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Effect of 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 on acute lung injury induced by lipopolysaccharide in mice

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Abstract

15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-prostaglandin J_2) has received attention for its anti-inflammatory properties. The present study investigated the efficacy of 15d-prostaglandin J_2 on acute lung injury induced by lipopolysaccharide in mice. ICR mice were administered with 15d-prostaglandin J_2 (10 μg/kg, 100 μg/kg, or 1 mg/kg) before intratracheal challenge with lipopolysaccharide (125 μg/kg). Treatment with 15d-prostaglandin J_2 did not ameliorate rather enhanced at a dose of 1 mg/kg the neutrophilic lung inflammation and pulmonary edema by lipopolysaccharide. The enhancement was concomitant with the increased lung expression of interleukin-1β, macrophage inflammatory protein-1α, and macrophage chemoattractant protein-1. 15d-prostaglandin J_2 increased the nuclear protein expression of peroxisome proliferator-activated receptor (PPAR)-γ and inhibited the nuclear localization of nuclear factor-κB related to lipopolysaccharide. 15d-prostaglandin J_2 increased the phosphorylation of c-Jun in the presence or absence of lipopolysaccharide. Our data suggest that 15d-prostaglandin J_2 may not be useful but potentially harmful for the therapeutic option of acute lung injury.

Keywords: Acute lung inflammation; Cytokine; Chemokine; Peroxisome proliferator-activated receptor-γ; Activator protein-1

1. Introduction

15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-prostaglandin J_2) is a derivative of the prostaglandin D_2 metabolism pathway and is a natural ligand for peroxisome proliferator-activated receptor (PPAR)-γ, which acts as a transcriptional nuclear receptor (Schoonjans et al., 1997; Ricote et al., 1998a; Forman et al., 1995; Lowell, 1999). Several studies have shown the role of 15d-prostaglandin J_2 and related cyclopentenone prostaglandins (prostaglandin A_1, prostaglandin A_2, and prostaglandin J_2) in the regulation of inflammatory process.

In vitro studies with monocytes/macrophages have suggested that 15d-prostaglandin J_2 could inhibit the expression of genes coding for interleukin-1β, tumor necrosis factor (TNF)-α, cyclooxygenase-2, nitric oxide synthase-2, and matrix metalloproteinases by preventing the activation of nuclear factor-κB (NF-κB)/Rel (Ricote et al., 1998b; Jiang et al., 1998). Recent studies in vivo have demonstrated that 15d-prostaglandin J_2 and thiazolidinediones, known as synthetic ligands for PPAR-γ, attenuate inflammation in the murine model of dextran sodium sulfate (DSS)-induced colitis (Su et al., 1999). More recently, we have demonstrated that 15d-prostaglandin J_2 and troglitazone, one of thiazolidinediones, ameliorate adjuvant-induced arthritis in rats (Kawahito et al., 2000). These observations raise the possibility that 15d-prostaglandin J_2 may be a potential therapeutic compound for treatment of inflammatory diseases.

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However, other studies have failed to observe an inhibitory effect of 15d-prostaglandin J_2 on the enhanced expression of TNF-α and interleukin-6 in freshly prepared human monocytes/macrophages (Thieringer et al., 2000). More recently, it has been reported that cyclopentenone prostaglandins at low concentrations potentiate proinflammatory cytokine expression at both transcriptional and posttranscriptional levels (Bureau et al., 2002). Thus, it remains controversial whether 15d-prostaglandin J_2 will be of therapeutic value as an anti-inflammatory agent, and should be elucidated on several experimental models of inflammatory diseases, especially, in which monocytes/macrophages play pivotal roles.

Intratracheal instillation of lipopolysaccharide produces a well-characterized model of acute lung injury, leading to the activation of alveolar macrophages and tissue infiltration of neutrophils (Brigham and Meyrick, 1986). Although the pathogenesis of acute lung injury is not fully understood, alveolar macrophages play, at least in part, a role in increased production of inflammatory mediators, such as interleukin-1β, followed by accumulation of large numbers of neutrophils and the development of interstitial edema (Jacobs et al., 1989; Ulich et al., 1991; Chollet-Martin et al., 1992). In addition, we and others have recently reported that PPAR-γ is expressed in normal lung tissues (Inoue et al., 2001; Michael et al., 1997). Based on these previous reports, we have decided to examine the effect of 15d-prostaglandin J_2 on the accepted murine model of acute lung injury.

In the present study, we showed that 15d-prostaglandin J_2 did not ameliorate but enhanced acute lung injury related to lipopolysaccharide at a dose of 1 mg/kg. This potentiation of acute lung injury was associated with the increased protein expression of proinflammatory cytokine and chemokines in the lung tissue supernatants. We also demonstrated that 15d-prostaglandin J_2 enhanced the protein expression of PPAR-γ and inhibited the nuclear localization of NF-κB related to lipopolysaccharide in the lung, whereas it increased the phosphorylation of c-Jun in the presence or absence of lipopolysaccharide.

