Prostaglandin as a Target Molecule for Pharmacotherapy of Allergic Inflammatory Diseases

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ABSTRACT
The purpose of this review is to summarize the role of prostaglandins (PGs) in allergic inflammation and to know the value of PGs, as a target molecule for an anti-allergic drug.

PGD2 is the major PG produced by the cyclooxygenase pathway in mast cells. Our and others findings indicate that PGD2 is one of the potent allergic inflammatory mediators and must be a target molecule of anti-allergic agent. From our data, one of PGD2 receptor antagonists show clear inhibition of airway hypersensitivity caused by allergic reaction. Concerning the role of PGE2 in allergic inflammation, conflicting results have been reported. Many experimental data suggest an individual role of each PGE2 receptor, EP1, EP2, EP3 and EP4 in allergic reaction. Our results indicate the protective action of PGE2 on allergic reaction via EP3. In addition, one of EP3 agonists clearly inhibits the allergic airway inflammation. These findings indicate the value of EP3 agonists as an anti-allergic agent.

In addition, some investigators including us reported that PGI2 plays an important role for the protection of the onset of allergic reaction. However, the efficacy of PGI2 analogue as an anti-allergic agent is not yet fully investigated.

Finally, the role of thromboxane A2 (TxA2) in allergic reaction is discussed. Our experimental results suggest a different participation of TxA2 in allergic reaction of airway and skin. In this review, the role of PGs in allergic inflammation is summarized and the value of PGs as a target molecule for developing a new anti-allergic agent will be discussed.

KEY WORDS
allergy, anti-allergic drug, PGD2, PGI2, prostaglandin
THE ROLE OF PGs IN ALLERGIC INFLAMMATION

Identifying a role of any mediator in pathological state is dependent on the collection of various types of evidence. Often, after the structure of a mediator is identified, and synthesized, the mediator is given to humans or experimental animals, to observe whether it can mimic signs or symptoms of the disease. Then when quantitative assays are available, efforts are made to measure it in the biological fluid, to determine whether it is released during a disease state. In addition, when the specific antagonists or inhibitors to the mediator, suppress the symptoms in animal disease model, the role of mediator is confirmed as a causative component in the disease.

From these points of view, the role of PGs in allergic inflammation has been examined by many researchers, including us, and various results were obtained. Most researchers agree with the production of several types of PGs during allergic reaction in human and experimental animals. But each researcher has failed to obtain consensus in terms of the effect of the PG inhibitors on allergic response and the magnitude of allergic response caused by each PG. So, in this review, we focused on the experimental results employing PG synthesis inhibitor, indomethacin, and PG receptor gene manipulated mice on allergic reaction in mice.

EFFECT OF INDOMETHACIN

In the first segment of experiment, in order to know the role of PGs in allergic inflammation, we tried to establish an allergic airway inflammation model in mice. Airway allergic inflammation was caused by repeated inhalation of aerosolized antigen into sensitized mice. Antigen provocation result in T helper 2 cell (Th2) polarized immune responses and eosinophilic airway inflammation (Fig. 1). Th2 polarized immune response is characterized by the elevation of serum IgE and the increase of Th2 cytokines, IL-4, 5 and 13 level and decrease of INF-γ level in bronchial alveolar lavage fluid (BALF). In addition, the airway responsiveness to acetylcholine is accelerated by repeated antigen provocation. This symptom is similar to many features of human airway hypersensitivity (AHR), one of typical asthmatic response.

In the next segment of experiment, to discover the role of PGs, the effect of indomethacin on this allergic airway inflammation and AHR was examined. As shown in Figure 2, indomethacin accelerates the production of Th2 cytokines, the accumulation of inflammatory cells in BALF and IgE antibody production (data not shown). The drug also shows the tendency to accelerate the AHR. These data suggest that the inhibition of PG production augments the allergic inflammation. This means the COX products, probably some PGs, suppress the Th2 dependent allergic inflammatory responses. On the other hand, some other studies so far reported suggest the existence of a pathological role of PGs in allergic inflammation. In fact, the existence of some PGs in allergic lesion has been recognized and some of them are able to mimic the symptoms of allergy. Therefore we carried out further experiments to elucidate the role of each PG in allergic reaction by employing each PG receptor gene deficient mice.

PGD₂

PGD₂ is the major PG produced by the COX pathway.

