

Epithelial cell–derived prostaglandin D₂ inhibits chronic allergic lung inflammation in mice

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ABSTRACT: The precise role of prostaglandin D (PGD)₂ in allergic lung inflammation remains controversial. Here, we aimed to clarify the role of PGD₂ in chronic allergic lung inflammation using hematopoietic PGD synthase (H-PGDS)–deficient mice. Repeated intranasal administration of ovalbumin (OVA) resulted in eosinophilic infiltration and mucin production in the lungs of wild type (WT) mice, leading to respiratory dysfunction. H-PGDS deficiency exacerbated these effects, which were accompanied by increased mRNA expression of TNF- α and eosinophil chemoattractants. The bronchial epithelium expressed both H-PGDS and TNF- α in the inflamed WT lung, and H-PGDS deficiency increased TNF- α expression further. In cultured bronchial tissue of WT mice, treatment with LPS elevated mRNA expression of TNF- α and eosinophil chemoattractants. H-PGDS deficiency promoted the expression of these factors, which was inhibited by treatment with PGD₂ receptor, D prostanoid (DP) receptor agonist, or PGD₂ metabolite 15-deoxy- $\Delta^{12,14}$ -PGJ₂ (15d-PGJ₂). Treatment with TNF- α receptor antibody inhibited eosinophil chemoattractant expression. *In vivo*, administration of DP agonist or 15d-PGJ₂ inhibited OVA-induced allergic lung inflammation. Bronchial epithelial cell–derived PGD₂ attenuated lung eosinophilic infiltration with chronic allergic inflammation; these phenomena are at least partly attributed to the inhibition of TNF- α production *via* DP activation or 15-deoxy- $\Delta^{12,14}$ -PGJ₂ signaling.—Maehara, T., Nakamura, T., Maeda, S., Aritake, K., Nakamura, M., Murata, T. Epithelial cell–derived prostaglandin D₂ inhibits chronic allergic lung inflammation in mice. FASEB J. 33, 000–000 (2019). www.fasebj.org

KEY WORDS: allergy • eosinophil • 15d-PGJ₂ • DP receptor

Prolonged allergic lung inflammation leads to asthma and chronic obstructive pulmonary inflammation (1). Although corticosteroids and bronchodilators have been widely used in the treatment of these conditions, over 3000 people die of severe asthma a year in the United States alone (2). Therefore, therapeutic innovations, especially for chronic and severe allergic lung inflammation, are indispensable.

There are 2 types of mouse models that are used to mimic human allergic lung inflammation or asthma (3). The acute model involves intratracheal or nasal instillation of

allergens 3–5 times within 2 wk to induce eosinophilic infiltration in the lung and mild airway hyperreactivity. The institution of allergens 8–20 times over a longer period (4–12 wk) causes more severe pathologic conditions, reproducing features of clinical allergic lung inflammation or asthma such as sustained inflammation, lung dysfunction, and poor responsiveness to therapy. It is important that investigators select the appropriate model, considering the symptom observed and the signaling pathways involved.

Prostaglandin D (PGD)₂ is produced from arachidonic acid by cyclooxygenase and hematopoietic PGD synthase (H-PGDS). PGD₂ exerts its biologic function through 2 distinct PGD₂ receptors: the D prostanoid (DP) receptor and the chemoattractant receptor–homologous molecule expressed on type 2 T helper cells (CRTH2). Further, the dehydration product of PGD₂, 15-deoxy- $\Delta^{12,14}$ -PGJ₂ (15d-PGJ₂) exerts its biologic function *via* peroxisome proliferator–activated receptor- γ (PPAR- γ)–dependent or –independent signaling (4–6).

PGD₂ synthase and PGD₂ receptors were originally detected in cells that play a major role in allergic inflammation, such as mast cells and eosinophils (7, 8). In addition, much higher concentrations of PGD₂ have been

ABBREVIATIONS: 15d-PGJ₂, 15-deoxy- $\Delta^{12,14}$ -PGJ₂; BAL, bronchoalveolar lavage; CCL, C-C motif chemokine; CRTH2, chemoattractant receptor–homologous molecule expressed on type 2 T helper cells; DP, D prostanoid; HE, hematoxylin and eosin; H-PGDS, hematopoietic PGD synthase; OVA, ovalbumin; PGD, prostaglandin D; PGE₂, prostaglandin E₂; PPAR, peroxisome proliferator–activated receptor; SpO₂, saturation of peripheral oxygen; TNFR, TNF receptor; WT, wild type

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detected in the bronchoalveolar lavage (BAL) fluids of patients with asthma than those of healthy volunteers (9). There are several reports suggesting the contribution of PGD₂ receptor-mediated signaling to allergic lung inflammation. Gene deficiency of DP or CRTH2 has been shown to reduce eosinophil infiltration in the ovalbumin (OVA)-induced acute murine asthma model (10). Currently, clinical trials of CRTH2 antagonists are being conducted for adult asthma (11). However, other groups have shown DP deficiency exacerbates type 2 T helper cell responses by modulating dendritic cell activity in the OVA-induced acute asthma model (12). Thus, PGD₂ signaling is likely to play a complex role by being both pro- and anti-inflammatory depending on the type of producing cell, receiving cell, mediating receptor, and disease stage. Detailed studies revealing the pathophysiological role of PGD₂ *in vivo* are necessary for an improved understanding and therapeutic application of its effects in allergic lung inflammation.

Here, we investigated the role of PGD₂ in chronic allergic lung inflammation using H-PGDS-deficient mice. We demonstrated H-PGDS-derived PGD₂ plays anti-inflammatory roles by inhibiting eosinophilic infiltration and mucin overproduction.

MATERIALS AND METHODS

Animals

All animal experiments were performed with the approval of the institutional care and use committee of The University of Tokyo (Tokyo, Japan). We used 8–12-week-old male mice for experiments. H-PGDS-deficient mice (*H-pgds*^{−/−}) were on a BALB/c background and generated as previously described (13, 14).