2. Materials and methods

2.1. Chemicals

Lipopolysaccharide from *Escherichia coli* B55:05 were purchased from Difco Lab (Detroit, MI). 15d-prostaglandin J_2 was purchased from Calbiochem (Band Soden, Germany).

2.2. Animal and study protocol

We used ICR male mice that have been reported to be highly responsive to lipopolysaccharide rather than Balb/c, C3H/He, and A/J mice (Takano et al., 2002). They were fed and housed as previously described (Takano et al., 2002).

The animals were randomized into six experimental groups as follows: (1) the vehicle group: vehicle (intratracheal administration: i.t. + intravenous administration: i.v.) + vehicle (i.t.); (2) the lipopolysaccharide group: vehicle (i.t. + i.v.) + lipopolysaccharide (125 μg/kg, i.t.); (3) the 15d-prostaglandin J_2 (10 μg/kg) + lipopolysaccharide group: 15d-prostaglandin J_2 (10 μg/kg, i.t. + 10 μg/kg, i.v.) + lipopolysaccharide (125 μg/kg, i.t.); (4) the 15d-prostaglandin J_2 (100 μg/kg) + lipopolysaccharide group: 15d-prostaglandin J_2 (100 μg/kg, i.t. + 100 μg/kg, i.v.) + lipopolysaccharide (125 μg/kg, i.t.); (5) the 15d-prostaglandin J_2 (1 mg/kg) + lipopolysaccharide group: 15d-prostaglandin J_2 (1 mg/kg, i.t. + 1 mg/kg, i.v.) + lipopolysaccharide (125 μg/kg, i.t.); (6) the 15d-prostaglandin J_2 group: 15d-prostaglandin J_2 (1 mg/kg, i.t. + 1 mg/kg, i.v.) + vehicle (i.t.). Phosphate-buffered saline (PBS) at pH 7.4 (Nissui Pharmaceutical, Tokyo, Japan) was used as vehicle for lipopolysaccharide, and PBS at pH 7.4 containing 4% dimethyl sulfoxide (DMSO) for 15d-prostaglandin J_2. 15d-prostaglandin J_2 or vehicle was administered intratracheally 3 h before the intratracheal challenge with lipopolysaccharide. 15d-prostaglandin J_2 or vehicle was also administered intravenously just before lipopolysaccharide treatment. Lipopolysaccharide or 15d-prostaglandin J_2 was dissolved in 75-μl aliquots and administered intratracheally or intravenously. Intratracheal inoculation was conducted using a polyethylene tube under anesthesia with 4% halothane (Hoechst Japan, Tokyo, Japan).

2.3. Bronchoalveolar lavage, lung water content, and histological evaluation

Bronchoalveolar lavage and cell counts in bronchoalveolar lavage fluid were conducted as previously described (Takano et al., 1997). The lung water content was evaluated as previously described (Ichinose et al., 1995). The lungs were fixed and stained with hematoxylin and eosin and periodic acid—Schiff (PAS) as previously described (Takano et al., 1997).

2.4. Measurement of interleukin-1β and chemokines in the lung tissue supernatants

The lungs were homogenized and centrifuged as previously described (Takano et al., 1997). Enzyme-linked immunosorbent assays (ELISA) for interleukin-1β (Endogen, Cambridge, MA), macrophage inflammatory protein-1α (MIP-1α; R&D systems, Minneapolis, MN), macrophage chemoattractant protein-1 (MCP-1; R&D systems), and keratinocyte chemoattractant (KC; R&D systems) in the lung tissue supernatants were conducted according to the manufacturer's instruction.

2.5. Preparation of nuclear protein extracts and Western blot analysis

We prepared nuclear protein extracts using methods described previously (Takano et al., 2002). Nuclear proteins were electrophoresed and blotted onto polyvinylidene difluoride membrane. The membrane was incubated with a rabbit anti-p50 antibody (Upstate Biotechnology, Lake Placid, NY), a rabbit anti-p65 antibody (Santa Cruz Biotechnology, Santa Cruz, CA), a rabbit anti-phospho-c-Jun antibody (Cell Signaling Technology, Beverly, MA), and a rabbit anti-PPAR-γ (Santa Cruz Biotechnology). After washes, the membrane was incubated with horseradish peroxidase-conjugated goat anti-rabbit antibody. After washes, the membrane was developed using the enhanced chemiluminescence light detecting kit (ECL-plus, Amersham Pharmacia, Buckinghamshire, UK) according to the manufacturer's recommended instructions. For quantification, bands in photographs were scanned by a densitometer linked to a computer analysis system (Densitograph, Atto, Japan).