Table 1  Prostaglandins and thromboxane receptors and physiological actions

<table>
<thead>
<tr>
<th>PGs</th>
<th>Receptor</th>
<th>Cellular signaling</th>
<th>Action</th>
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<tbody>
<tr>
<td>PGD₂</td>
<td>DP</td>
<td>c-AMP ↑</td>
<td>Platelet aggregate ↓, Allergic inflammation ↑, Sleep ↑, Eosinophils ↑</td>
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<td></td>
<td>CRTH2</td>
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<tr>
<td>PGE₂</td>
<td>EP₁</td>
<td>Ca²⁺↑</td>
<td>Smooth muscle ↑, Stress ↑, Ovattan follicle ↑</td>
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<tr>
<td></td>
<td>EP₂</td>
<td>c-AMP↑</td>
<td>Vasodilation ↑, Blood pressure ↓</td>
</tr>
<tr>
<td></td>
<td>EP₃</td>
<td>c-AMP↓</td>
<td>Pyrexia ↑, Gastric secretion ↓, Pain sensation ↑</td>
</tr>
<tr>
<td></td>
<td>EP₄</td>
<td>c-AMP↑</td>
<td>Smooth muscle ↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Patent ductus arteriosus ↑, Oscillation ↑, Immune response ↓</td>
</tr>
<tr>
<td>PGF₂α</td>
<td>FP</td>
<td>IP₃/DG↑</td>
<td>Labor ↑, Smooth muscle ↑, Intraocular pressure ↓</td>
</tr>
<tr>
<td>GL₂</td>
<td>IP</td>
<td>c-AMP↑</td>
<td>Blood pressure ↓, Platelet aggregation ↓, Renal blood flow ↑</td>
</tr>
<tr>
<td>TxA₂</td>
<td>TP</td>
<td>IP₃/DGA</td>
<td>Platelet aggregation ↑, Smooth muscle ↑</td>
</tr>
<tr>
<td></td>
<td>c-AMP</td>
<td></td>
<td>Thrombosis ↑</td>
</tr>
<tr>
<td>LTC₄, D₄, E₄</td>
<td></td>
<td>cys LT₁ Ca²⁺↑</td>
<td>Airway smooth muscle ↑, Eosinophils ↑, Permeability ↑</td>
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↓, down regulation; ↑, up regulation.
in mast cells.28 Significant quantities of PGD2 are not produced by the immunological activation of basophils.29,30 Generally, PGD2 is produced by either L-type (lipocalin type) or H-type (hematopoetic type) PGD2 synthetase. L-type PGD2 synthetase exists in mainly central nervous system and H-type PGD2 synthetase exists in peripheral tissues and immune cells including mast cells, antigen-presenting cells and Th2 cells. In addition, recent studies have revealed two G protein-coupled receptor for PGD2, DP and chemottractant receptor homologous molecule expressed on Th2 cells (CRTH2).31,32

Concerning the role of PGD2 in allergic bronchial asthma, there are some clinical evidence to suggest the patho-physiological role of PGD2. PGD2 is detected in BALF from asthmatic patients and it constricts human bronchial smooth muscle in vitro.33-35 Despite the recognition of the existence and action of PGD2 during bronchial asthma, basic research about the role of PGD2 in allergic inflammation is still lagging.

Therefore, we investigated the role of PGD2 in allergic inflammation by employing DP gene deficient mice. Consequently, we demonstrated that PGD2 plays a role in an allergic asthma as a mediator.24 Our results are summarized below briefly. Sensitization and aerosol challenge of the homozygous mutant DP gene deficient mice with ovalbumin induced increases in the serum concentration of IgE similar to those in wild-type mice subjected to this model of allergic asthma. However, the concentration of Th2 cytokines (IL-4 and IL-5) and the extent of lymphocyte accumulation in the antigen challenged lung of DP gene deficient mice significantly decreased compared to those in wild type mice. DP gene deficient mice showed only marginal infiltration of eosinophils and failed to develop AHR. Thus, PGD2 functions as a mast cell derived mediator, to trigger asthmatic responses. Our results and related evidence regarding the pharmacological action of PGD2 are summarized in Table 2.

