitch and Tobias, 1999). Lipopolysaccharide interacts with host immune cells by binding to surface CD14 molecules or by forming a complex with soluble CD14 and binding to toll-like receptor-4 on monocytes, macrophages and dendritic cells (Cauwels et al., 1999; Verbon et al., 2001). Lipopolysaccharide and its lipid A component are potent inducers of human Th1-like cytokines and of T-cell proliferation (Mattern et al., 1994, 1998). The activation and proliferation of T-cells by lipopolysaccharide occurs in a monocyte-dependent and requires direct cell-to-cell contact (Mattern et al., 1994, 1998; Chirathaworn et al., 2002; Gosset et al., 2001). In addition, lipopolysaccharide induces TNF-α production in monocytes and T-cells. Previously, we reported that the cell-to-cell interaction through CD28 and lymphocyte function-associated antigen (LFA)-1 on the T-cell and their counter receptors, intercellular adhesion molecule (ICAM)-1, B7.1 and B7.2 on monocytes, was involved in lipopolysaccharide-induced TNF-α production (Morichika et al., 2003).

Through its microvascular and anti-inflammatory actions (Schrror, 1991; Carlson and Olsson, 1976; Yamanaka et al.,

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1997), prostaglandin E_1 is of therapeutic value in sepsis in animal and human models (Zhang et al., 1994; Eierman et al., 1995). Prostaglandin E_1 inhibits ischemia-reperfusion injury of the liver (Kishimoto et al., 2000), and in human peripheral blood mononuclear cells (PBMC) stimulated with lipopolysaccharide it significantly suppresses both the mRNA expression and the production of TNF-α in a dose-dependent manner over the range of 10^{-8}–10^{-6} M (Ishikawa et al., 1998). Serum levels of prostaglandin E_2 are significantly increased in patients suffering from septic shock and sepsis (Astiz et al., 1996). Prostaglandin E_2 is produced in macrophages and monocytes stimulated with lipopolysaccharide (Lee et al., 1992; Hempel et al., 1994; Demasi et al., 2000), and secreted prostaglandin E_2 also inhibits the lipopolysaccharide-induced production of TNF-α by macrophages through an increase in intracellular cyclic adenosine monophosphate (cAMP) (Mauel et al., 1995; Dooper et al., 2002). Prostaglandin E2 contributes to immune suppression by inhibiting T-cell proliferation as well as various macrophage functions (Taffet and Russell, 1981; Vogel et al., 1981; Chouaib et al., 1985). Thus, it regulates the innate immune-response involved in sepsis. In contrast, prostaglandin E_1 has a high affinity for the prostanoid IP-receptor in addition to the specific membrane-bound G protein-coupled prostanoid EP-receptors EP_1, EP_2, EP_3 and EP_4 (Coleman et al., 1994). Also, lipopolysaccharide enhances IP-receptor mRNA expression in monocytes/macrophages (Plum et al., 2002). However, the pharmacological actions of prostaglandins E_1 and E_2 on lipopolysaccharide-induced adhesion molecules expression on monocytes is poorly understood.

In the present study, we investigated the effect of prostaglandin species metabolized from dihomo-gamma-linolenic acid (prostaglandin E_1) and arachidonic acid (prostaglandin E_2) on the lipopolysaccharide-induced expression of ICAM-1, B7.1, B7.2, CD40 and CD40L on monocytes as well as T-cell proliferation and TNF-α production. We also analyzed the relative contribution of prostaglandin receptor subtypes to the activity of prostaglandin E_1 and E_2, and the effects of antibodies against adhesion molecules on T-cell proliferation and TNF-α production.