Allergic lung inflammation model

Mice were sensitized against OVA by intraperitoneal injections of 100 μg of OVA (MilliporeSigma, Burlington, MA, USA) and 1 mg of aluminum potassium sulfate in 100 μl saline on d 0 and 7. On d 14, mice were challenged by intranasal injection of 100 μg of OVA diluted in physiologic saline (20 μl) every 4 d for a total of 10 times under anesthesia with isoflurane (3% induction, 1.5% maintenance). In some experiments, the DP receptor agonist BW245C (Cayman Chemicals, Ann Arbor, MI, USA) (200 μg/kg) or 15d-PGJ₂ (Cayman Chemicals) (100 μg/kg) was administered intranasally 30 min before each OVA challenge.

Saturation of peripheral oxygen measurement

Saturation of peripheral oxygen (SpO₂) levels were measured as previously described by Kobayashi *et al.* (15). SpO₂ levels were measured using a mouse pulse oximeter (Starr Life Sciences, Oakmont, PA, USA). Mice were anesthetized with isoflurane (3% induction, 1–2% maintenance), and heart rates were maintained for 400–500 bpm when the reading of pulse oximetry were measured.

Collection of BAL fluids

A total of 24 h after the 10th OVA challenge, the BAL fluid was collected. Mice were anesthetized with 100 mg/kg pentobarbital *via* intraperitoneal injection. A 24-gauge needle hub was gently

inserted into the trachea, and 100 μl of saline was administrated and aspirated slowly through the needle hub. The collected BAL fluid was applied to the slide using a centrifuge (700 rpm for 5 min). The number of eosinophils was counted on the Wright-Giemsa-stained section.

Measurement of PGD₂ and prostaglandin E₂ levels

The concentration of PGD₂ and prostaglandin E₂ (PGE₂) in lung tissue was measured as previously described by Nakamura *et al.* (16). Briefly, the lung tissue was homogenized using an amalgam mixer. The homogenized lung tissue was stored in ethanol containing 0.02% HCl, and samples were separated by HPLC. Quantification was performed using an LCMS-8030 Triple Quadrupole Mass Spectrometer (Shimadzu, Kyoto, Japan).

RNA isolation and real-time PCR

Real-time PCR analysis was performed using an Aria MX Real-Time PCR System (Agilent Technologies, Santa Clara, CA, USA). The total RNA extraction was performed using Trizol reagent, and reverse transcription was performed using ReverTra Ace (Toyobo, Osaka, Japan). The cDNA was amplified for 48 cycles of 95°C for 15 s and 59°C for 1 min. The list of primers used in this study is shown in Supplemental Table S1.

Alcian blue staining

The lung tissue was fixed with Carnoy's solution for 4 h, embedded in paraffin, and cut into 4-μm sections. The slides were immersed into 2% acetic acid for 2 min and then stained with Alcian blue solution (pH 2.5; Fujifilm, Tokyo, Japan) for 1 h. After staining with Alcian blue solution, the slides were rinsed with distilled water for 5 min followed by hematoxylin staining for 5 min.

TNF-α and H-PGDS staining

The lung tissue was fixed with 4% paraformaldehyde for 24 h, embedded in paraffin, and cut into 4-μm sections. To block endogenous peroxidase activity, the slides were incubated with 0.03% hydrogen peroxide for 30 min at room temperature and then incubated with 0.1% trypsin and 0.1% calcium chloride in Tris-buffered saline for 15 min at 37°C. After washing, the slides were permeabilized with 0.05% Triton X-100 and 2% BSA in PBS for 30 min. The permeability solutions were washed twice with PBS for 10 min. After washing, incubation with rabbit anti-mouse H-PGDS antibody (10004348, 04264141, 1:500; Cayman Chemicals) or rat anti-mouse TNF-α antibody (AF410NA, NQ13, 1:200; R&D Systems, Minneapolis, MN, USA) took place overnight at 4°C. The preparations were labeled with 1:200 diluted goat anti-rat IgG secondary antibody (BA-9401; Vector Laboratories, Burlingame, CA, USA) or goat anti-rabbit antibody (BA-1000, 2B1007; Vector Laboratories) for 2 h at room temperature. The slides were incubated with the avidin-biotin complex reagents for 30 min at room temperature and were washed twice with PBS for 10 min. The slides were stained with 3,3'-diaminobenzidine for 3 min.

Bronchial tissue culture

Bronchial tissues were isolated from mice and cut in DMEM. Tissues were treated with 100 ng/ml LPS, and 0.1 and 1 μM of BW245C was added 5 min before LPS treatment. Treatment with 0.1 and 1 μM of 15d-PGJ₂ and TNF receptor (TNFR)-1/2 antibody (BioLegend, San Diego, CA, USA) was added after 1-h

LPS treatment. Tissues were incubated for 12 h at 37°C in 5% CO₂. After incubation, bronchial tissues were homogenized using an amalgam mixer and analyzed by real-time PCR.

Statistical analysis

All results are shown as means $\pm$ SEM. An unpaired Student's *t* test was used to compare 2 groups, and 1-way ANOVA with Tukey's or Dunnett's test was used for comparisons among more than 3 groups. Values of *P* < 0.05 were considered statistically significant.

RESULTS

H-PGDS deficiency enhances OVA-induced eosinophil infiltration and lung dysfunction

We measured arterial oxygen saturation, SpO₂, which is an index of respiratory function. Without OVA challenge,

there was no difference in SpO₂ levels between wild-type (WT) and *H-pgds*^{-/-} mice. Intranasal challenge with 100 μ g of OVA decreased SpO₂ levels by 5% in WT (Fig. 1A; 24 h after the 10-times OVA challenge). H-PGDS deficiency decreased SpO₂ levels further by 25% (Fig. 1A). There were no differences in serum OVA-specific IgE levels between the 2 lines of mice following a 10-times OVA challenge (Fig. 1B).

