15-Deoxy- $\Delta^{12,14}$ -Prostaglandin J_2 Inhibits the IL- 1β -Induced Expression of Granulocyte-Macrophage Colony-Stimulating Factor in BEAS-2B Bronchial Epithelial Cells

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KUMAGAI, M., IMAIZUMI, T., SUZUKI, K., YOSHIDA, H., TAKANASHI, S., OKUMURA, K., SUGAWARA, K., and SATOH, K. *15-Deoxy- $\Delta^{12,14}$ -Prostaglandin J_2 Inhibits the IL- 1β -Induced Expression of Granulocyte-Macrophage Colony-Stimulating Factor in BEAS-2B Bronchial Epithelial Cells.* Tohoku J. Exp. Med., 2004, **202** (2), 69-76 — 15-Deoxy- $\Delta^{12,14}$ -prostaglandin J_2 (15d-PGJ₂) is an agonist for peroxisome proliferator-activated receptor- γ (PPAR- γ), which plays an important role in various biological processes including inflammatory responses. We have addressed the effect of 15d-PGJ₂ on the expression of granulocyte-macrophage colony-stimulating factor (GM-CSF) in a cell line derived from human bronchial epithelial cells (BEAS-2B). Besides being a hematopoietic growth factor, GM-CSF activates mature leukocytes and is involved in regulation of inflammatory responses. Cultures of BEAS-2B were stimulated with interleukin- 1β (IL- 1β), and the expressions of GM-CSF mRNA and protein were analyzed by reverse transcription-polymerase chain reaction and enzyme-linked immunosorbent assay, respectively. IL- 1β stimulated the expression of GM-CSF in BEAS-2B cells in concentration- and time-dependent manners. When the cells were pretreated with 15d-PGJ₂ for 1 hour, the IL- 1β -induced GM-CSF expression was inhibited in a concentration-dependent manner (2-50 μ M). Ciglitazone, another agonist of PPAR- γ , did not affect the IL- 1β -induced GM-CSF expression in BEAS-2B cells. A PPAR- γ antagonist, bisphenol A diglycidyl ether (BADGE), did not reverse the inhibitory effects of 15d-PGJ₂ on GM-CSF expression. 15d-PGJ₂ regulates GM-CSF expression in the bronchial epithelium, which may be mediated through a mechanism unrelated to PPAR- γ . ——— 15d-PGJ₂; GM-CSF; BEAS-2B; IL- 1β ; BADGE

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Bronchial epithelial cells produce various factors involved in inflammatory reactions and hyperresponsiveness of the airway (Alder et al. 1994). Granulocyte-macrophage colony-stimulating factor (GM-CSF) was identified as a hematopoietic factor, and it also activates mature leukocytes including neutrophils, monocytes and eosinophils (Nagata et al. 1995). GM-CSF is produced by a variety of cell types including monocytes/macrophages, vascular endothelial cells, and fibroblasts (Munker et al. 1986; Agostini et al. 1992; Takahashi et al. 2001). In normal airways, GM-CSF is expressed at low or undetectable levels, but its expression is significantly enhanced in response to cytokines, infectious agents, environmental pollutants etc. (King et al. 1998; Takizawa et al. 2000; Kawasaki et al. 2001). GM-CSF level is increased in the epithelium of asthmatics (Sousa et al. 1993), and mice genetically rendered deficient in GM-CSF develop chronic infectious disease of the lung with lesions similar to pulmonary proteinosis (Stanley et al. 1994). These facts suggest an important role of GM-CSF in pulmonary physiology and resistance to infection. Interleukin- 1β (IL- 1β) is found in elevated levels in airways of asthmatics and has been shown to stimulate GM-CSF expression in bronchial epithelial cells (Broide et al. 1992). Induction of GM-CSF by IL- 1β is mediated predominantly through transcriptional mechanism (Adkins et al. 1998).

15-Deoxy- $\Delta^{12,14}$ -prostaglandin J_2 (15d-PGJ $_2$) is known to regulate expressions of various genes through the activation of peroxisome-proliferator activated receptor- γ (PPAR- γ) (Forman et al. 1995; Kliewer et al. 1995). However, recent studies revealed that 15d-PGJ $_2$ exerts diverse effects through PPAR- γ -dependent or -independent mechanisms (Chawla et al. 2001; Jozkowicz et al. 2001). The present study was undertaken to address the effect of 15d-PGJ $_2$ on the GM-CSF expression in a bronchial epithelial cell line stimulated with IL- 1β .

