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Pronounced Eosinophilic Lung Inflammation and Th2 Cytokine Release in Human Lipocalin-Type Prostaglandin D Synthase Transgenic Mice

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PGD₂ is a major lipid mediator released from mast cells, but little is known about its role in the development of allergic reactions. We used transgenic (TG) mice overexpressing human lipocalin-type PGD synthase to examine the effect of overproduction of PGD₂ in an OVA-induced murine asthma model. The sensitization of wild-type (WT) and TG mice was similar as judged by the content of OVA-specific IgE. After OVA challenge, PGD₂, but not PGE₂, substantially increased in the lungs of WT and TG mice with greater PGD₂ increment in TG mice compared with WT mice. The numbers of eosinophils and lymphocytes in the bronchoalveolar lavage (BAL) fluid were significantly greater in TG mice than in WT mice on days 1 and 3 post-OVA challenge, whereas the numbers of macrophages and neutrophils were the same in both WT and TG mice. The levels of IL-4, IL-5, and eotaxin in BAL fluid were also significantly higher in TG mice than in WT mice, although the level of IFN-γ in the BAL fluid of TG mice was decreased compared with that in WT mice. Furthermore, lymphocytes isolated from the lungs of TG mice secreted less IFN-γ than those from WT mice, whereas IL-4 production was unchanged between WT and TG mice. Thus, overproduction of PGD₂ caused an increase in the levels of Th2 cytokines and a chemokine, accompanied by the enhanced accumulation of eosinophils and lymphocytes in the lung. These results indicate that PGD₂ plays an important role in late phase allergic reactions in the pathophysiology of bronchial asthma. The Journal of Immunology, 2002, 168: 443–449.

Bronchial asthma is now well recognized as a persistent allergic disease in the lung. In asthmatics, inhalation of specific allergens causes a biphasic allergic reaction consisting of acute and late phase responses (1, 2). The acute allergic reaction comprises bronchoconstriction and plasma extravasation and manifests as an asthmatic attack and airflow obstruction. Late phase allergic reactions exhibit the major characteristic feature of infiltration of inflammatory cells in the lungs, particularly that of eosinophils and CD4⁺ Th cells (3, 4). Eosinophils have been demonstrated to cause mucosal injury and to contribute to the generation of altered lung physiology, including airway hyper-responsiveness and airway remodeling (5). Highly polarized Th cells, designated Th2 cells, which produce predominantly IL-4 and IL-5, infiltrate into the mucosa of the airways, help B cells to develop into Ab-producing cells, and aid in the maturation of eosinophils (6).

Mast cells, residing in mucosal airways, are believed to orchestrate allergic reactions through the production of various cytokines and lipid mediators after activation by crossing of their high affinity IgE receptors (7). PGD₂ is a major cyclooxygenase product formed in activated mast cells (8–10). Indeed, the PGD₂ level in bronchoalveolar lavage (BAL) fluid increases in response to Ag provocation and, therefore, is used as a marker for activation of mast cells in vivo (11, 12). PGD₂ shows a variety of biological actions (13–16), including vasodilation and bronchoconstriction. Thus, PGD₂ is thought to be involved in the acute phase allergic reaction. However, relatively little is known about the role of PGD₂ in the late phase reaction of the allergic response, such as eosinophilic lung inflammation in bronchial asthma. In mice with a null mutation of the D-type prostanoid (DP) receptor gene, eosinophil and lymphocyte accumulation in the lungs and the concentration of Th2 cytokines were reduced compared with those in their wild-type (WT) littermates after Ag challenge in a murine asthma model (17), indicating that PGD₂ signaling is involved in the regulation of allergic inflammation.

PGD₂ is formed from arachidonic acid by successive enzyme reactions: oxygenation of arachidonic acid to PGH₂, a common precursor of various prostanoids, catalyzed by cyclo-oxygenase, and isomerization of PGH₂ to PGD₂ by PGD synthase (PGDS). As recently reviewed (18), there are two distinct types of PGDS: one is the lipocalin-type PGDS (L-PGDS) (19–21), and the other is hematopoietic PGDS (H-PGDS) (22, 23). We isolated the cDNA
and the gene for each enzyme and determined the tissue distribution profile and cellular localization in several animal species. L-PGDS is localized in the CNS (24, 25), ocular tissues (26), and male genital organs (27) of various mammals and the human heart and is secreted into cerebrospinal fluid (28, 29), seminal plasma, plasma (30), and urine (31). The human enzyme was identified as \( \beta \)-trace, which is a major protein in human cerebrospinal fluid. This enzyme is considered to be a dual function protein; it acts as a PGD2-producing enzyme and also as a lipophilic ligand-binding protein, because the enzyme binds retinoids, thyroids, and bile pigments with high affinity. H-PGDS is widely distributed in the peripheral tissues and is localized in APC, mast cells (22), and megakaryocytes (32). The hematopoietic enzyme is the first recognized vertebrate homologue of the sigma class of GST (23). X-ray crystallographic analyses and generation of gene-knockout recognized vertebrate homologue of the sigma class of GST (23).