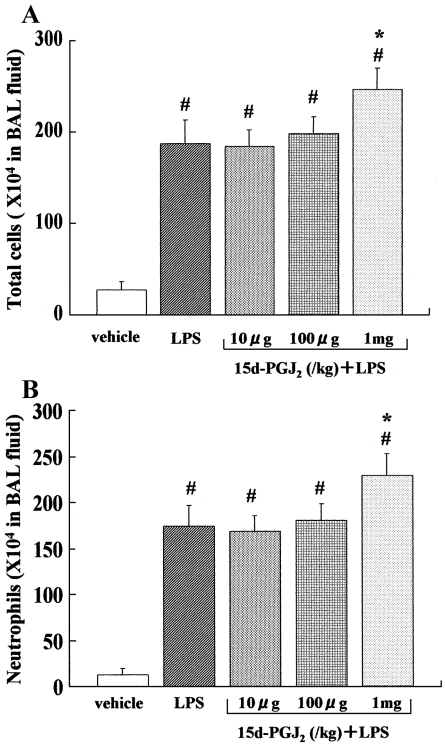

Fig. 1. Effect of 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) on total cells (A) and neutrophils (B) in bronchoalveolar lavage fluid in mice challenged with lipopolysaccharide (LPS: 125 μg/kg). Twenty-four hours after the intratracheal administration of LPS, lungs were lavaged for the analysis of bronchoalveolar lavage fluid. The total cell count was determined on a fresh fluid specimen using a hemocytometer. Differential cell counts were assessed on cytologic preparations stained with Diff-Quik. *$P<0.05$ versus the LPS group; #$P<0.01$ versus vehicle. Values are the mean ± S.E.M. of eight animals in each group.

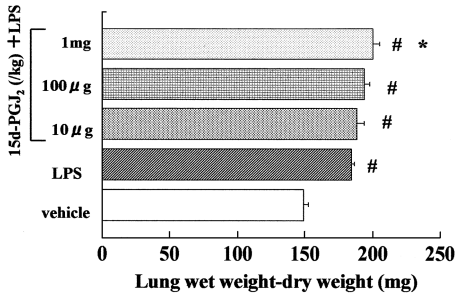

Fig. 2. Effect of 15d-PGJ_2 on lung water content in mice challenged with LPS. The bilateral lungs were weighted immediately after the exsanguinations 24 h after the administration of LPS, and dried in a oven at 95 °C for 48 h. Thereafter, lung water content was estimated. *$P<0.05$ versus the LPS group; #$P<0.01$ versus vehicle. Values are the mean ± S.E.M. of eight animals in each group.

2.6. Statistical analysis

Data were reported as mean ± S.E.M. Differences among groups were determined using analysis of variance (Stat

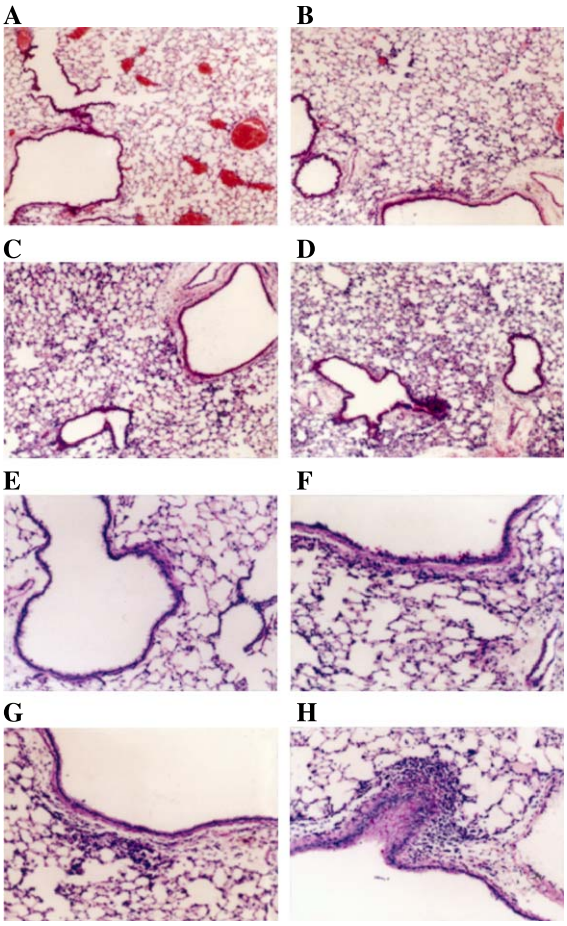

Fig. 3. Lung histology 24 h after LPS and 15d-PGJ_2 (1 mg/kg) administration to mice. Low-power photomicrographs of lung sections stained with hematoxylin and eosin (A–D), and periodic acid–Schiff stain (E–H). (A, E) the vehicle group, (B, F) the 15d-PGJ_2 group, (C, G) the LPS group, (D, H) the 15d-PGJ_2 (1 mg/kg) + LPS group. Original magnification, A–D; ×25, E–H; ×50.

view version 4.0; Abacus Concepts, Berkeley, CA) as previously described (Takano et al., 1997).