MATERIALS AND METHODS

Reagents

Dulbecco's modified Eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco-BRL (Gaithersburg, MD, USA). 15d-PGJ $_2$ was from Biomol (Plymouth Meeting, PA, USA) and dexamethasone (DEX) from Wako (Osaka). Ciglitazone and WY14643 were from Calbiochem (San Diego, CA, USA). Primer oligo(dT) $_{12-18}$ and M-MuLV reverse transcriptase were from Invitrogen (Carlsbad, CA, USA). An RNA isolation kit and Taq DNA polymerase were from Qiagen (Hilden, Germany). Recombinant human IL- 1β and a Quantikine enzyme-linked immunosorbent assay (ELISA) kit for GM-CSF were obtained from R&D Systems (Minneapolis, MN, USA). Bisphenol A diglycidyl ether (BADGE) was from Cayman Chemical (Ann Arbor, MI, USA). The oligonucleotide primers were synthesized by Greiner Japan (Atsugi).

Cells

BEAS-2B cells, a cell line derived from human bronchial epithelial cells (Amstad et al. 1998), were cultured using DMEM supplemented with 10% FBS, and tightly confluent monolayers were used for the following experiments.

Cells were preincubated for up to 2 hours with 15d-PGJ $_2$ (2-50 μ M) and then stimulated with 1 ng/ml IL- 1β for up to 24 hours. 15d-PGJ $_2$ was dissolved in ethanol and the final concentration of ethanol in culture medium was 1%. The control cells were treated with vehicle only. The effects of specific PPAR- α agonist WY14643 (Santilli et al. 1974) or PPAR- γ agonist ciglitazone (Sohda et al. 1982) on the IL- 1β -induced GM-CSF expression were examined in a similar manner. WY14643 and ciglitazone were also dissolved in ethanol. In order to clarify the role of PPAR- γ in the inhibitory activity of 15d-PGJ $_2$, the effect of BADGE, an antagonist for PPAR- γ (Hinz et al. 2003), was examined. BADGE was dissolved in ethanol and applied to cultures 30 minutes prior to 15d-PGJ $_2$ treatment. The

effect of DEX, an anti-inflammatory steroid, was also examined. DEX was dissolved in ethanol and added simultaneously with 15d-PGJ₂ 1 hour before the stimulation with IL-1 β .

Reverse transcription-polymerase chain reaction

Total RNA was extracted from BEAS-2B cells using an RNeasy RNA isolation kit, and single-strand cDNA for a polymerase chain reaction (PCR) template was synthesized from 1 μ g of the RNA using a primer oligo(dT)₁₂₋₁₈ and M-Mulv reverse transcriptase. Primers were designed from cDNA sequences for GM-CSF (Gene Bank Accession Number M11220), PPAR- α , PPAR- γ , IL-1 α , IL-1 β and glyceraldehydes-3-phosphate dehydrogenase (GAPDH); and each cDNA was amplified by PCR using Taq DNA polymerase. The sequences of the primers were as follows:

GM-CSF-F (5'-GGCTGCAGAGCCTGCTGCTCT-3'),
 GM-CSF-R (5'-GCTTGTACAGCTCCAGCGGGTC-3'),
 PPAR- α -F (5'-CATCACGGACACGCTTTCACC-3'),
 PPAR- α -R (5'-GTTCTTCAAGTAGGCCTCTAG-3'),
 PPAR- γ -F (5'-CCATTCTGGCCCAACCAAC-3'),
 PPAR- γ -R (5'-CTGAAACCGACAGTACTG-3'),
 IL-1 α -F (5'-CAAGGAGAGCATGGTGGTAGCAACCAACG-3'),
 IL-1 α -R (5'-TAGTGCCGTGAGTTTCCCA GAAGAAGAGGAGG-3'),
 IL-1 β -F (5'-AAACAGATGAAGTGCTCCTTCCA-3'),
 IL-1 β -R (5'-GAGAACACCACTTGTGCTCCA-3'),
 IL-8-F (5'-AGGAGTGCTAAAGAACTTCGA-3'),
 IL-8-R (5'-TGAATTCTCAGCCCTCTTCAA-3'),
 GAPDH-F (5'-CCACCCATGGCAAATTCATGGCA-3'),

and GAPDH-R (5'-TCTAGACGGCAGGTCAGGTCCACC-3').