2. Materials and methods

2.1. Reagents and drugs

Lipopolysaccharide from *Escherichia coli* (L8274, serotype 026:B6, purification more than 97%) was purchased from Sigma Chemical (St. Louis, MO) and pure water produced by MILLIPORE (MILLIPORE JAPAN, Tokyo, Japan) was used as its solvent solution for LPS. 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 E2), 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 (Tokyo, Japan). FITC-conjugated and non-conjugated mouse immunogloblin (Ig) G_1 monoclonal antibodies against ICAM-1/CD54 and PE-conjugated anti-CD14 monoclonal antibody were purchased from DAKO (Glostrup, Denmark), and FITC-conjugated and non-conjugated mouse IgG_1 monoclonal antibodies against B7.1/CD80 (MAB104) were purchased from IMMUNOTECH (Marseille, France). FITC-conjugated and non-conjugated mouse IgG_1 monoclonal antibodies against B7.2/CD86 and CD40 were acquired from Pharmingen (SanDiego, CA), and non-conjugated mouse IgG_1 monoclonal antibody against CD40L was purchased from Ancel (Bayport, MN). Finally, FITC-conjugated and non-conjugated IgG_1 class matched control were obtained from Sigma Chemical. [^{3}H] thymidine was purchased from Amersham (Braunschweig, Germany). The endotoxin concentrations in prostaglandins E_1 and E_2 along with ONO-1301, ONO-DI-004, ONO-AE1-259-01, ONO-AE-248, ONO-AE1-329 and Abs solutions were under the detection limit of 0.06 EU/ml as measured using an the Endospecy kit (Seikagaku Kogyo, Tokyo, Japan).

2.2. Isolation of PBMC

Normal PBMC were obtained from ten human volunteers with their oral informed consent. Samples of 50 ml of peripheral blood were withdrawn from a forearm vein, after which PBMC were isolated and then suspended at a final concentration of 1×10^6 cells/ml in the medium as previously described (Takahashi et al., 2003). The endotoxin concentrations in the medium described above were under the detection limit of 0.06 EU/ml as measured using the Endospecy kit.

2.3. Preparation of monocytes and T-cells

PBMC were prepared as described in *Isolation of PBMC*. Monocytes isolated from PBMC were enriched by counter-flow centrifugal elutriation using a SRR6Y elutriation system (Hitachi Koki, Tokyo, Japan) as previously described (Takahashi et al., 2003). Briefly, PBMC were injected at an initial flow rate of 14 and 16 ml/min at 4 °C with a rotor speed of 2000 rpm. The cell population which was determined by flow cytometry with FITC-conjugated anti-CD 14 antibody (monocytes), PE-conjugated anti-CD3 antibody (T-cells) and PE-conjugated anti-CD19 antibody (B-cells), contained 85% monocytes and less than 5% T- and B-cells. T-cells were then enriched from PBMC by passing them through a nylon wool column to a purity of contained 85% T-cells as determined by flow cytometry with FITC-conjugated anti-CD3 antibody.

2.4. Flow cytometric analysis

Changes in the expression of human leukocyte antigens B7.1 and CD40 on monocytes were examined by double-labeling flow cytometry using a combination of PE-conjugated anti-CD14 antibody and FITC-conjugated anti-B7.1 or anti-CD40 antibody. The cultured cells at 5×10^5 cells/ml were prepared as previously described (Takahashi et al., 2003) and analyzed using a FACSCalibur (Becton Dickinson, Biosciences, San Jose, CA), after which the data was processed using the CELL QUEST program (Becton Dickinson Biosciences). The results were expressed as means±S.E.M. for five independent experiments.

2.5. Cytokine assay

PBMC at 1×10^6 cells/ml were incubated with prostaglandins E_1, E_2, prostanoid IP- and EP-receptor agonists in the presence or absence of lipopolysaccharide for 48 h at 37 °C in a humidified atmosphere of 5% CO_2 in air. After culturing, the cell-free supernatant fractions were assayed for TNF-α protein by enzyme-linked immunoadsorbent assay (ELISA) as previously described (Morichika et al., 2003).

2.6. Measurement of cellular proliferation

As described in Preparation of monocytes and T-cells, monocytes and T-cells isolated from the same donor were the mixed to produce the reconstituted cells. The reconstituted cells were incubated for 48 h including 16 h of pulsing with [^{3}H] thymidine. The cells were then divided into 96-well microplates at 200 μl/well resulting in 1 μCi [^{3}H] thymidine per well, and harvested using a the Micro-Mate 196 Cell Harvester (Perkin Elmer Life Science, Boston, MA, USA). Thymidine incorporation was measured by beta-counter (Matrix 9600, Perkin Elmer Life Science).