As described in Table 2, Fujitani et al.36 confirmed a role of PGD2 in allergic inflammation by employing L-type PGD2 synthetase gene over-expressed mice. The overproduction of PGD2 causes an increase in the levels of Th2 cytokines and chemokines, accompanied by the enhanced accumulation of eosinophils and lymphocytes in the lung. The findings of Fujitani
et al.\textsuperscript{36} and our studies indicate that PGD\textsubscript{2} plays an important role for the accumulation of eosinophils into allergic lesion. Moreover, Honda \textit{et al.}\textsuperscript{37} revealed the mechanism of PGD\textsubscript{2} induced eosinophil infiltration. They have described the mediation of macrophage-derived chemokine from airway epithelial cells for PGD\textsubscript{2} inducing local eosinophilia.

In addition to DP, recent studies suggest the participation of CRTH2 receptor in allergic inflammation.\textsuperscript{38,39} Some groups have demonstrated that CRTH2 selective agonists induce eosinophilic airway and skin inflammation in animal models.\textsuperscript{40-44} These data support the hypothesis that CRTH2 may play a critical role in allergic inflammation; however, more
Prostaglandin as a Target for Remedy

**Fig. 3** Chemical structure of PGD\(_2\) receptor (DP) antagonist and the effect of DP antagonist on antigen-induced airway hyperresponsiveness in mice. Each experiment consists of the mean ± SE on 5–7 mice. AUC; area under the curve (range; 31.25–2000 mg/Kg), Ach; acetylcholine.

*, **, *** p < 0.05, 0.01 and 0.001, respectively (vs Sal; Student’s test).

#, ## p < 0.05 and 0.01 respectively (vs OA; Student’s t-test).

†, †† p < 0.05 and 0.01, respectively (vs OA; Dunnett’s multiple comparison test).

data are necessary to clarify the precise role of CRTH2 in allergic diseases.

From the clinical point of view, whereas extensive efforts have been made to elucidate the role of PGD\(_2\) in allergic diseases, adequate data are not yet forthcoming. Two of the important references suggest a close relationship between the onset of allergic asthma and polymorphism of haematopoietic PGD\(_2\) synthetase and DP gene.\(^{45,46}\) These are important findings to investigate the role of PGD\(_2\) in human allergic diseases.

The above-noted clinical and basic researches stimulate the studies to develop a new anti-DP agent as a remedy for allergy. Mitsumori et al.\(^{47,48}\) and Arimura et al.\(^{49,50}\) have reported the efficacy of DP antagonist on allergic diseases, especially triggered by mast cell activation. Using the past findings as a background, we also tried to examine the effect of DP antagonist on the allergic AHR in mice. Figure 3 indicates the chemical structure of one of a potent DP antagonist and the results of the experiments to measure the AHR. The DP antagonist showed a clear inhibition of allergic AHR. The increase of eosinophils in the airway is also inhibited, but the elevation of serum IgE and Th2 cytokine level in BALF are not affected by this agent. In summary, above data suggest that PGD\(_2\) is one of the potent allergic inflammatory mediators and must be a target molecule of anti-allergic agent.

**PGE\(_2\)**

PGE\(_2\) is commonly considered to be a potent proinflammatory mediator and is involved in several inflammatory diseases including rheumatoid arthritis (RA). The activity of PGE\(_2\) is mediated by four receptors, termed E prostanoid receptors (EP\(_1\)–\(_4\)). Activation of EP\(_2\) and EP\(_4\) increases intracellular cAMP whereas the EP\(_1\) receptor mediates the elevation of intracellular calcium. The different isoforms of the EP\(_3\) receptor couple to multiple G proteins producing either inhibition of adenylate cyclase and calcium mobilization or stimulation of adenylate cyclase activity. These differences are caused by the condition of employed cells and circumstances.
Regarding the role of PGE2 in allergic reaction, conflicting results have been reported by some researchers, as shown in Table 3. Parord et al. and other investigators have reported that PGE2 inhibits the antigen-induced allergic asthmatic responses, but other researchers have shown an augmentation of IgE production and the enhancement of immunological release of mast cell mediators.