Hematoxylin and eosin (HE)-stained sections showed accumulation of immune cells, mostly eosinophils characterized by acidophilic cytoplasm and nuclear segmentation (Fig. 1C lower left panel shows high-power fields), around the bronchus and blood vessels in the 10-times OVA-challenged WT lungs (indicated by arrowhead in Fig. 1C, upper panel). In *H-pgds*^{-/-} lungs, a greater degree of eosinophilic infiltration was observed, and eosinophils were found to fill some bronchial lumens (indicated by the dot circle in Fig. 1C, lower right pane).

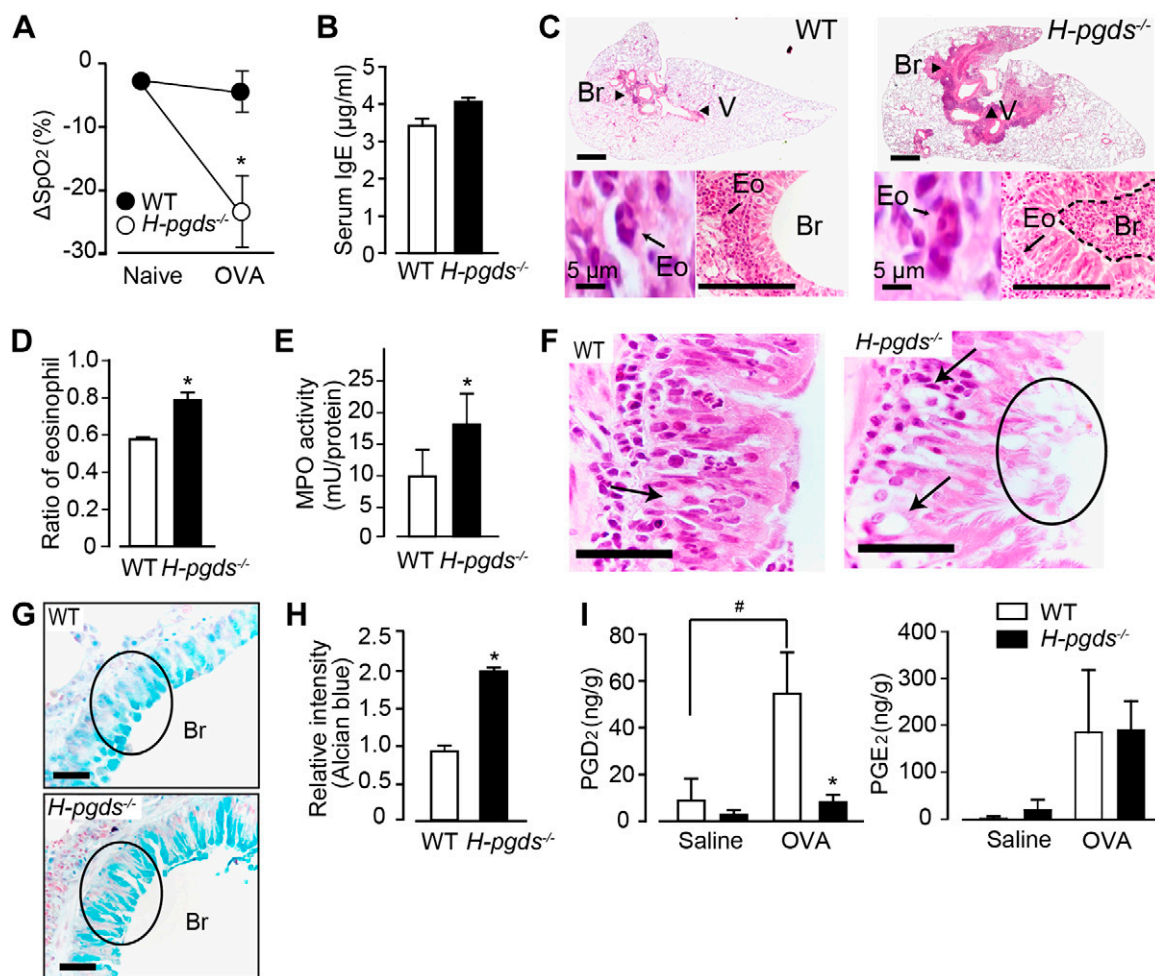


Figure 1. *H-pgds* deficiency aggravated allergic lung inflammation. A) SpO₂ levels at 24 h after 10-times OVA challenge of H-PGDS-deficient (*H-pgds*^{-/-}) mice and WT mice; the difference in SpO₂ between naive and OVA-challenged mice is shown (*n* = 5–9). B) OVA-specific IgE levels of 10-times OVA-challenged mice in serum were measured by ELISA (*n* = 4 each). C) Representative pictures of HE sections of 10-times OVA-challenged mice lungs (*n* = 4 each). Scale bars, 100 μ m. D) The proportion of eosinophils in the BAL fluid of 10-times OVA-challenged mice was determined by examination of Wright-Giemsa-stained sections (*n* = 5 each). E) Myeloperoxidase activity following 10-times OVA challenge was significantly higher in lung tissues from *H-pgds*^{-/-} mice compared with those from WT mice (*n* = 3). F, G) HE- (F) and Alcian blue (G)-stained sections of mouse bronchial epithelium at 24 h after 10-times OVA challenge (*n* = 4) (Scale bars, 25 μ m.). H) The pixel density of Alcian blue in *H-pgds*^{-/-} mice was normalized to the pixel density in WT mice (*n* = 4 each). I) PGD₂ and PGE₂ content of lung tissue was measured by liquid chromatography with tandem mass spectrometry (*n* = 4 each). Br, bronchus; Eo, eosinophil; V, vessel. Results are expressed as means $\pm$ SEM. **P* < 0.05 vs. WT mice. #*P* < 0.05 vs. saline-treated WT.