3. Results

3.1. 15d-prostaglandin J_2 enhances acute lung injury induced by bacterial endotoxin

To determine the effect of 15d-prostaglandin J_2 on the neutrophilic lung inflammation induced by bacterial endotoxin, we investigated the cellular profile of bronchoalveolar lavage fluid 24 h after the second intratracheal instillation of vehicle or lipopolysaccharide. The lipopolysaccharide group showed a marked increase in the numbers of total cells (Fig. 1A) and neutrophils (Fig. 1B) as compared with the vehicle group ($P<0.01$). 15d-prostaglandin J_2 (10 μg/kg, 100 μg/kg, or 1 mg/kg) combined with lipopolysaccharide increased the numbers of bronchoalveolar lavage total cells (Fig. 1A) and neutrophils (Fig. 1B) as compared with lipopolysaccharide administered alone ($P<0.05$; the 15d-prostaglandin J_2 (1 mg/kg) + lipopolysaccharide group versus the lipopolysaccharide group).

To quantitate the pulmonary edema, we evaluated the lung water content (Fig. 2) 24 h after the second intratracheal instillation. The lung water content was significantly greater in the lipopolysaccharide group than in the vehicle group ($P<0.01$). The combined administration of 15d-prostaglandin J_2 and lipopolysaccharide showed increases in the lung water content as compared with lipopolysaccharide administered alone ($P<0.05$; the 15d-prostaglandin J_2 (1 mg/kg) + lipopolysaccharide group versus the lipopolysaccharide group).

To determine the histological changes, we evaluated the lung specimens stained with hematoxylin and eosin (Fig. 3A–D) and PAS (Fig. 3E–H) 24 h after the second intratracheal instillation. The lipopolysaccharide group showed the moderate infiltration of neutrophils around the airways and vessels (Fig. 3C). The combined treatment with 15d-prostaglandin J_2 (1 mg/kg) and lipopolysaccharide led to a marked recruitment of neutrophils and interstitial edema (Fig. 3D) and the presence of mucin-producing cells (Fig. 3H). Interestingly, the 15d-prosta-