This enzyme is considered to be a dual function protein; it acts as an important role of PGD2 in the development of allergic inflammation. For this purpose, we used TG mice overexpressing human L-PGDS (33) as an in vivo model of bronchial asthma, because human H-PGDS TG mice showed weaker eosinophilic lung inflammation than L-PGDS TG mice. L-PGDS TG mice showed a pronounced infiltration of eosinophils and lymphocytes and up-regulation of Th2 cytokine production in response to OVA provocation, demonstrating an important role of PGD2 in the development of allergic inflammation.

Materials and Methods

Mice

TG mice overexpressing human L-PGDS were generated in the inbred FVB strain as described previously (33). Among five independent founder mice, we used three founders (B7, B17, and B20) in this study. Mice were maintained under specific-pathogen-free conditions in isolated cages.

Northern blot analysis

Mice were anesthetized with pentobarbital (Abbott Laboratories, Abbott Park, IL). Lungs were quickly isolated and homogenized in ISOGEN (Nippon Gene, Tokyo, Japan). Total RNA was extracted according to the manufacturer’s instructions, separated (10 \( \mu \)g/lane) on a 1% agarose/2.2 M formaldehyde gel, and transferred to a Biodyem membrane (Pall Ultrafine Filtration, Glen Cove, NY). The blot was hybridized with the \(^{32}P\)-labeled cDNA for human L-PGDS. After being stripped of L-PGDS probe, the blot was hybridized with the \(^{32}P\)-labeled mouse 3GPDH cDNA.

Enzyme assay

Lungs were harvested and homogenized in 1 ml ice-cold PBS with a Polytron homogenizer (Brinkmann, Westbury, NY). The homogenates were centrifuged at 100,000 \( \times \) g at 4 °C for 30 min, and the supernatants were used for the enzyme assay, as described previously (20). Protein concentration was determined by use of bicinechonic acid reagent (Pierce, Rockford, IL) with BSA as a standard following the manufacturer’s protocol.

Western blot analysis

L-PGDS in the lungs of WT and TG mice was partially purified by acid treatment and ammonium sulfate fractionation as reported previously (19). In brief, the 100,000 \( \times \) g supernatants of the lung homogenates were acidified to pH 4.5 with glacial acetic acid and centrifuged at 10,000 \( \times \) g for 10 min. The resultant clear supernatant was adjusted to pH 7.0 and fractionated by 55–80% saturated ammonium sulfate. The resultant pellet was dissolved in PBS and subjected to SDS-PAGE. The proteins were transferred to a polyvinylidene difluoride nylon membrane (Millipore, Bedford, MA) and immunostained with polyclonal rabbit anti-human L-PGDS Ab (34) as reported previously (19).

Histologic analysis

Mice were perfused with PBS and subsequently with 4% paraformaldehyde (pH 3.8) through the left ventricle. The lungs were removed and embedded in paraffin. Sections were then cut and stained with H&E. Immunoperoxidase staining was performed as reported previously (35) with rabbit polyclonal Ab against human L-PGDS (34).