Consistently, the ratio of eosinophils in BAL fluid of *H-pgds*^{-/-} mice was higher than that in WT (Fig. 1D). In the lungs of patients with relatively severe asthma, neutrophils also play a crucial role in disease progression. The activity of myeloperoxidase, an index of neutrophil infiltration, was also significantly increased in inflamed *H-pgds*^{-/-} lungs compared with that in WT lungs (Fig. 1E).

Infiltrating eosinophils and neutrophils damage the lung epithelium by secreting a variety of bioactive substances. As shown in Fig. 1F, HE-stained sections showing the disruption of cilia and ciliated epithelial cells (indicated by circles in Fig. 1F) were observed in the case of *H-pgds*^{-/-} mice but not in WT. As often seen in patients with asthma, hyperplasia of goblet cells (indicated by an arrow in Fig. 1F) and excessive mucin production were also observed only in *H-pgds*^{-/-} lungs (indicated by circles in Fig. 1G, H). The OVA challenge increased the concentration of PGD₂ in the lung tissue of WT mice but not in *H-pgds*^{-/-} mice (Fig. 1I). In contrast, OVA challenge did not influence the production of a major inflammatory PG (PGE₂) in both lines of mice.

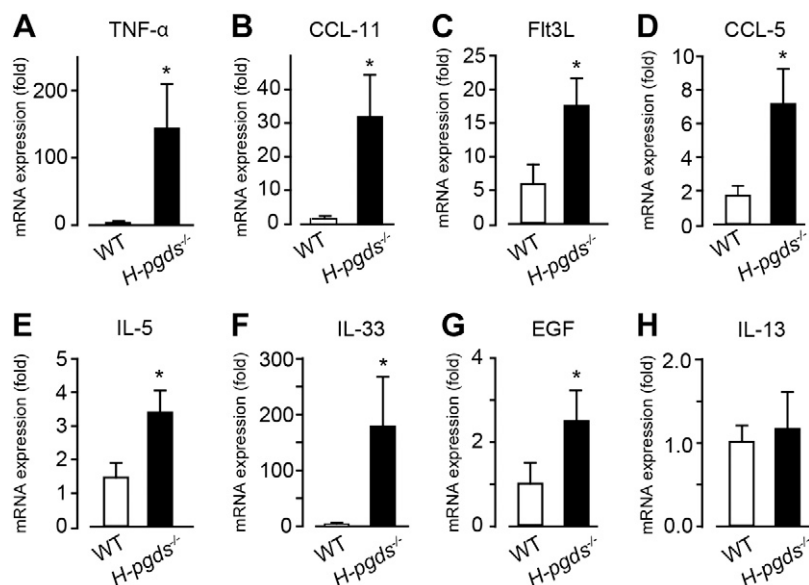
These results suggest that the deficiency of H-PGDS-derived PGD₂ aggravates allergic lung inflammation and mucin overproduction.

H-PGDS deficiency increases the expression of TNF- α , eosinophil chemoattractants, and epidermal growth factor

A major inflammatory mediator, TNF- α , induces epithelial damage and stimulates mucin secretion. The mRNA expression of TNF- α was elevated almost 150-fold in the inflamed *H-pgds*^{-/-} lungs compared with that in the WT lungs (Fig. 2A).

Several chemoattractants of eosinophils are known: epithelial cells produce C-C motif chemokine (CCL)-11 and FMS-like tyrosine kinase 3 ligand, whereas lymphocytes produce CCL-5 and IL-5. H-PGDS deficiency significantly increased the mRNA expression of these chemoattractants (Fig. 2B–E).

Figure 2. *H-pgds* deficiency promotes mRNA expression of eosinophil chemoattractants and mucin secretory cytokines. The mRNA expression in 10-times OVA-challenged mouse lungs was assessed by real-time PCR. Eosinophil chemoattractants (A–E), epithelial damage marker IL-33 (F), and mucin secretory cytokines (G, H) of 10-times OVA-challenged mouse lungs was assessed. Results are shown as means $\pm$ SEM. **P* < 0.05 vs. WT mice (*n* = 4–7).



As shown in Fig. 1F, morphologic observations showed that H-PGDS deficiency promoted epithelial damage. Consistent with these phenomena, an index of epithelial damage, IL-33 expression, was about 200 times higher in *H-pgds*^{-/-} mice than in WT mice (Fig. 2F).

Epidermal growth factor and IL-13 stimulate mucin production from goblet cells and clara cells (17–19). H-PGDS deficiency additionally significantly increased the expression of epidermal growth factor (Fig. 2G) but not that of IL-13 (Fig. 2H) in the inflamed lungs.

Bronchial epithelial cells express H-PGDS and TNF- α

Immunostaining showed that the protein expression of H-PGDS was not detected in the naive (Fig. 3A) and the once OVA-challenged WT lung (Fig. 3B). The 10-time OVA challenges strongly induced H-PGDS expression (Fig. 3C) in bronchial epithelial cells (Fig. 3C, right upper panel). Some mast-like cells (Fig. 3C, right lower panel indicated by white arrowhead) and macrophage-like cells in the lungs (Fig. 3C, right lower panel indicated by black arrowhead), which were recognized by cytoplasmic granules, large cytoplasm, and large nuclei, also weakly expressed H-PGDS (Fig. 3C, right lower panel). In the 10-times OVA-challenged *H-pgds*^{-/-} lung, H-PGDS expression was not detected (Fig. 3D).

The protein expression of TNF- α was slightly detected in once OVA-challenged WT and *H-pgds*^{-/-} lungs (Fig. 3E, left panels). The 10-times OVA challenges induced TNF- α expression on epithelial cells in WT lungs. H-PGDS deficiency increased this expression further (Fig. 3E, right panels).