The reaction condition for GM-CSF, IL-1 α , IL-1 β , IL-8 and GAPDH was 1 $\times$ (94°C, 1 minute), 30 $\times$ (94°C, 1 minute; 60°C, 1 minute; 72°C, 1 minute), and 1 $\times$ (72°C, 10 minutes). The condition for PPAR- α was 1 $\times$ (94°C, 1 minute), 30 $\times$ (94°C, 1 minute; 62°C, 1 minute; 72°C, 1 minute), and 1 $\times$ (72°C, 10 minutes). The condition for PPAR- γ was 1 $\times$ (94°C, 1 minute), 33 $\times$ (94°C, 1 minute; 58°C, 1 minute; 72°C, 1 minute), and 1 $\times$ (72°C, 10 minutes). The products were analyzed by electrophoresis on a 1.5% agarose gel containing ethidium bromide. The size of the PCR products for GM-CSF, PPAR- α , PPAR- γ , IL-1 α , IL-1 β , IL-8 and GAPDH was 239 bp, 450 bp, 479 bp, 598 bp, 389 bp, 219bp and 696 bp, respectively. Because all of these primers were designed from different exons, the products with the expected size were amplified from single-strand cDNA but not from contaminating genomic DNA. The PCR products were confirmed to be specific for each cDNA by sequencing.

ELISA for GM-CSF

Levels of GM-CSF protein in conditioned medium of BEAS-2B cells were measured using an ELISA kit. After the stimulation, the cells were washed with serum-free DMEM and incubated for 1 hour in DMEM containing 0.1% human serum albumin. Then the conditioned medium was recovered and subjected to centrifugation at 10 000 $\times$ g for 10 minutes. The supernatant solutions were used as samples for ELISA.

Statistical analysis

Data were presented as mean $\pm$ S.D., and statistical significance was analyzed by Student's *t*-test. Probability less than 0.05 was considered statistically significant.

RESULTS

Effect of 15d-PGJ₂ on the IL-1 β -induced GM-CSF mRNA expression in BEAS-2B cells is shown in Fig. 1. The IL-1 β -induced GM-

Fig. 1. Effect of 15d-PGJ₂ on the IL-1 β -induced GM-CSF mRNA expression in BEAS- 2B cells. (A) 15d-PGJ₂ (25 μ M) was added to the cells before (–2 hours, –1 hour), simultaneously (0 hour) or after (+1 hour, +2 hours) stimulation with IL-1 β . (B) BEAS-2B cells were treated with 2–50 μ M 15d-PGJ₂ for 1 hour prior to stimulation with 1 ng/ml IL-1 β . (C) BEAS-2B cells were treated with 10 μ M DEX for 30 minutes prior to addition of 15d-PGJ₂ followed by IL-1 β stimulation.

CSF expression was significantly inhibited when the cells were incubated with 25 μ M 15d-PGJ₂ 1 hour prior to the stimulation with 1 ng/ml IL-1 β , and the inhibitory effect declined after 2 hours (Fig. 1A). The effect of 15d-PGJ₂ on the IL-1 β -induced expression of GM-CSF was observed in a concentration-dependent manner as shown

in Fig. 1B. IL-1 β also induced the expression of IL-1 α , IL-1 β and IL-8, and 15d-PGJ₂ inhibited the expression of these genes in a similar manner (Fig. 1B). Co-treatment of the cells with DEX and 15d-PGJ₂ had a synergistic inhibitory effect on the IL-1 β -induced GM-CSF expression (Fig. 1C).

RT-PCR analyses on the effects of specific

Fig. 2. Effects of PPAR agonists and BADGE on the IL-1 β -induced GM-CSF expression. BEAS-2B were treated for 1 hour with 10-100 μ M WY14643 (A) or 2-50 μ M ciglitazone (B) prior to stimulation with 1 ng/ml IL-1 β . (C) Constitutional expression of PPAR- α and PPAR- γ in unstimulated BEAS-2B cells was confirmed by RT-PCR analysis. (D) The cells were pretreated with 50 μ M BADGE for 30 minutes prior to the addition of 15d-PGJ₂ followed by IL-1 β stimulation. RNA was extracted and subjected to RT-PCR.

agonists for PPAR- α or PPAR- γ are shown in Figs. 2A and 2B. Pretreatment of the cells with PPAR- α agonist WY14643 or PPAR- γ agonist ciglitazone had no effect on the GM-CSF expression in response to IL-1 β (Figs. 2A and 2B). In parallel experiments, constitutional expressions of both PPAR- α and PPAR- γ are confirmed by RT-PCR (Fig. 2C). PPAR- γ antagonist BADGE did not affect the inhibitory effect of 15d-PGJ₂ on the

IL-1 β -induced GM-CSF expression (Fig. 2D).