Immunization and Ag challenge of mice

TG and WT mice (females, 25–30 g, 14–16 wk old) were immunized i.p. with 10 \( \mu \)l OVA (grade V, Sigma, St. Louis, MO) in 0.2 ml alun (Serva, Heidelberg, Germany) on days 0 and 14. On day 21 the mice were exposed to aerosolized OVA (50 mg/ml sterile saline) for 20 min. At specified times after the OVA challenge the mice were anesthetized with sodium pentobarbital. The trachea was cannulated, and BAL was performed by three repeated lavages with 0.3 ml saline injected into the lungs via the tracheal cannula. Total cells were counted, and differential cell counts with 500 cells were performed based on standard morphologic criteria. For determination of the amounts of Th2 cytokines, eotaxin, and total IgE, BAL fluid and serum were collected 24 h after OVA provocation. The levels of IL-4, IL-5, eotaxin, and IFN-\( \gamma \) were determined with the respective ELISA kits (BioSource, Camarillo, CA). Total IgE titers were determined with an ELISA kit (Yamasa, Chiba, Japan). OVA-specific IgE was quantified by incubation of mouse serum in ELISA plates precoated with monoclonal anti-mouse IgE followed by incubation with biotinylated OVA (BD Pharmingen, San Diego, CA) and alkalinephosphatase-conjugated streptavidin (BD Pharmingen).

Measurement of PGs in the lung

The amounts of PGD2 and PGE2 in the lungs were determined as described previously (36). Briefly, the lungs were homogenized in 2 ml ethanol containing 0.25 N HCl with a Polytron homogenizer. \(^{[3]H}\)PGD2 and PGE2 (NEN Life Science Products, Boston, MA) were added to each homogenate as tracers to determine the recovery during the purification procedure. PGs in the ethanol extract were applied onto Sep-Pak C18 cartridges (Waters Associates, Milford, MA), eluted with ethyl acetate, and then fractionated by HPLC. The amounts of PGs were measured with the respective kits (Cayman Chemical, Ann Arbor, MI).

Quantitative RT-PCR of mRNAs for DP and CRTH2 receptors in the lung

First-strand cDNA was transcribed from 1 \( \mu \)g total RNA obtained from the lung, with random primers by AMV reverse transcriptase (Takara Shuzo, Kyoto, Japan). For quantitative PCR, we amplified DNA using a LightCycler (Roche, Mannheim, Germany), a LightCycler-DNA Master SYBR Green I Kit (Roche), and primers specific for mouse G3PDH, (5'-TGAACGGGAAGCTCACTGG-3' and 5'-TACAGCAACAGGTTGGTT GGA-3'), mouse DP receptors (5'-TTTGGGAAGCTTGCAGTACT-3' and 5'-GCCATGGGCGTGGAGTAGA-3') (36), and mouse CRTH2 receptors (5'-TGGCCCTTCTTCACAGCGT-3' and 5'-AGCCAGATGG GGAATCTCG-3') (37). The reactions were cycled 40 times with denaturation at 95°C for 3 s, annealing for 30 s, and elongation at 72°C for 3 s. The cycles were repeated for 20, 25, and 30°C, respectively. Fluorescence was acquired after heating at 95°C for 10 s. Temperature gradients for denaturation, annealing, and elongation were 20–25°C, and 20–25°C, respectively. Quantification data were analyzed with LightCycler analysis software. All PCR products were sequenced to confirm their origin from the intended mRNAs.

In vitro T cell cytokine secretion

The lungs were perfused via the right ventricle with 5 ml PBS containing 100 U/ml heparin to remove any blood and intravascular leukocytes. The lungs were then removed and placed into DMEM containing 10% FCS, 2-ME (50 \( \mu \)M), l-glutamine (2 mM), sodium pyruvate (1 mM), HEPES (10 mM), and gentamicin (50 \( \mu \)g/ml). They were then gently homogenized, the cell suspension was filtered through a 70- \( \mu \)m pore size filter, and the lymphocyte population was enriched by centrifugation on a single-step Ficoll gradient. The lymphocyte fraction was plated at a concentration of 2 \( \times \)10^6 cells in 96-well plates coated with anti-CD3 Ab (50 \( \mu \)g/ml; BD Pharmingen). The cells were cultured for 3 days in the presence of human IL-2 (200 U/ml; BD Pharmingen). The supernatants were harvested, and cytokine production (IL-4 and IFN-\( \gamma \)) was determined by ELISA.

Statistical analysis

The results of the experiments were expressed as the mean ± SEM. Student’s t test was used for the statistical analysis in cases in which the variance was homogeneous, and Welch’s test was used when the variance was heterogeneous. A value of \( p < 0.05 \) was considered significant.
**Results**

**Human L-PGDS is overexpressed in the lungs of TG mice**

As previously reported we had established five independent founders of TG mice (33). Based on the level of transgene expression, we chose three lines of TG mice (B7, B17, and B20) for this study. As shown in Fig. 1A, human L-PGDS mRNA was expressed in the lungs of all three lines of TG mice. The L-PGDS-immunoreactive protein was also detected to be overexpressed in the lungs of TG mice (Fig. 1B). To confirm that the expressed transgene is functional in the lung, we measured PGDS activity in lung homogenates with PGH₂ as a substrate. As shown in Fig. 1C, PGDS activities in the lung homogenates from the three lines of TG mice, i.e., B7, B17, and B20, were 3.2-, 2.0-, and 3.6-fold higher, respectively, than that from WT mice. These results indicate the functional expression of the transgene in the lungs of these mice.