PGD₂ inhibits TNF- α and eosinophil chemoattractant expression

LPS activates nuclear factor kappa B- and myeloid differentiation primary response 88-mediated signaling,

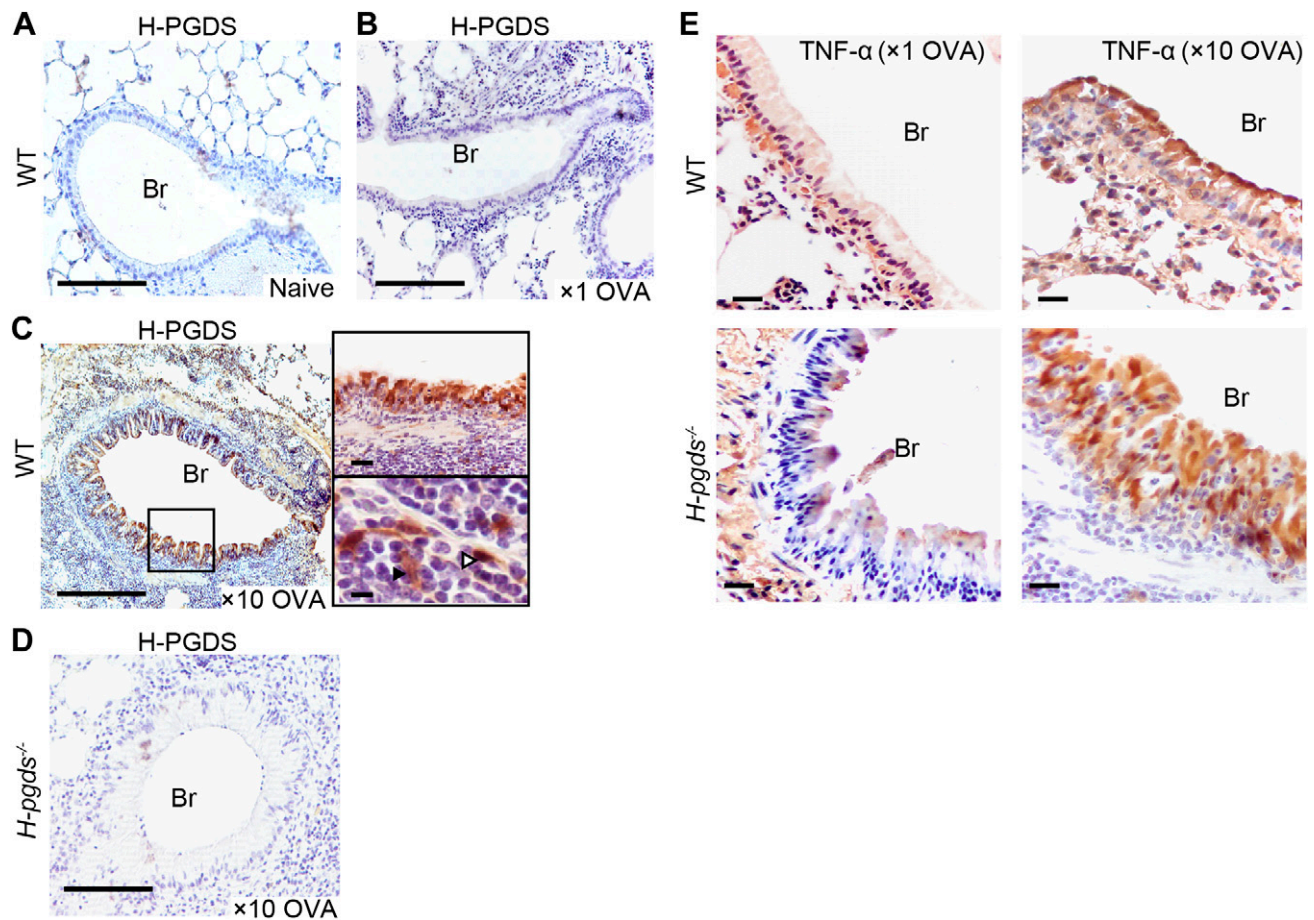


Figure 3. Bronchial epithelial cells express H-PGDS and TNF- α . A–D) Representative pictures of H-PGDS-stained sections of naive (A), 1-time OVA-challenged WT (B), 10-times OVA-challenged WT (C), or *H-pgds*^{-/-} mouse lung (D) ($n = 4$ each). Epithelial cells strongly expressed H-PGDS, [(C) right upper panel], macrophages [(C) right lower panel indicated by black arrowhead], and mast cells [(C) right lower panel indicated by white arrowhead] express H-PGDS at low levels. Scale bars, 100 μ m. E) Representative pictures of TNF- α -stained lung sections from 1-time and 10-times OVA-challenged WT and *H-pgds*^{-/-} mice ($n = 4$ each); Br, bronchus. Scale bars, 25 μ m.

which increases TNF- α expression in various types of cells (20). To investigate the mechanisms by which epithelial cell-derived H-PGDS inhibits TNF- α expression, we examined organ-cultured bronchus tissue treated with LPS.

Twelve-hour incubation with 100 ng/ml LPS increased TNF- α expression in bronchial tissue isolated from WT (Fig. 4A). H-PGDS deficiency increased this TNF- α expression further by about two-fold compared with that from WT. Treatment with 1 μ M, but not with 0.1 μ M, of DP agonist BW245C significantly inhibited LPS-induced TNF- α expression in WT tissues. In *H-pgds*^{-/-} tissue, these treatments also significantly inhibited the elevation of TNF- α expression, and this expression is not different between WT and *H-pgds*^{-/-} tissue.