2.7. Statistical analysis

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

3. Results

3.1. The dose–response relationship of the effects of prostaglandins E1 and E2 as well as IP- and EP-receptor agonists on B7.1 and CD40 expression on human monocytes

The effects of prostaglandins E_1 and E_2 between 0 and 10^{-6} M on changes in the expression of B7.1 and CD40 were determined by double-labeling flow cytometry after 48 h incubation of PBMC (Fig. 1). Previously, we reported that lipopolysaccharide at 1 ng/ml enhances the expression of ICAM-1, B7.1 and B7.2 on monocytes (Morichika et al., 2003). The same concentration of lipopolysaccharide up-regulated the expression of CD40 (Fig. 1A) and CD40L (data not shown) on monocytes. Prostaglandin E_1 inhibited lipopolysaccharide-induced B7.1 and CD40 expression in a concentration-dependent manner (Fig. 1A), but had no effect on the expression of B7.1, B7.2 and CD40L (data not shown). The IC50 values for the inhibitory effect of prostaglandin E_1 on the expression of B7.1 and CD40 induced by lipopolysaccharide were estimated to be 5 and 3 nM, respectively. Prostaglandin E_2 had no effect on any type of adhesion molecule expression in the presence or absence of lipopolysaccharide (data not shown). To determine which prostaglandin receptor subtypes among IP, EP_1, EP_2, EP_3, EP_4 are responsible for the effects of prostaglandin E_1 on the expression of B7.1 and CD40, we examined the effects of prostanoid IP- and EP-receptor agonists between 0 and 10^{-6} M on ICAM-1, B7.1 B7.2, CD40 and CD40L expression on monocytes in the presence of lipopolysaccharide at 1 ng/ml after 48 h incubation. ONO-1301, a prostanoid IP-receptor agonist (Imawaka and Sugiyama, 1998) prevented the

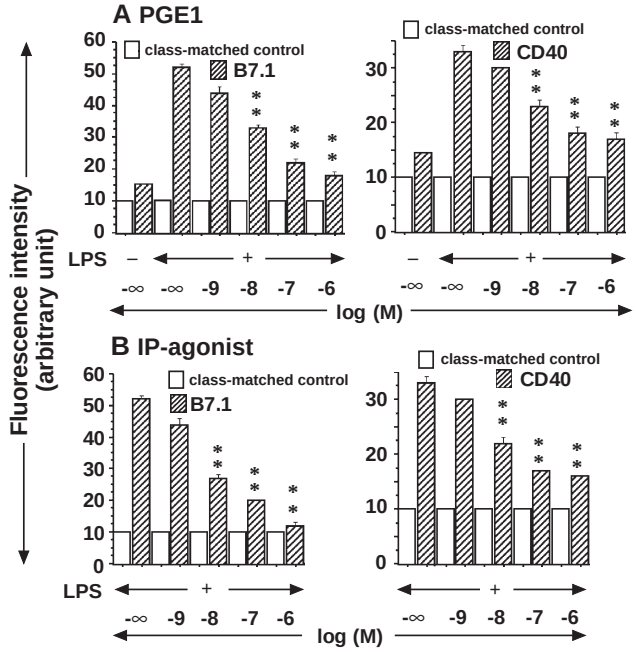

Fig. 1. The effects of prostaglandin E_1 and ONO-1301 on lipopolysaccharide-induced B7.1 and CD40 expression on the surface of monocytes in PBMC. PBMC at 1×10^6 cells/ml were cultured in medium containing lipopolysaccharide at 1 ng/ml, prostaglandin E_1 and ONO-1301, a prostanoid IP-receptor agonist, between 10^{-9} and 10^{-6} M for 48 h. At the end of culturing, PBMC at 5×10^5 cells/ml were double-stained for monocyte-specific antigens (CD14) and B7.1 or CD40 as described in Materials and methods, and the expression of B7.1 and CD40 on monocytes was analyzed by flow cytometric analysis. Mouse IgG_1 was use as a class-matched control. The results are the means±S.E.M. for five independent experiments involving five donors. $^{**}P<0.01$ compared with the corresponding value for the presence of lipopolysaccharide alone.