The transgene was expressed in airway epithelium, sub-bronchial and ventricular smooth muscle cells, and alveolar lining cells of three lines of TG mice, as examined by immunostaining with anti-human L-PGDS Ab. Typical results obtained with B20 mice are shown in Fig. 1, D and E.

**Allergic airway inflammation is enhanced in TG mice**

These TG mice were then used in an OVA-induced lung inflammation model. No eosinophils, neutrophils, or lymphocytes were observed in the BAL fluid of either WT or TG mice before and after saline challenge. The numbers of alveolar macrophages before and after saline challenge were not significantly different among WT, B7, B17, and B20 mice (4.2 ± 0.7 and 5.2 ± 0.6, 6.8 ± 1.8 and 4.9 ± 0.5, 3.7 ± 1.9 and 4.7 ± 0.6, and 8.5 ± 2.0 and 4.3 ± 0.4 × 10⁴ cells/ml, respectively; n = 6–7). BAL fluid was collected on days 1 and 3 after OVA challenge. One day after OVA challenge the numbers of macrophages in BAL fluid were almost the same as that in the saline-challenged group (Fig. 2). The number of macrophages in BAL fluid was increased in both WT and TG mice on day 3 postchallenge without any significant difference between WT and TG mice. Infiltration of neutrophils into the lungs was observed on day 1 postchallenge and was resolved on day 3, but the cell numbers were unchanged between WT and TG mice. However, infiltration of eosinophils in B17 and B20 mice was increased by 4.0- and 3.9-fold (12.9 ± 2.9 and 12.5 ± 2.5 × 10⁴ cells/ml) on day 1 and by 2.2- and 1.6-fold (31.8 ± 4.9 and 22.8 ± 3.9 × 10⁴ cells/ml) on day 3, respectively, compared with that in WT mice (3.2 ± 1.7 and 14.2 ± 2.7 × 10⁴ cells/ml on days 1 and 3, respectively). B7 mice also appeared to show higher eosinophilia (7.2 ± 2.4 and 16.3 ± 3.0 × 10⁴ cells/ml on days 1 and 3, respectively) than WT mice, although the difference was not statistically significant. Furthermore, the number of lymphocytes was increased in all three lines of TG mice by 7.4-, 5.7-, and 6.8-fold (0.56 ± 0.1, 0.43 ± 0.05, and 0.52 ± 0.11 × 10⁴ cells/ml; B7, B17, and B20 mice, respectively) compared with that in WT mice (0.08 ± 0.05 × 10⁴ cells/ml) on day 1. Three days post OVA challenge, B17 and B20 mice showed a significant increase in lymphocytes compared with WT mice.

Histologic observation revealed that OVA challenge induced infiltration of inflammatory cells into peribronchial, perivascular, and alveolar spaces in TG mice more significantly than in WT mice. Typical results with WT and B17 mice are shown in Fig. 3. Eosinophils infiltrated into the peribronchial region of B17 mice more significantly than in WT mice.

**FIGURE 1.** Expression of human L-PGDS in the lungs of TG mice. A. Total RNA (10 μg/ lane), isolated from lungs of WT and TG mice, was hybridized with the ³²P-labeled cDNA probes for either human L-PGDS or mouse G3PDH. B. Western blot analysis of the lung extracts of WT and TG mice with anti-L-PGDS Ab. The position of the 27-kDa marker protein is shown to the left. C. PGDS activity of the lung homogenates of WT and TG mice was measured with [¹⁴C]PGH₂ as a substrate. D. Immunoperoxidase staining of human L-PGDS in the lungs of WT and B20 TG mice. Human L-PGDS was immunostained in brown. Scale bar = 100 μm. E. High magnification views of human L-PGDS immunoreactive bronchial epithelial cells (double arrow), sub-bronchial smooth muscle cells (arrow), and ventricular smooth muscle cells (arrowhead). Scale bar = 20 μm.