Treatment with LPS additionally increased the expression of eosinophil chemoattractants CCL-5 and CCL-11 in bronchial tissue isolated from WT (Fig. 4B, C). The expression of these factors was much higher in *H-pgds*^{-/-} mice than in WT. Treatment with 1 μ M, but not with 0.1 μ M, of BW245C or 15d-PGJ₂ almost completely inhibited the elevation of CCL-5 and CCL-11 expression

in both lines of mice. We also investigated whether BW245C or 15d-PGJ₂ inhibit these expressions *via* affecting DP or PPAR- γ . Pretreatment with a DP antagonist, BWA868C, further increased the LPS-induced TNF- α and CCL-5 expressions in the bronchus tissue of WT but not in that of *H-pgds*^{-/-} (Supplemental Fig. S1A, B). These data suggest endogenous production of PGD₂ by LPS stimulation, which autocrinally attenuates cytokine and chemokine expressions *via* DP. Pretreatment with BWA868C canceled the inhibitory effect of BW245C. Moreover, pretreatment with 15d-PGJ₂ inhibited the LPS-induced TNF- α and CCL-5 expressions, which were inhibited by pretreatment with the PPAR- γ antagonist GW9662 (Supplemental Fig. S1C, D).

We next assessed the contribution of TNF- α signaling to the increase in chemoattractant expression. The administration of 10 ng/ml, but not that of 1 ng/ml, of anti-TNF-R1/2 antibodies suppressed the expression of CCL-5 (Fig. 4B) and CCL-11 (Fig. 4C) without affecting the expression of TNF- α (Fig. 4A).

These results suggest that PGD₂ and DP and 15d-PGJ₂ inhibit eosinophil chemoattractant expression in bronchial tissue by inhibiting TNF- α expression.

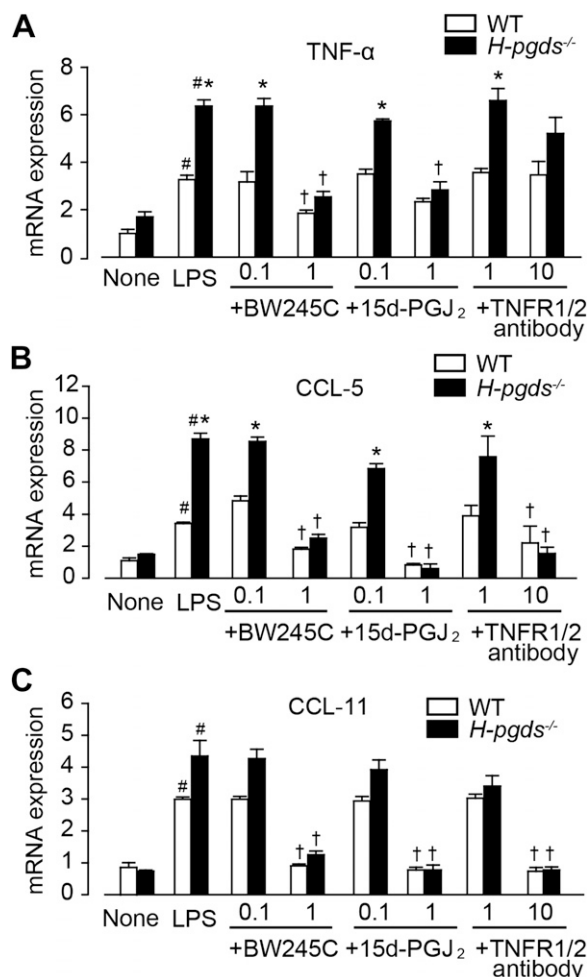


Figure 4. Bronchial epithelial cell-derived PGD₂ inhibited TNF-α and eosinophil chemoattractant expression. The expression of TNF-α (A), CCL-5 (B), or CCL-11 (C), as measured by real-time PCR, in isolated bronchial tissue cultured with 100 ng/ml LPS (*n* = 4 each); in some experiments, pretreatment with BW245C (0.1, 1 μM) and TNFR1/2 antagonist (1, 10 ng/ml) was performed 15 min before stimulation with LPS; pretreatment with 15d-PGJ₂ (0.1, 1 μM) was performed 1 h before LPS stimulation. Treatment with LPS resulted in an increase in the expression of TNF-α, CCL-5, and CCL-11 in bronchial tissue isolated from WT mice. (*n* = 4 each). Results are shown as means ± SEM. [#]*P* < 0.05 vs. none, ^{*}*P* < 0.05 vs. WT, [†]*P* < 0.05 vs. LPS.

Enhancement of PGD₂ signaling attenuates allergic lung inflammation *in vivo*

We examined the therapeutic potential of DP agonists BW245C or 15d-PGJ₂ using WT. As described above, in naive WT, SpO₂ levels were observed to be about 99%. The 10-times OVA challenge decreased SpO₂ levels by 5% (Fig. 5A). The administration of BW245C (200 μg/kg, intranasally, every fourth day) or 15d-PGJ₂ (100 μg/kg, intranasally, every fourth day) recovered decreased SpO₂ levels by 2% (Fig. 5A).

HE-stained sections exhibited the presence of macrophages, which were recognized by their large cytoplasm and round nuclei; these were mainly detected in non-treated mice lungs (Fig. 5B, left upper panel). The 10-time

OVA challenges increased the infiltration of eosinophils around bronchi and blood vessels (Fig. 5B, right upper panel). The administration of BW245C and 15d-PGJ₂ inhibited eosinophilic infiltration to similar extents (Fig. 5B, lower panels).

The 10-times OVA challenges further resulted in increased proportions of eosinophils in BAL fluid (Fig. 5C). The administration of BW245C decreased the proportion of eosinophils by almost half. The administration of 15d-PGJ₂ decreased the eosinophil proportion by a greater extent relative to BW245C administration.