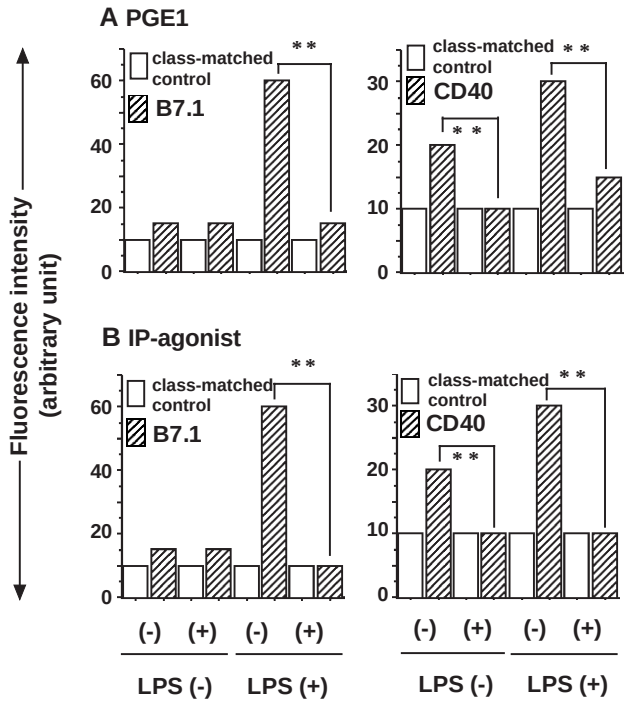

Fig. 2. The effects of prostaglandin E_1 and ONO-1301 on B7.1 and CD40 expression on isolated monocytes. Isolated monocytes at 1×10^6 cells/ml were cultured in medium containing lipopolysaccharide at 1 ng/ml, prostaglandin E_1 (A) and ONO-1301, a prostanoid IP-receptor agonist (B) at 10^{-6} M for 48 h. At the end of culturing, isolated monocytes at 5×10^5 cells/ml were stained with anti-B7.1 and anti-CD40 antibodies as described in Materials and methods, and the expression of B7.1 and CD40 on monocytes was analyzed by flow cytometric analysis. Mouse IgG_1 was use as a class-matched control. The results are the means±S.E.M. for five independent experiments involving five donors. $^{**}P<0.01$ compared with the corresponding value for the absence of prostaglandin E_1 and ONO-1301.

expression of B7.1 and CD40 (Fig. 1B), but had no effect on the expression of B7.1, B7.2 and CD40L (data not shown). The IC50 values for the inhibitory effect of ONO-1301 on the expression of B7.1 and CD40 were estimated to be 3 and 5 nM. However, ONO-DI-004, a prostanoid EP_1-receptor agonist, ONO-AE1-259-01, a prostanoid EP_2-receptor agonist, ONO-AE-248, a prostanoid EP_3-receptor agonist and ONO-AE1-329 a prostanoid EP_4-receptor agonist (Suzawa et al., 2000; Kitagawa et al., 2001) had no effect on the expression of these adhesion molecules (data not shown).

3.2. The effect of prostaglandin E1 and prostanoid IP-receptor agonist on B7.1 and CD40 expression on isolated monocytes

The effects of prostaglandin E_1 and prostanoid IP-receptor agonist at 10^{-6} M on the expression of B7.1 and CD40 on isolated monocytes in the presence of lipopolysaccharide at 1 ng/ml were examined after 48 h incubation. ONO-1301 as well as prostaglandin E_1 was found to have prevented the expression of B7.1 and CD40 (Fig. 2).

3.3. The dose–response relationship of the effects of prostaglandin E1 and E2, as well as prostanoid IP- and EP-receptor agonists on TNF-α production in PBMC

The effect of prostaglandin E_1 and E_2 along with prostanoid IP- and EP-receptor agonists between 0 and 10^{-6} M on the production of TNF-α in PBMC treated with lipopolysaccharide at 1 ng/ml was determined by ELISA after 48 h incubation. Prostaglandin E_1 and E_2 as well as ONO-1301, ONO-AE1-259-01 and ONO-AE1-329 inhibited lipopolysaccharide-induced TNF-α production in a concentration-dependent manner (Fig. 3), but ONO-DI-004 and ONO-AE-248 had no effect (data not shown). The IC50 values for the inhibitory effect of prostaglandins E_1 and E_2 on the production of TNF-α was 20 and 30 nM, respectively.