**FIGURE 2.** Lung inflammation after OVA challenge in sensitized WT and TG mice. The OVA-sensitized mice were challenged with aerosolized OVA and sacrificed on days 1 and 3 post-OVA challenge. BAL fluids were collected, and differential cell counts were performed. Results are expressed as the mean ± SEM of six or seven mice at each time point. *p < 0.05; **p < 0.01 (compared with WT mice).
Pronounced lung inflammation was observed in all three independent TG lines, indicating that such a phenotype was not due to the effect(s) of insertion of the transgene in a specific locus of the mouse genome. Taken together, these results indicate that the overexpression of human L-PGDS caused a pronounced allergic lung inflammation revealed by the enhanced infiltration of eosinophils and lymphocytes into BAL fluid after OVA challenge. Since B17 mice developed the most pronounced inflammation at both time points, this founder was used for additional experiments.

**PGD₂ content is increased in the lung during the late phase allergic reaction in TG mice**

The PGD₂ content in the lungs of WT and TG mice was determined before and after OVA challenge. As shown in Fig. 4, the PGD₂ content in the lungs of the unchallenged WT and B17 mice was low and statistically unchanged (0.51 ± 0.01 and 0.72 ± 0.40 ng/lung, WT and B17 mice, respectively). However, the PGD₂ content significantly increased 30 min after OVA challenge in both WT and TG mice (1.47 ± 0.18 and 1.26 ± 0.06 ng/lung, respectively), although no significant difference was observed between WT and TG mice. The production of PGD₂ at 30 min may be attributed to mast cells and macrophages that express endogenous H-PGDS. Accumulation of the other inflammatory cells, including eosinophils and neutrophils, began approximately 3 h after challenge, and these cells expressing L-PGDS in the TG mice contributed to the higher production of PGD₂ assessed 6 h after challenge. The PGD₂ content reached its peak at 6 h post-OVA challenge, at which time point the content in TG mice (4.38 ± 0.88 ng/lung) was 2.3-fold higher than that in WT mice (1.94 ± 0.88 ng/lung). Furthermore, 24 h after OVA challenge, the content remained significantly higher in TG mice (3.0 ± 0.34 ng/lung) than in WT mice (1.11 ± 0.50 ng/lung). In contrast to PGD₂, the level of PGE₂, another inflammatory prostanoid, in the lungs was the same before and after OVA challenge in either group. These results indicate that the human L-PGDS overexpressed in the lungs of TG mice functioned to overproduce PGD₂ in response to the OVA challenge without affecting PGE₂ biosynthesis.

**The mRNA for DP receptors, but not CRTH2 receptors, is up-regulated in the lungs of WT and TG mice after OVA challenge**

We then examined the possible changes in the expression level of PGD₂ receptors by the overproduction of PGD₂. There are two distinct types of PGD₂ receptors, i.e., Gₛ-coupled DP receptors (36, 38) and Gᵢ-coupled CRTH2 receptors (39, 40). We determined by RT-PCR the contents of mRNAs for DP and CRTH2 receptors in the lungs of WT and TG mice before and after OVA challenge. As shown in Fig. 5, the mRNA for DP receptors was increased 2- to 3-fold in the lungs of WT and TG mice 2 days after OVA challenge compared with WT mice.
challenge. However, the DP mRNA content was statistically unchanged between WT and TG mice either 1 or 2 days after OVA challenge. On the other hand, the content of mRNA for CRTH2 receptors in the lungs of WT and TG mice before OVA challenge was 100-fold or higher than that of mRNA for DP receptors. The CRTH2 mRNA content was almost unchanged or was slightly decreased in both mice after OVA challenge.