The 10-times OVA challenges increased mRNA expression of TNF-α (Fig. 5D), but not that of CCL-5 (Fig. 5E), in lung tissue. The administration of BW245C or 15d-PGJ₂ attenuated the increase in the expression of these factors. Treatment with BW245C and 15d-PGJ₂ almost completely inhibited the mRNA expressions of TNF-α and CCL-5. However, this regimen did not inhibit eosinophil migration strongly. Other chemokines, such as CCL-7 and CCL-13, might also be involved in eosinophil migration in this model (21). As mentioned above and shown in Fig. 5F, the 10-times OVA challenges disrupted ciliated epithelial cells. The administration of BW245C, but not that of 15d-PGJ₂, ameliorated this damage. Alcian blue staining showed that 10-times OVA challenges promoted mucin production by epithelial cells; this was inhibited by treatment with BW245C but not 15d-PGJ₂ (Fig. 5G).

These results indicate that enhancement of PGD₂ and DP signaling ameliorates allergic lung inflammation and that 15d-PGJ₂ administration inhibits eosinophilic infiltration *in vivo*.

DISCUSSION

In the present study, we utilized a murine model of allergic inflammation induced by 10-times OVA challenges for 6 wk. This model at least partly reproduced the features of clinical allergic lung inflammation or asthma with sustained inflammation and respiratory dysfunction. We revealed that PGD₂ exerts anti-inflammatory effects in chronic allergic lung inflammation. In detail, damaged epithelium releases PGD₂, and DP signaling activation inhibits eosinophil infiltration, epithelial damage, and mucin hypersecretion directly or through inhibition of TNF-α production. The administration of 15d-PGJ₂ additionally inhibits eosinophilic infiltration, at least partly through the inhibition of TNF-α production.

Consistent with observations in patients with asthma (7), a much higher concentration of PGD₂ was detected in the lung tissues of WT with allergic inflammation than the lung tissue of WT with saline administration. However, this was almost abolished by systemic gene deficiency of H-PGDS. Under the endogenous PGD₂-deficient condition, production of the major proinflammatory cytokine TNF-α was significantly increased in the allergic lung. Several reports suggest the involvement of TNF-α in chronic allergic lung inflammation. In patients with asthma, anti-TNF-α therapy improves lung dysfunction (22). In