3.4. Inhibition of lipopolysaccharide-induced TNF-α production by anti-B7.1 and anti-CD40 antibodies

In the previous study, we found that lipopolysaccharide-induced TNF-α production in PBMC is blocked by anti-ICAM-1, anti-B7.1 and anti-B7.2 antibodies at 10 and 100 μg/ml (Morichika et al., 2003). In addition, the effects of anti-CD40 and anti-CD40L antibodies between

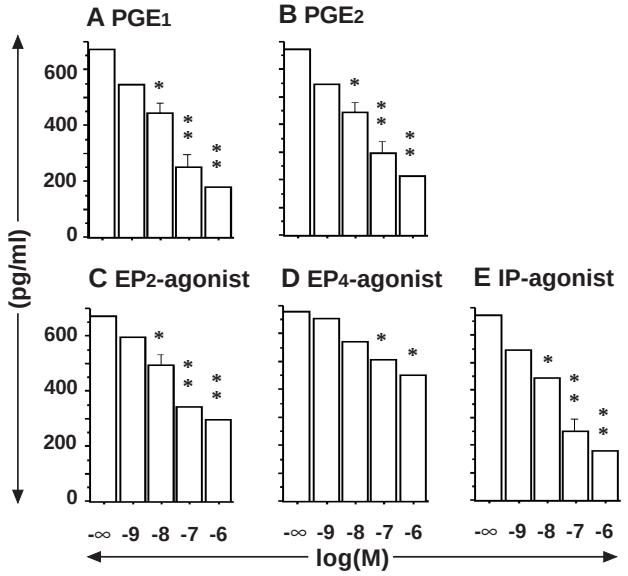

Fig. 3. The effects of prostaglandins E_1 and E_2 as well as prostanoid IP- and EP-receptor agonists on lipopolysaccharide-induced TNF-α production. PBMC at 1×10^6 cells/ml were incubated with increasing concentrations of prostaglandin E_1 (A), prostaglandin E_2 (B), ONO-AE1-259-01, a prostanoid EP_2-receptor agonist (C), ONO-AE1-329, a EP_4-receptor agonist (D) and ONO-1301, a prostanoid IP-receptor agonist (E) between 10^{-9} and 10^{-6} M in the presence of lipopolysaccharide at 1 ng/ml for 48 h, and the levels of TNF-α in the conditioned media were determined by ELISA as described in Materials and methods. The results are the means±S.E.M. for five independent experiments involving five donors. $^{*}P<0.05$, $^{**}P<0.01$ compared with the corresponding value for the presence of LPS alone.

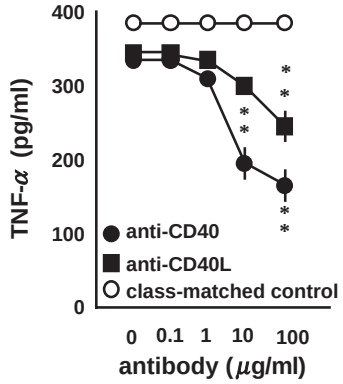

Fig. 4. The inhibition of lipopolysaccharide-induced TNF-α production by anti-CD40 and anti-CD40L antibodies. PBMC at 1×10^6 cells/ml as described in Materials and methods were incubated with lipopolysaccharide at 1 ng/ml in the presence of different concentrations of anti-CD40, anti-CD40L antibodies and class-matched control between 0 and 100 μg/ml for 48 h, and the levels of TNF-α in the conditioned media were determined by ELISA as described in Materials and methods. Mouse IgG_1 was use as a class-matched control. The results are means±S.E.M. for five independent experiments involving five donors. $^{**}P<0.01$ compared with the corresponding value for the presence of lipopolysaccharide alone. Error bars smaller than the symbols are not shown.

0 and 100 μg/ml on TNF-α production in the presence and absence of lipopolysaccharide were measured by ELISA after 48 h incubation (Fig. 4). Anti-CD40 and anti-CD40L antibodies inhibited lipopolysaccharide-induced TNF-α production in a concentration-dependent manner but the class-matched non-relevant antibody at the concentration of 100 μg/ml had no effect (Fig. 4).