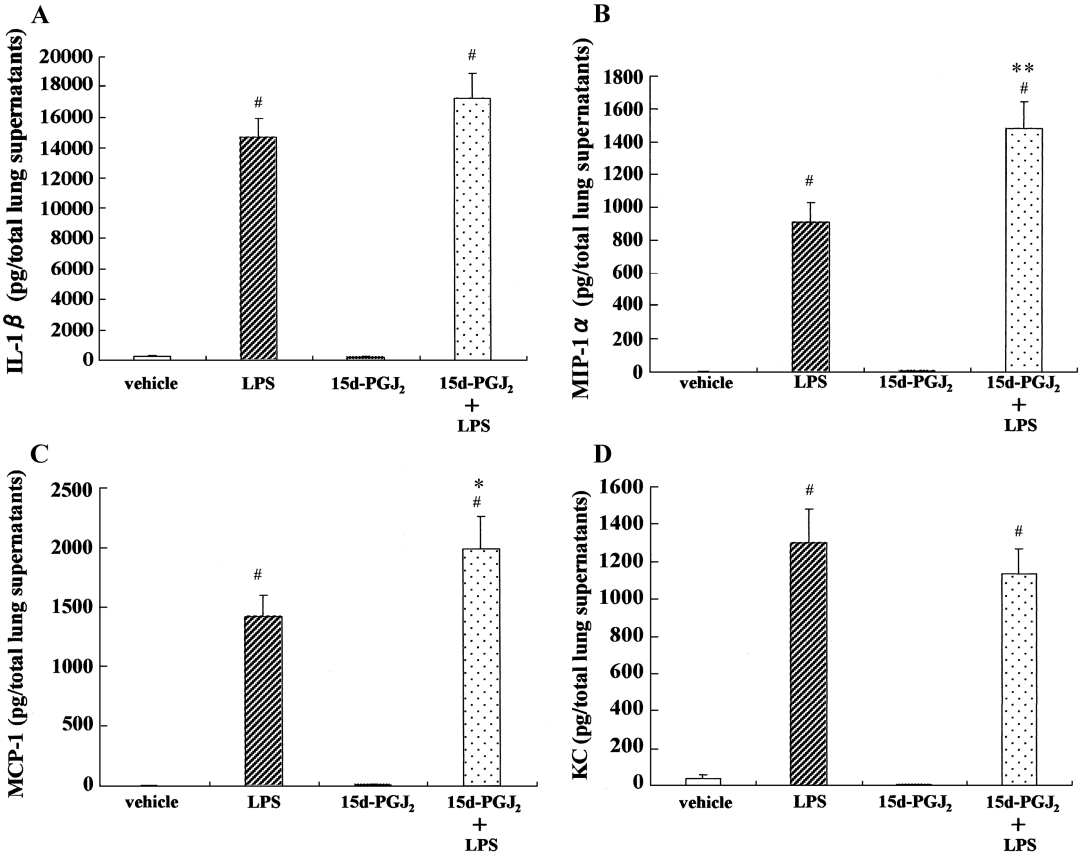

Fig. 4. Effect of 15d-PGJ_2 (1 mg/kg) on protein levels of chemokines and interleukin-1β (IL-1β) in murine lung tissue supernatants after challenge with LPS. Lungs from mice ($n=8$ in each group) were obtained 24 h after the LPS administration. IL-1β (A), macrophage inflammatory protein (MIP)-1α (B), macrophage chemoattractant protein (MCP)-1 (C), and keratinocyte chemoattractant (KC) (D) levels in the lung tissue supernatants were measured by enzyme-linked immunosorbent assays. #$P<0.01$ versus the vehicle group and the 15d-PGJ_2 group; *$P<0.05$ versus the LPS group; **$P<0.01$ versus the LPS group. Values are the mean ± S.E.M. in each group.

glandin J_2 group also showed the presence of mucin-producing cells (Fig. 3F) with slight neutrophilic infiltration (Fig. 3B). Vehicle administration alone caused no histological changes (Fig. 3A and E).

3.2. 15d-prostaglandin J_2 enhances the expression of proinflammatory proteins related to lipopolysaccharide

To elucidate the role of proinflammatory proteins in the enhancing effect of 15d-prostaglandin J_2 (1 mg/kg) on the lung injury related to lipopolysaccharide, we quantitated protein levels of interleukin-1β (Fig. 4A), MIP-1α (Fig. 4B), MCP-1 (Fig. 4C), and KC (Fig. 4D) in the lung tissue supernatants 24 h after the second intratracheal instillation. The protein levels of all these chemokines and interleukin-1β were significantly higher in the lipopolysaccharide group than in the vehicle group ($P<0.01$) and in the 15d-prostaglandin J_2 group ($P<0.01$). The levels of MIP-1α and MCP-1 in the 15d-prostaglandin J_2 (1 mg/kg) + lipopolysaccharide group were significantly greater than those in the lipopolysaccharide group ($P<0.01$ and $P<0.05$, respectively). Although the levels of interleukin-1β in the 15d-prostaglandin J_2 (1 mg/kg) + lipopolysaccharide group was greater than those in the lipopolysaccharide group, the difference was not significant. The protein levels of KC were not significantly different between the 15d-prostaglandin J_2 (1 mg/kg) + lipopolysaccharide group and the lipopolysaccharide group. On the other hand, treatment with 15d-prostaglandin J_2 (1 mg/kg) alone showed negligible levels of these molecules.

3.3. 15d-prostaglandin J_2 modulates activation of nuclear transcription factors related to lipopolysaccharide

To examine the role of nuclear transcription factors in the increased expression of these proinflammatory molecules, we investigated the nuclear localization of NF-κB and the phosphorylation of c-Jun 2 h after the second intratracheal administration. As compared with the vehicle group, the lipopolysaccharide group showed an increase in the nuclear localization of p50 subunit of NF-κB ($P<0.05$ versus the vehicle group), whereas the 15d-prostaglandin J_2 (1 mg/kg) + lipopolysaccharide group resulted in a slight decrease in its nuclear localization (Fig. 5A). Administration of 15d-prostaglandin J_2 (1 mg/kg) alone did not affect the nuclear localization of p50 protein (Fig. 5A). On the other hand, lipopolysaccharide treatment caused the intense nuclear localization of p65 subunit of NF-κB as compared to vehicle treatment ($P<0.05$: Fig. 5B). The combined treatment with 15d-prostaglandin J_2 (1 mg/kg) + lipopolysaccharide resulted in a decrease in the nuclear localization of p65 as compared to lipopolysaccharide treated alone. Administration of 15d-prostaglandin J_2 (1 mg/kg) alone showed a slight increase in the nuclear localization of p65 protein as compared to vehicle administration (Fig. 5B). The cytoplasmic localization of p50 and p65 was not significantly