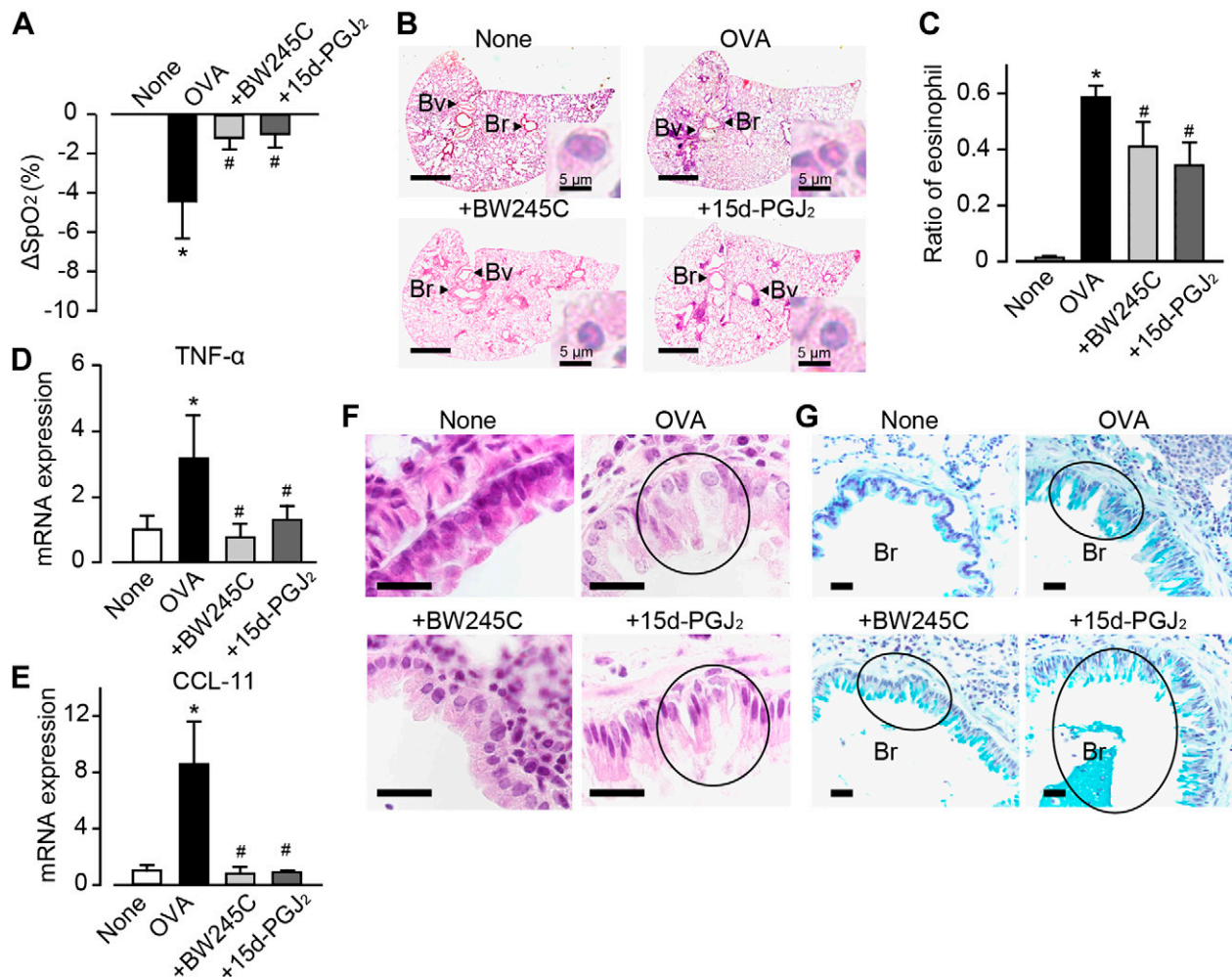


Figure 5. DP stimulation and 15d-PGJ₂ treatment ameliorated allergic lung inflammation. **A)** SpO₂ levels at 24 h after 10-times OVA challenges in WT mice; the difference in SpO₂ levels between naive and OVA-challenged mice is shown as ΔSpO_2 . A DP agonist, BW245C (200 $\mu\text{g}/\text{kg}$), or 15d-PGJ₂ (100 $\mu\text{g}/\text{kg}$) was intranasally administered to WT mice 30 min before each OVA (100 μg) challenge. **B)** Representative pictures of HE sections of lung tissue (Scale bars, low-power fields, 100 μm ; high-power fields, 5 μm). Macrophages were detected in mouse lungs, and eosinophils were detected in 10-times OVA-challenged BW245C- and 15d-PGJ₂-treated mice in high magnification. **C–E)** Ratio of eosinophil in BAL fluid (**C**), mRNA expression of TNF- α (**D**), and CCL-11 (**E**) of 10-times OVA-challenged mouse lungs. **F, G)** The representative pictures of HE-stained (**F**) and Alcian blue-stained sections (**G**). Scale bars, 25 μm . Results are shown as means $\pm$ SEM. * $P < 0.05$ vs. no treatment. # $P < 0.05$ vs. OVA challenge ($n = 5$ each).

OVA-induced murine lung inflammation, additive administration of TNF- α accelerates the infiltration of eosinophils and neutrophils into lungs and promotes airway hyperreactivity (23). Here, we showed that damaged epithelial cell-produced PGD₂ acts as an anti-inflammatory mediator, inhibiting secondary and chronic inflammatory reactions that are at least partly attributable to TNF- α .

We here reported the gene deficiency of H-PGDS accelerated chronic allergic lung inflammation in mice induced by 10-time intranasal administration of 100 μg OVA. In contrast, Matsuoka *et al.* (10) reported that gene deficiency of DP attenuated asthmatic symptoms in mice, suggesting an exacerbating role for PGD₂-DP signaling in asthma. They utilized a relatively acute asthmatic model induced by 3-time inhalations of 1% OVA. Arimura *et al.* (24) reported that administration of DP antagonist prevented eosinophil migration in lungs induced by 1-time

inhalation of 1% OVA in guinea pigs. The differences in used model, disease stage, and severity may be responsible for this discrepancy.

Pharmacological activation of DP signaling inhibited eosinophilic infiltration and mucin overproduction and protected the epithelium. Several reports show that bronchiolar cells express DP in the OVA-induced murine lung inflammation model. It is also reported that DP stimulation reduced OVA-induced inflammatory cell inflammation in a skin model of allergic inflammation (25). DP couples with G-proteins, and its activation causes the elevation of intracellular cAMP levels and subsequent PKA activation (26). Activation of cAMP and PKA signaling inhibits LPS-induced TNF- α expression in macrophages (27). Previous studies have shown that the activation of this signaling pathway significantly decreases the mRNA expression of eosinophil chemoattractants in bronchial epithelial cells (28–30). Activation

of DP signaling may exert beneficial effects in chronic allergic inflammation *via* both TNF- α -dependent and -independent signaling. In addition, we previously reported that DP stimulation improves the barrier function of vascular endothelial cells, thus attenuating the progression of acute lung inflammation and tumor growth (31). Although we did not focus on the endothelial barrier function in this study, we propose that the anti-inflammatory effects of PGD₂ signaling may additionally be attributable to this response.

Exogenous administration of the degraded product of PGD₂ and 15d-PGJ₂ also corrected the excessive eosinophilic infiltration and increased expression of inflammatory mediators observed under H-PGDS-deficient conditions. However, in comparison with DP stimulation, this regimen did not attenuate epithelial damage and mucin overproduction. It has been shown that 15d-PGJ₂ acts as a ligand of PPAR- γ , which inhibits proinflammatory transcription factors such as nuclear factor kappa B (4–6). Previous reports have shown that PPAR- γ is expressed strongly in eosinophils; accordingly, 15d-PGJ₂ may affect PPAR- γ -expressing eosinophils, but not epithelial cells, in the present mouse model of chronic disease. Further investigation is needed to explore the roles of 15d-PGJ₂ in allergic lung inflammation.

Clinical studies have additionally shown that oral administration of CRTH2 antagonists decreases the number of eosinophils in the blood in several patients with asthma; however, in the majority of patients, improved lung function was not observed (32). In our preliminary experiments, CRTH2 deficiency attenuated eosinophilic infiltration and respiratory dysfunction during the early phase but not the chronic phase of allergic lung inflammation (Supplemental Fig. S1).

Here, we showed that PGD₂ exerts anti-inflammatory effects through DP and 15d-PGJ₂ signaling during the progression of allergic lung inflammation; these proinflammatory reactions may be induced *via* the stimulation of CRTH2, especially during the early phase of allergic inflammation. In humans, asthma is a multifactorial disorder involving a variety of bioactive substances and signaling; therefore, further detailed investigations that take into consideration disease type and stage are required to elucidate the contribution of PGD₂ and develop improved therapeutic strategies.

To our knowledge, the present study demonstrates endogenous PGD₂ signaling plays an anti-inflammatory role in chronic allergic lung inflammation. The present findings indicate that enhancement of PGD₂ and DP signaling and 15d-PGJ₂ treatment may exert therapeutic effects in allergic pulmonary inflammation. **[F]**

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AUTHOR CONTRIBUTIONS

T. Maehara designed and carried out experiments, analyzed data, and wrote the manuscript; T. Nakamura and S. Maeda carried out experiments and analyzed data; K. Aritake and M. Nakamura provided experimental tools; M. Nakamura and T. Murata drafted the manuscript for important intellectual content and designed experiments; and T. Maehara, T. Nakamura, S. Maeda, K. Aritake, M. Nakamura, and T. Murata made final approval of the version to be published.

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