3.5. The dose–response relationship of the effects of prostaglandins E1 and E2 as well as prostanoid IP- and EP-receptor agonists on T-cell proliferation

The effects of prostaglandins E_1 and E_2 along with prostanoid IP- and EP-receptor agonists between 0 and 10^{-6} M on T-cell proliferation in the presence or absence of lipopolysaccharide were measured via the incorporation of [^{3}H] labeled thymidine after 48 h incubation. As described in Preparation of monocytes and T-cells, monocytes at 10^6 cells/ml and T-cells at 10^6 cells/ml isolated from the same donor were mixed to produce reconstituted cells, which the reconstituted cells were then incubated for 48 h including 16 h of pulsing with [^{3}H] thymidine. Prostaglandins E_1 and E_2 as well as ONO-1301, ONO-AE1-259-01 and ONO-AE1-329 inhibited lipopolysaccharide-induced T-cell proliferation in a concentration-dependent manner, but had no effect in the absence of lipopolysaccharide (Fig. 5). In addition, ONO-DI-004 and ONO-AE-248 had no effect (data not shown). The IC50 value for the inhibitory effect of prostaglandin E_1 and E_2 as well as ONO-AE1-259-01, ONO-AE1-329 and ONO-1301 on lipopolysaccharide-induced T-cell proliferation were 5, 30, 10, 10 and 3 nM, respectively.

3.6. Inhibition of lipopolysaccharide-induced T-cell proliferation by anti-ICAM-1, B7.1, B7.2, CD40 and anti-CD40L antibodies

The effects of anti-ICAM-1, anti-B7.1, B7.2, CD40 and CD40L antibodies between 0 and 100 μg/ml on T-cell

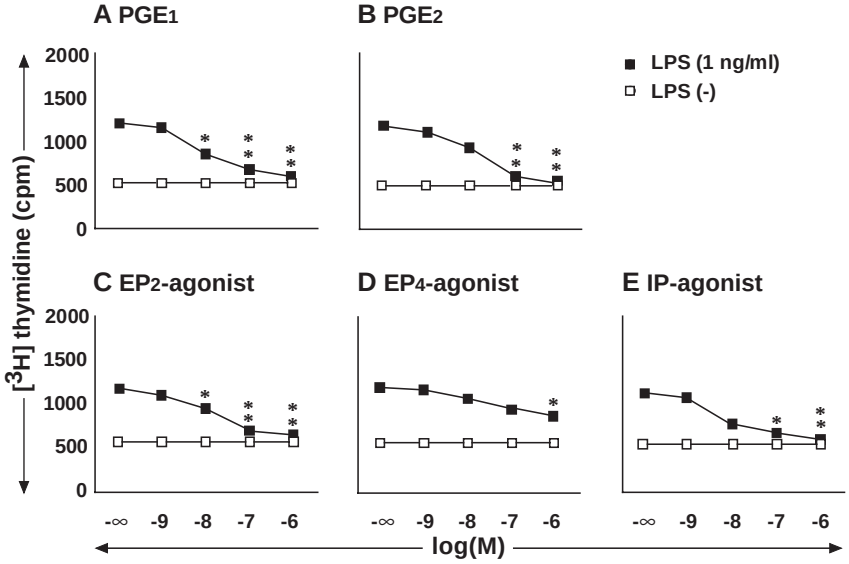

Fig. 5. The effects of prostaglandins E_1, E_2 along with prostanoid IP- and EP-receptor agonists on lipopolysaccharide-induced T-cell proliferation. Reconstituted cells at 1×10^6 cells/ml as described in Materials and Methods were incubated with different concentrations of prostaglandin E_1 (A), prostaglandin E_2 (B), ONO-AE1-259-01, a prostanoid EP_2-receptor agonist (C), ONO-AE1-329, a prostanoid EP_4-receptor agonist (D) and ONO-1301, a prostanoid IP-receptor agonist (E) between 10^{-9} and 10^{-6} M in the presence (filled squares) or absence (open squares) of lipopolysaccharide at 1 ng/ml for 48 h. After incubation, [^{3}H] thymidine uptake by T-cells among the reconstituted cells was determined. The results are the means±S.E.M. for five independent experiments involvingg five donors. $^{*}P<0.05$, $^{**}P<0.01$ compared with the value for the presence of lipopolysaccharide alone. Error bars smaller than the symbols are not shown.