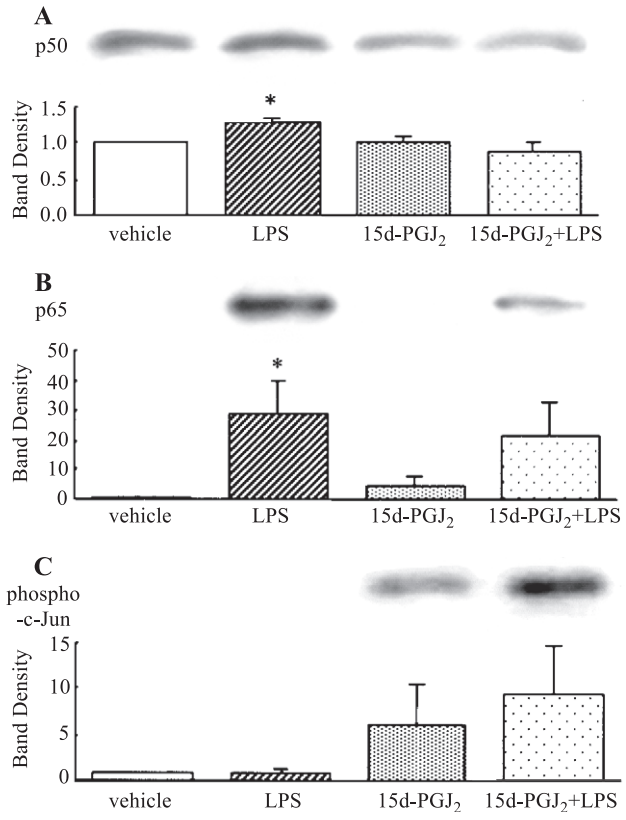

Fig. 5. Effect of 15d-PGJ_2 (1 mg/kg) on activation of nuclear transcriptional factors related to LPS. Nuclear localization of p50 (A) and p65 (B) of nuclear factor-κB, and phosphorylation of c-Jun (C) were investigated 2 h after the intratracheal administration using Western blot analysis. The top panel shows actual membrane pictures of p50, p65, and phosphorylated c-Jun. The bottom panel shows band density for p50, p65, and phosphorylated c-Jun. Each density represents the mean ± S.E.M. of at least five animals per group. *$P<0.05$ versus the vehicle group.

different among the four experimental groups (data not shown). Lipopolysaccharide treatment did not affect the phosphorylation of c-Jun in the nucleus as compared to vehicle treatment (Fig. 5C). 15d-prostaglandin J_2 (1 mg/kg) treated alone showed an increase in the phosphorylation of c-Jun, and the combined treatment with 15d-prostaglandin J_2 (1 mg/kg) + lipopolysaccharide resulted in a further increase in its phosphorylation (Fig. 5C).

3.4. Modulation of PPAR-γ by lipopolysaccharide and 15d-prostaglandin J_2 in the lung

To examine the regulation of PPAR-γ by 15d-prostaglandin J_2 and/or lipopolysaccharide, we investigated the expression of PPAR-γ protein in the nucleus 2 h after the second intratracheal administration. Expression of PPAR-γ protein was observed in the lungs. The expression was down-regulated by treatment with lipopolysaccharide. Treatment with 15d-prostaglandin J_2 (1 mg/kg) alone increased its protein level as compared to vehicle treatment, whereas the combined administration of 15d-prostaglandin J_2 (1 mg/kg) + lipopolysaccharide decreased the protein

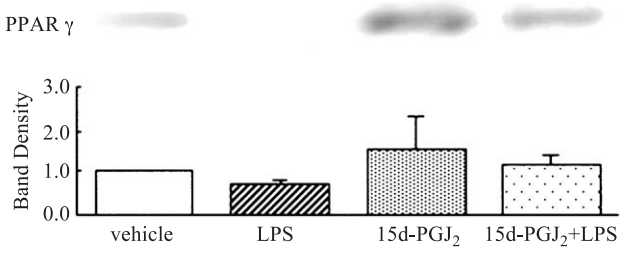

Fig. 6. Effect of 15d-PGJ_2 (1 mg/kg) on PPAR-γ expression related to LPS. Nuclear expression of PPAR-γ was investigated 2 h after intratracheal administration using Western blot analysis. The top panel shows actual membrane pictures of PPAR-γ. The bottom panel shows band density for PPAR-γ. Each density represents the mean ± S.E.M. of at least five animals per group.

level as compared with 15d-prostaglandin J_2 (1 mg/kg) administered alone (Fig. 6).

4. Discussion

The present study has demonstrated that 15d-prostaglandin J_2 (10 μg/kg, 100 μg/kg, and 1 mg/kg) does not ameliorate but significantly enhances acute lung injury induced by lipopolysaccharide at a dose of 1 mg/kg. The lung injury comprises neutrophilic inflammation and interstitial edema. In addition, 15d-prostaglandin J_2 administration induces the presence of mucin-producing cells, which is further enhanced by the combination with lipopolysaccharide. The enhancement in the lung injury related to lipopolysaccharide by 15d-prostaglandin J_2 is associated with the increased local expression of interleukin-1β, MIP-1α, and MCP-1. The enhancement is accompanied by the increased phosphorylation of c-Jun in the lung. 15d-prostaglandin J_2 up-regulates the protein expression of PPAR-γ and inhibits the nuclear localization of NF-κB.