Levels of Th2 cytokines and chemokines are enhanced in the BAL fluid of TG mice

Given the important role of Th2 cytokines and chemokines in allergic inflammation, we determined the levels of IL-4, IL-5, IFN-γ, and eotaxin in the BAL fluid of WT and TG mice after OVA provocation. Low levels of IL-4 and IL-5 (<10 pg/ml) were detected in the BAL fluid of WT and TG mice after saline challenge (Fig. 6A). After OVA provocation, however, Th2 cytokine levels were increased in both WT and TG mice. IL-4 and IL-5 in BAL fluid of the challenged TG mice (IL-4, 298.9 ± 119.6 pg/ml; IL-5, 358.7 ± 48.9 pg/ml) were 3.9- and 2.4-fold, respectively, higher than those in WT mice (IL-4, 77.2 ± 27.2 pg/ml; IL-5, 152.2 ± 54.4 pg/ml). Furthermore, the eotaxin level in TG mice (36.5 ± 1.7 pg/ml) was significantly higher than that in WT mice (22.1 ± 1.2 pg/ml) after OVA challenge. In contrast to Th2 cytokines and eotaxin, IFN-γ content in BAL fluid of TG mice (10.0 ± 1.6 pg/ml) was significantly decreased compared with that in WT mice (21.0 ± 1.7 pg/ml). To determine the extent of systemic immune modulation by OVA challenge, we determined serum levels of IL-5, total IgE, and OVA-specific IgE (Fig. 6B). Although serum IL-5 levels in WT and TG mice increased after OVA challenge, there was no significant difference between the two groups of mice. Furthermore, the total IgE level and the OVA-specific IgE level in the serum were the same in WT and TG mice after either saline or OVA challenge.

Secretion of cytokines from lymphocytes isolated from the lungs of TG mice is shifted to Th2-declined phenotype

Since Th2 cytokine levels were increased in the BAL fluid of TG mice, we isolated lung lymphocytes and stimulated them with anti-CD3 Ab to examine their cytokine secretion patterns. As shown in Fig. 7, lung lymphocytes from both WT and TG mice challenged with OVA produced both IL-4 and IFN-γ. Although there was no significant difference in IL-4 secretion between WT and TG mice (312.0 ± 35.0 and 259.0 ± 27.0 pg/ml, respectively), IFN-γ secretion from TG mice (4.2 ± 0.6 pg/ml) was significantly lower than that from WT mice (11.8 ± 0.6 pg/ml), suggesting that lymphocytes in lungs from TG mice showed a Th2-declined phenotype.

Discussion

Here we demonstrate that OVA challenge to OVA-sensitized mice overexpressing human L-PGDS caused overproduction of PGD2 and exacerbated allergic responses, such as infiltration of eosinophils and lymphocytes, and Th2 cytokine production. PGD2 is a major prostanoid produced from mast cells; however, its role in the pathogenesis of asthma has not been clarified, except that PGD2 contracts airway smooth muscle cells via thromboxane A2 receptor (41). Mast cells are key effector cells for the pathophysiology of bronchial asthma. In murine asthma models with Ag sensitization and aeroantigen challenge, eosinophilia in BAL fluid and lung and airway hyper-responsiveness were reduced in genetically mast cell-deficient W/Wv mice (42, 43), and the eosinophilia and the airway hyper-responsiveness in W/Wv mice were restored by adoptive transfer of bone marrow-derived mast cells (42, 43). Thus, the results in this study suggest that the eosinophilia in BAL fluid and lung of those asthma model animals is partly, if not

FIGURE 5. Levels of mRNA for DP and CRTH2 receptors in the lungs of WT and TG mice after OVA challenge.

FIGURE 6. Levels of IL-4, IL-5, IFN-γ, eotaxin, total IgE, and OVA-specific IgE. A. Mice were sacrificed 1 day after saline or OVA challenge. The levels of IL-4, IL-5, IFN-γ, and eotaxin in BAL fluids were then measured. Results are expressed as the mean ± SEM of six or seven mice in each group. *, p < 0.05; **, p < 0.01 (compared with WT mice). B. Mice were sacrificed 1 day after saline or OVA challenge. Serum levels of IL-5, total IgE, and OVA-specific IgE were measured. Results are expressed as the mean ± SEM of six or seven mice in each group. *, p < 0.05 compared with WT mice.

FIGURE 7. IL-4 and IFN-γ production from lung lymphocytes. Lung lymphocytes were purified from mice 1 day after OVA challenge. Lymphocytes were plated on 96-well culture plate (2 × 10^5 cells/well) and stimulated with anti-CD3 Ab for 3 days. Data are shown as the mean ± SEM of three to five individual cultures obtained from a pool of lungs from six mice in each group. **, p < 0.01 compared with WT mice.
completely attributed to PGD₂ released from mast cells. This idea is supported by a previous report that mice lacking DP receptors did not develop asthmatic responses, including eosinophilic lung inflammation and airway hyper-reactivity (17).