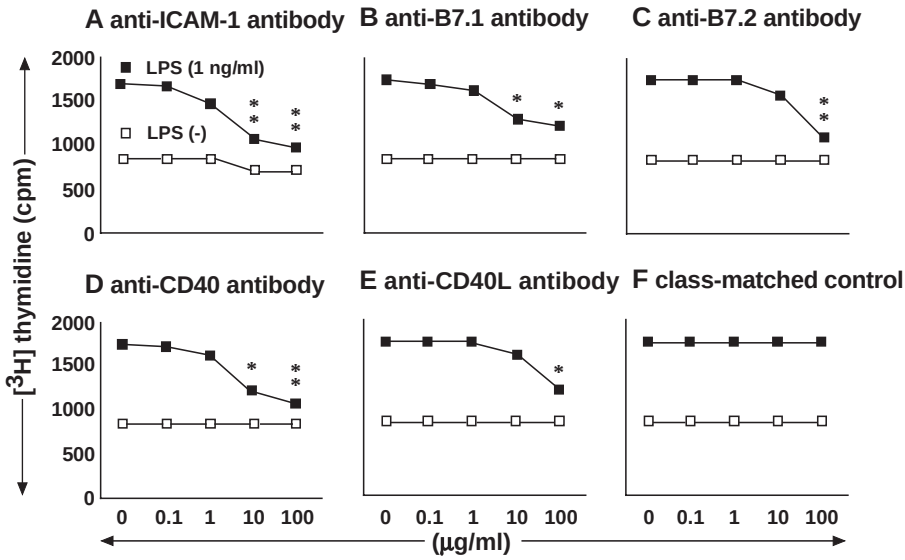

Fig. 6. The inhibition of lipopolysaccharide-induced T-cell proliferation by anti-ICAM-1, B7.1, B7.2, CD40 and CD40L antibodies. Reconstituted cells at 1×10^6 cells/ml as described in Materials and methods were incubated with different concentrations of anti-ICAM-1 (A), B7.1 (B), B7.2 (C), CD40 (D), and CD40L antibodies (E) or class-matched control (mouse IgG_1) (F) between 0 and 100 μg/ml in the presence (filled squares) or absence (open squares) of lipopolysaccharide at 1 ng/ml for 48 h. After incubation, [^{3}H] thymidine uptake by T-cells among the reconstituted cells was determined. The results are the means±S.E.M. for five independent experiments for five donors. *P<0.05, **P<0.01 compared with the value for the presence of lipopolysaccharide alone. Error bars smaller than the symbols are not shown.

proliferation in the presence or absence of lipopolysaccharide were measured by [^{3}H] thymidine incorporation after 48 h incubation. All antibodies inhibited lipopolysaccharide-induced T-cell proliferation in a concentration-dependent manner, but had no effect in the absence of lipopolysaccharide (Fig. 6). The class-matched non-relevant antibody at a concentration of 100 μg/ml had no effect on lipopolysaccharide-induced T-cell proliferation (Fig. 6).