Accumulating lines of evidence have demonstrated that cyclopentenone prostaglandins and well-recognized anti-diabetic agents, thiazolidinediones, have anti-inflammatory properties in vitro. However, little is known concerning the efficacy of these agents in vivo. PPAR-γ ligands can attenuate inflammation in DSS-induced colitis, which has been evidenced by a disease activity index score and histological evaluations (Su et al., 1999). In addition, 2,4,6-trinitrobenzene sulfonic acid-induced colitis is significantly reduced by the administration of ligands for PPAR-γ and retinoid X receptor, heterodimeric partner of PPAR-γ (Desreumaux et al., 2001). Recently, we have demonstrated that PPAR-γ agonists ameliorate adjuvant-induced arthritis in rats, a well-known model of rheumatoid arthritis (Kawahito et al., 2000). More recently, Diab et al. (2002) have reported that 15d-prostaglandin J_2 ameliorates experimental autoimmune encephalomyelitis, an animal model for multiple sclerosis, by suppressing the expression of IFN-γ, interleukin-10, and interleukin-4.

On the other hand, Thieringer et al. (2000) have demonstrated that treatment with thiazolidinedione does not suppress the increased circulatory proinflammatory cytokines in *db/db* mice challenged with intravenous lipopolysaccharide, suggesting that activation of PPAR-γ might not be useful for the treatment of endotoxemia. However, the workers have not referred to the other data including inflammatory responses and histological evaluations. In the present study, the administration of 15d-prostaglandin J_2 (10 μg/kg, 100 μg/kg, or 1 mg/kg) failed to reverse acute lung injury caused by lipopolysaccharide and instead aggravated neutrophilic inflammation and edema at its dose of 1 mg/kg. These results make it doubt that administration of 15d-prostaglandin J_2 would be an effective intervention for animal models of acute lung injury caused by bacterial endotoxin.

In the present study, treatment with 15d-prostaglandin J_2 induced the presence of mucin-producing cells in the bronchial epithelium, which was further enhanced by the combination with lipopolysaccharide. Increased secretion of mucin derived from the mucin-producing cells might be, at least partly, implicated in the potentiation of acute lung injury by 15d-prostaglandin J_2 in the presence of lipopolysaccharide. It has been reported that several prostaglandins including prostaglandin D_2, prostaglandin J_2, and thromboxane A_2 administered locally in the conjunctiva induce conjunctival redness, edema, and mucous cell discharge (Woodward et al., 1995). Further studies are needed to elucidate the mechanisms by which prostaglandins increases mucous production in vivo.

The pathogenesis of acute lung injury involves increased production of proinflammatory cytokines including interleukin-1β (Jacobs et al., 1989; Ulich et al., 1991; Chollet-Martin et al., 1992). In addition, C–X–C chemokines regulate tissue sequestration of neutrophils in acute lung injury (Shanley et al., 1995; Standiford et al., 1995; Brieland et al., 1992; Jones et al., 1992; Leonard and Yoshimura, 1990). MIP-1α has been shown to contribute to leukocyte recruitment and lung injury induced by deposition of IgG immune complexes (Shanley et al., 1995) and by airway instillation of lipopolysaccharide (Shanley et al., 1995; Standiford et al., 1995). MIP-1α seems to function as an autocrine stimulator of macrophages, causing enhanced secretion of interleukin-1β under these conditions. MCP-1 is derived from mononuclear cells and other cell sources including alveolar macrophages, and has chemotactic activity for monocytes (Brieland et al., 1992; Jones et al., 1992; Leonard and Yoshimura, 1990). In a model of lung injury induced by IgA immune complexes, blockade of MCP-1 attenuates the injury, suggesting importance of MCP-1 (Jones et al., 1992). In addition, since an anti-interleukin-8 antibody treatment has prevented the pulmonary edema with neutrophil infiltration induced by lipopolysaccharide and heat-killed *Streptococcus pyogenes*, interleukin-8 can have a significant role in the induction of lung injury associated with lipopolysaccharide (Yokoi et al., 1997). In fact, our previous study confirmed that interleukin-1β, MIP-

1α, MCP-1, and KC were significantly elevated in the lung tissue supernatants by the intratracheal challenge with lipopolysaccharide, which was concomitant with the magnitude of lung injury (Takano et al., 2002). Also in the present study, the lung expression of these proinflammatory proteins was consistent with the severity of lung injury with respect to overall trends.

In previous studies in vitro, 15d-prostaglandin J_2 can inhibit the release of interleukin-1β and MCP-1 (Ricote et al., 1998b; Jiang et al., 1998). In contrast, it reportedly potentiates lipopolysaccharide-induced gene expression of interleukin-8 mRNA in human monocytes/macrophages in vitro (Zhang et al., 2001). Furthermore, cyclopentenone prostaglandins, when used at concentrations substantially lower than required for NF-κB inhibition, significantly potentiate the inflammatory response to TNF-α (Bureau et al., 2002). In addition to these controversial studies in vitro, there is no study in vivo on the regulation by 15d-prostaglandin J_2 of these proinflammatory cytokines and chemokines. In the present study, 15d-prostaglandin J_2 (1 mg/kg) treatment increased the lung expression of interleukin-1β, MIP-1α, and MCP-1 in the presence of lipopolysaccharide in vivo, which was concomitant with the integrity of lung injury. Moreover, the 15d-prostaglandin J_2 group showed negligible levels of these molecules. Our results indicate that the enhancement in acute lung injury related to lipopolysaccharide by 15d-prostaglandin J_2 might be explained, at least partly, by the enhanced expression of these chemokines and interleukin-1β, and that the effect of 15d-prostaglandin J_2 on lipopolysaccharide-related lung injury is specific and synergistic.