The levels of CC chemokines, such as eotaxin, RANTES, monocyte chemoattractant protein-3, and monocyte chemoattractant protein-4, which are selective chemoattractants for eosinophils (44), are increased in BAL fluid and lungs in response to Ag provocation (45, 46). In asthma, Th2 lymphocytes are increased in number in the airways after an Ag challenge (47, 48). Th2 cells produce IL-4 and IL-5, both cytokines contribute to the development of IgE-producing B cells, and IL-5 supports the proliferation of eosinophils. We show that PGD₂ overproduction increased IL-4, IL-5, and eotaxin and down-regulated IFN-γ in BAL fluid of TG mice (Fig. 6), suggesting that PGD₂ produces a decrease in Th2 in the lung microenvironment after Ag provocation. Indeed, lung lymphocytes, isolated from TG mice, showed a decreased Th2 cytokine production pattern in vitro; its IFN-γ secretion was specifically decreased compared with that in WT mice (Fig. 7).

Most recently, PGD₂ was identified as a ligand for a G protein-coupled, seven-transmembrane-type receptor, CRTH2 (39), which is preferentially expressed in Th2 cells, eosinophils, and basophils, and PGD₂ was shown to induce chemotaxis of these cells through CRTH2, but not through DP receptors (40). Thus, the direct chemotactant action of PGD₂ on CRTH2-possessing cells may explain the pronounced infiltration of eosinophils and lymphocytes and the increased Th2 cytokines in the BAL fluid of L-PGDS TG mice upon OVA challenge. Furthermore, these observations address the possibility that PGD₂ functions as an allergic mediator through both CRTH2 and DP receptors and, more importantly, highlight the role of the PGD₂ biosynthesis pathway in allergic lung inflammation.

In asthmatic airways, cyclooxygenase-2, an inducible isoform of cyclo-oxygenase, is up-regulated (49, 50), suggesting the possible involvement of cyclooxygenase products, including PGD₂, in the pathogenesis of this disease. In an experimental animal model of bronchial asthma, nonsteroidal anti-inflammatory drugs, such as indomethacin and aspirin, were found to have a negative impact on Ag-induced eosinophilic lung inflammation (51). However, the inhibition of cyclooxygenase by nonsteroidal anti-inflammatory drugs abrogates the production of all PGs. Especially PGE₂ and prostacyclin are bronchodilatory PGs with an anti-inflammatory effect (52). Thus it is necessary to use specific inhibitors of PGD₂ receptors or PGDS to determine the role of PGD₂ in this model.

It is well known that mast cells express H-PGDS and secrete PGD₂ in response to Ag stimulation (8). It was also reported that Th2 cells, but not Th1 cells, express H-PGDS and produce PGD₂ after TCR activation (53). Thus, it is very likely that both mast cells and Th2 cells, which infiltrate into the allergic inflammatory site, produce PGD₂ upon Ag stimulation to regulate the allergic conditions. Recently, it was reported that HQL-79, an inhibitor of H-PGDS, efficiently inhibited Ag-induced eosinophilic lung inflammation in guinea pigs (54, 55). Thus, H-PGDS will be a target for drugs designed for bronchial asthma and other allergic diseases.

The cysteinyl leukotrienes have been implicated in the pathophysiology of bronchial asthma. They elicit contraction of bronchial smooth muscle cells and vascular smooth muscle cells. The latter induces extravasation of plasma protein and accumulation of inflammatory cells such as Th2 cells and eosinophils. Activation of constitutive mast cells residing in the lung to release PGD₂ would cause accumulation of Th2 cells and eosinophils via CRTH2 with generation of polarized cytokines, which, in turn, expand pheno-

typically distinct mucosal mast cells (reactive mast cells) shifted to the cysteinyl leukotriene pathway (56). PGD₂ also elicits relaxation of smooth muscle cells and endothelial cells via DP and may induce lung inflammation, as proposed in the study of DP receptor knockout mice (17). These inflammatory cells, including reactive mast cells, further generate PGD₂, contributing to a positive feedback of the allergic and asthmatic inflammation where a range of cytokines and chemokines are produced so as to sustain the Th2-dependent inflammatory allergic and asthmatic responses.

In summary, we used TG mice overexpressing human L-PGDS to examine the effect of overproduction of PGD₂ on the development of allergic inflammation with a murine asthma model. We show that overproduction of PGD₂ caused an increase in the levels of Th2 cytokines and a chemokine accompanied by the enhanced accumulation of eosinophils and lymphocytes in the lung. These results indicate that PGD₂ plays an important role in late phase allergic reactions in the pathophysiology of bronchial asthma.

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