4. Discussion

In the presence of lipopolysaccharide at 1 ng/ml, prostaglandin E_1 inhibited B7.1 and CD40 expression in a concentration-dependent manner (Fig. 1A), and the prostanoid IP-receptor agonist mimicked the effect of prostaglandin E_1 on lipopolysaccharide-induced B7.1 and CD40 expression (Fig. 1B). Moreover, we confirmed that prostaglandin E_1 and ONO-1301 inhibited lipopolysaccharide-induced B7.1 and CD40 expression on monocytes by directly acting on purified monocyte preparation (Fig. 2). These results indicated that the IP-receptor might be involved in the inhibitory effect of prostaglandin E_1 on B7.1 and CD40 expression. In addition to a report showing that prostaglandin E_1 between 10^{-8} and 10^{-6} M significantly suppresses the production of TNF-α in PBMC stimulated with lipopolysaccharide (Ishikawa et al., 1998), prostaglandin E_1 between 10^{-9} and 10^{-6} M suppressed enhanced T-cell proliferation induced by lipopolysaccharide (Fig. 5). The results in Figs. 3 and 5 indicate that IP-receptors are involved in the decrease in TNF-α production and T-cell proliferation. The engagement of ICAM-1/LFA-1, B7/CD28 and CD40/CD40L is involved in lipopolysaccharide-induced T-cell proliferation and TNF-α production (Morichika et al., 2003, Figs. 4 and 6), and T-cell proliferation is induced by the stimulation of CD28 and CD40L (Chirathaworn et al., 2002). The inhibitory effect of prostaglandin E_1 on TNF-α production and T-cell proliferation might be mediated by the reduction of the expression of B7.1 and CD40 on monocytes.

On the other hand, prostaglandin E_2 had no effect on adhesion molecule expression in the presence of lipopolysaccharide at 1 ng/ml (data not shown), and the prostanoid EP_1-, EP_2- EP_3- and EP_4-receptor agonists also had no effect (Fig. 1B). However, prostaglandin E_2 suppressed the enhanced TNF-α production and T-cell proliferation induced by lipopolysaccharide in PBMC (Dooper et al., 2002, Fig. 5). In a rat and mouse model, prostaglandin E_2 suppresses T-cell proliferation through the stimulation of EP_2- and EP_4-receptors on T-cells (Nataraj et al., 2001; Choudhry et al., 2002). As shown in Figs. 3 and 5, EP_2- and EP_4-receptors were involved in the decrease in TNF-α production and T-cell proliferation. Thus, the inhibitory effect of prostaglandin E_2 on TNF-α production and T-cell proliferation might occur in an adhesion molecules-independent manner. Therefore, the mechanism responsible for the effect of prostaglandin E_1 on TNF-α production and T-cell proliferation is different from that of prostaglandin E_2.

T-cells activation occurs during the early stage of sepsis, and the later stages are associated with the impairments of T-cell function. Lipopolysaccharide at 10 μg/ml, which has been used as an in vitro model for studying the late stage of sepsis, suppresses T-cell proliferation in PBMC (Yaqub et al., 2003), but at a

concentration of 1 ng/ml induced T-cell proliferation (Fig. 5). The addtion of lipopolysaccharide at between 0.01 and 10 μg/ml to human blood ex vivo causes the release of prostaglandin E_2 of between 20 and 60 nM (Yaqub et al., 2003). However, the level of prostaglandins E_1 and E_2 induced by lipopolysaccharide at 1 ng/ml in the culture medium was under the detection limit of 10 pg/ml analyzed by ELISA (data not shown), suggesting that the distinct effects of lipopolysaccharide on T-cell proliferation from those of lipopolysaccharide might be due to a lack of production of endogenous prostaglandins E_1 and E_2.

Previously, we reported that prostaglandins E_1 and E_2 suppressed interleukin-18-elicited expression of ICAM-1, B7.2 and CD40 through the stimulation of IP/EP_2-/EP_4- and EP_2/EP_4-receptors (Takahashi et al., 2003; Takahashi et al., 2002). Since the level of interleukin-18 induced by lipopolysaccharide at 1 ng/ml was under the detection limit of 10 pg/ml (Morichika et al., 2003), endogenous interleukin-18 production might has been slightly associated with lipopolysaccharide-induced responses under the present conditions.

In lipopolysaccharide-treated murine bone marrow-derived dendritic cells (DC), cAMP-elevation induced by the stimulation of EP_2-, IP- and DP-receptors dose-dependent-manner inhibits the release of interleukin-6, TNF-α and interleukin-12, and enhances the release of interleukin-10, with TNF-α secretion being the most strongly affected (Jozefowski et al., 2003). The present study shows that prostaglandin E_1 suppresses lipopolysaccharide-induced TNF-α production and T-cell proliferation through the inhibition of B7.1 and CD40 expression and that these effects are distinct from those of prostaglandin E_2. Thus, IP-receptor stimulation may suppress the cytokine production in monocytes/DC stimulated with LPS.

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