There is no established insight on the effects of 15d-prostaglandin J_2 on the interactions between the transcriptional factors related to bacterial endotoxin and PPAR-γ, especially in vivo. NF-κB plays a pivotal role in the activation of genes related to inflammatory responses (Ghosh et al., 1998). To date, two roles of cyclopentenone prostaglandins in regulation of the NF-κB pathway have been proposed (Ricote et al., 1998b; Jiang et al., 1998). First, cyclopentenone prostaglandins have been suggested to exert their anti-inflammatory activity through the activation of PPAR-γ (Ricote et al., 1998b). On the other hand, workers have demonstrated that 15d-prostaglandin J_2 directly inhibits NF-κB DNA binding ability (Cernuda-Morollon et al., 2001). However, there is no study how 15d-prostaglandin J_2 modulates NF-κB in vivo. In the present study, the nuclear localization of both p50 and p65 subunits of NF-κB were enhanced by lipopolysaccharide, whereas they were decreased by the combination with 15d-prostaglandin J_2 (1 mg/kg). Therefore, our present results in vivo are consistent with the previous studies concerning the role of 15d-prostaglandin J_2 on this transcriptional factor in vitro. Furthermore, the nuclear expression of PPAR-γ was enhanced by 15d-prostaglandin J_2 treatment (1 mg/kg) as compared to vehicle treatment, suggesting that 15d-prostaglandin J_2 induce PPAR-γ activation in the murine lung. In the present study, inhibition of the NF-κB activation might be mediated via the activation of PPAR-γ. Despite the activation of PPAR-γ and the inhibition of NF-κB in vivo, however, 15d-prostaglandin J_2 enhanced the expression of the chemokines and interlukin-1β and aggravated the subsequent acute lung injury in the present study. The striking results might be explained, at least in part, by activator protein-1 (AP-1) pathway, another transcriptional factor.

Apart from the NF-κB activation, the AP-1 activation plays a role in the expression of proinflammatory chemokines (Mezzano et al., 2001; Lim and Garzino-Demo, 2000; Wang et al., 2000; Lucio-Cazana et al., 2001). Transcription of the chemokines has been reported to be differently regulated by NF-κB and AP-1 (Wang et al., 2000). However, the action of 15d-prostaglandin J_2 on the AP-1 signaling pathway has been less studied in vitro, and none in vivo. 15d-prostaglandin J_2 potentiates the effects of interleukin-1β on the translocation of c-Jun in the nucleus in human chondrocytes (Boyault et al., 2001), whereas 15d-prostaglandin J_2 inhibit the activation of AP-1 as well as NF-κB (Fahmi et al., 2001; Subbaramaiah et al., 2001). Simonin et al. (2002) have demonstrated that troglitazone increases lipopolysaccharide-induced AP-1 activation in rat synovial fibroblasts. Their data suggest that PPAR-γ activation induce AP-1 activation. In the present study, phosphorylation of c-Jun was increased by 15d-prostaglandin J_2 (1 mg/kg) alone, which was further enhanced by the combination with lipopolysaccharide. AP-1 activation by 15d-prostaglandin J_2 in the presence of NF-κB activation related to lipopolysaccharide might play a role in the enhanced expression of chemokines and interleukin-1β related to lipopolysaccharide and in the subsequent aggravation of acute lung injury, since NF-κB and AP-1 lead to synergistic activation of the chemokine promoter (Lim and Garzino-Demo, 2000).

In conclusion, 15d-prostaglandin J_2 (10 μg/kg, 100 μg/kg, or 1 mg/kg) does not improve but significantly enhances acute lung injury related to lipopolysaccharide at a dose of 1 mg/kg. 15d-prostaglandin J_2 induces the presence of mucin-producing cells, which is further enhanced by the combination with lipopolysaccharide. The enhancement in the acute lung injury is associated with elevated local expression of proinflammatory chemokines and interleukin-1β. 15d-prostaglandin J_2 leads to an increase in the phosphorylation of c-Jun. These findings suggest that 15d-prostaglandin J_2 may not be useful but potentially harmful for the therapeutic option for acute lung injury related to lipopolysaccharide, and the action of 15d-prostaglandin J_2 on acute lung injury might be caused by the activation of AP-1 pathways.

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