Effect of 15d-PGJ₂ on the IL-1 β -induced GM-CSF protein production is shown in Fig. 3. BEAS-2B cells produced 72 ± 9.3 pg/10⁶ cells ($n=3$) of GM-CSF when stimulated with 1 ng/ml IL-1 β , and the preincubation with 25 μ M 15 d-PGJ₂ significantly decreased the IL-1 β -induced GM-CSF production by about 40%: 43 ± 8.6 pg/10⁶ cells ($n=3$; $p<0.05$, based on t -test).

Fig. 3. Effect of 15d-PGJ₂ on the production of GM-CSF protein in BEAS-2B cells stimulated with IL-1 β . The cells were treated for 1 hour with 2–25 μ M 15d-PGJ₂ prior to stimulation with 1 ng/ml IL-1 β for 1 hour. The cells were washed and incubated for 2 hours in DMEM containing 0.1% human serum albumin. The medium was collected and assayed for GM-CSF by ELISA. Means ($\pm$ S.D.) of three experiments are shown. (* p <0.05, based on t -test). 15d-PGJ₂; ■, 0 μ M; ▨, 2 μ M; ▩, 10 μ M; □, 25 μ M.

DISCUSSION

GM-CSF is one of major hematopoietic factors that have growth-promoting activity on precursor cells of granulocytes and monocytes, and it also exerts agonistic activities towards mature granulocytes (Nagata et al. 1995). Therefore the production of GM-CSF in the non-hematopoietic tissues may have a potential effect on inflammatory responses, and bronchial epithelial cells may be involved in the control of inflammatory responses in the bronchial trees through the production of various factors including GM-CSF (Fujimoto et al. 2001). In fact, a gene targeting study disclosed that GM-CSF-deficient mice develop pulmonary abnormality characterized by chronic inflammatory changes, without major hematopoietic perturbation (Stanley et al. 1994). GM-CSF expression in cellular components of the lung is enhanced by various pro-inflammatory stimuli (King et al. 1998; Takizawa et al. 2000;

Kawasaki et al. 2001); and there is evidence for therapeutic effectiveness of GM-CSF for respiratory dysfunction in severe sepsis (Lieschke and Burgess 1992; Presnelli et al. 2002).

15d-PGJ₂, a metabolite of PGD₂, was first identified as a factor that induces adipocyte differentiation by activating a nuclear receptor PPAR- γ (Forman et al. 1995; Kliewer et al. 1995). It also exerts a regulatory effect on the expressions of various genes related to inflammatory reactions (Jiang et al. 1998; Ricote et al. 1998). PPARs are a family of nuclear hormone receptor and three subtypes, PPAR- α , - β and - γ , have been described. Each subtype regulates expression of specific genes in response to specific agonists. Recent studies demonstrated that part of 15d-PGJ₂ activities is also mediated through mechanisms unrelated to PPAR- γ (Chawla et al. 2001; Jozkowicz et al. 2001). It is shown that 15d-PGJ₂ inhibits the signal transduction via nuclear-factor κ B (NF- κ B) or signal transducer and activator of transcription (STAT-1) in a PPAR- γ -independent manner (Boyault et al. 2001; Imaizumi et al. 2003).

It is reported that PPAR- γ regulates the expression of IL-8 in airway epithelial cells (Wang et al. 2001). In the present study, pretreatment of BEAS-2B cells with 15d-PGJ₂ inhibited the expression of GM-CSF induced by IL-1 β . However, another specific PPAR- γ agonist, ciglitazone, did not affect the IL-1 β -induced expression of GM-CSF in BEAS-2B cells. BADGE, a PPAR- γ antagonist (Hinz et al. 2003), had no effect on the inhibitory effect of 15d-PGJ₂ on the IL-1 β -induced GM-CSF expression. These results suggest that the effect of 15d-PGJ₂ may be mediated through a mechanism independent on PPAR- γ . Specific PPAR- α agonist WY14643 (Santilli et al. 1974) was inert. Anti-inflammatory steroid DEX had a synergistic effect on the inhibition of GM-CSF expression by 15d-PGJ₂. IL-1 β enhanced the expressions of IL-1 α , IL-8 and IL-1 β itself; and 15d-PGJ₂ also inhibited the expressions of these cytokines. Autocrine expression of IL-1 and activation of cytokine network may be involved

in the expression of GM-CSF, and 15d-PGJ₂ may inhibit the GM-CSF expression by regulating these pathways.

We conclude that 15d-PGJ₂ inhibits the IL-1 β -induced expression of GM-CSF in BEAS-2B cells and the inhibitory effect may be unrelated to PPAR- γ . This may represent part of regulatory activity of this eicosanoid on inflammatory responses.

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