As for the protective effect of PGE2, when PGE2 solution is inhaled by the asthmatic patients, it prevents allergen-induced airway response and airway inflammation. Other researchers also reported that PGE2 prevents the early and late phase antigen-induced bronchoconstriction through the relaxation of airway smooth muscles and inhibition of the release of mast cell mediators including histamine, leukotrienes and PGD2. Moreover, PGE2 protects the allergen-induced AHR by the reduction of inflammatory cells, especially eosinophils recruitment. In fact, they indicate that the inhalation of PGE2 by asthmatic patients markedly attenuates the increase of eosinophils and metachromatic cells detectable in sputum. Despite the accumulation of such data, the mechanism of PGE2 action and participating receptors still remains to be fully elucidated. While there is little evidence about the role of EP1 in allergic reaction, Chan et al. have indicated the relationship between EP2 and anti-allergic responses including the relaxation of airway smooth muscle and the inhibition of histamine release from mast cells. In addition to EP2, the role of EP3 in allergic reaction has been extensively studied. Our recent study employing EP3 gene deficient mice indicates the importance of EP3 in the recruitment of eosinophils in the lung during antigen-induced airway inflammation. When allergic airway inflammation is caused by repeated allergen inhalation in EP3 gene deficient mice, allergic airway eosinophilia, IgE antibody production, Th2 cytokines (IL-4, 5 and 13) production are accelerated significantly when compared to those in wild type mice. These data suggest that an EP3 agonist can be one of the ingredients of a new anti-allergic drug. Then we examined the effect of EP3 receptor selective agonist, ONO-AE-248, on the allergic airway inflammation in mice. ONO-AE-248 shows an inhibitory effect on antigen-induced airway allergic inflammation as indicated in Table 4. The EP3 agonist clearly inhibits the elevation of airway sensitivity to methacholine and eosinophilia without affecting IgE antibody production. These data indicate that the lack of EP3 gene accelerates the allergic responses and the EP3 agonist suppresses an allergic inflammation. This probably means that PGE2 has an anti-allergic inflammatory action through EP3 receptor. Regarding the main target cell for EP3-induced anti-allergic effect, our further experiments indicate the importance of mast cells. As indicated in Table 4, passive cutaneous anaphylaxis, immunological histamine release and IL-13 release from mast cells are accelerated by the depletion of EP3 gene. Simultaneously, EP3 agonist clearly inhibits the antigen induced histamine release from sensitized mast cells. These data suggest that mast cell is an effector cell for the EP3 agonist. Finally, while the interest on the role of EP3 receptor in allergic reaction is increasing, but unfortunately clear evidence is still lacking. In conclusion, although PGE2 has proinflammatory properties, it also possesses a bronchodilating and anti-allergic actions probably through one of four different receptor subtypes. From our data, EP3 agonist may lead to a new approach for the treatment of allergic diseases.

**PGL2**

PGL2 is mainly produced by vascular endothelial cells and prevents platelet aggregation caused by a variety of stimuli. Some observations indicated the production of PGL2 by the local tissues and blood vessels through an acute allergic inflammation and anaphylaxis. In the allergic reaction in lung, produced PGL2 suppresses the generation of leukotrienes and causes the relaxation of airway smooth muscle. These evidences suggest the role of PGL2 in allergic inflammation and bronchial asthma. Therefore, we carried out the experiments to trace the role of PGL2 in allergic reaction by employing allergic airway inflammation model in IP gene lacking mice. In IP gene deficient mice, the elevation of airway eosinophilia, Th2 cytokine production and leakage of serum albumin into BALF are significantly augmented by repeated allergen inhalation. These data suggest that PGL2 may play a suppressive role in an allergic airway inflammation through IP.

To analyze the mechanism of above response to allergic inflammation in IP gene deficient mice, Th1 and Th2 response of isolated splenocytes were compared in gene deficient mice and wild type mice. While the IL-4 production by antigen stimulation is accelerated in the gene deficient mice, IFN-γ production is not altered, when compared to wild type mice. When the anti-CD3 and anti-CD28 induced cytokine production by isolated CD4+ T cells from nonsensitized mice were examined, the production of IL-4 was not altered but IFN-γ production was signific-

<table>
<thead>
<tr>
<th>Table 3 Effect of PGE2 on allergic responses</th>
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<tr>
<td>Anti-allergic actions of PGE2</td>
</tr>
<tr>
<td>1. Inhibition of antigen-induced asthmatic responses (Bronchoconstriction, Airway hyperresponsiveness, Eosinophilia, Edema)</td>
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<tr>
<td>2. Inhibition/augmentation of the immunological release of allergic mediators from mast cells</td>
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<tr>
<td>3. Inhibition of allergic eosinophil recruitment</td>
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<td>4. Augmentation of IgE production</td>
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The role of PGI2 in the chronic airway allergic inflammation was investigated. Using such background information as pegs, we investigated the role of TxA2 in allergic inflammation.63-65 Various reports also indicate the release of TxA2 during allergic reaction and TxA2 causes a potent bronchoconstriction in vivo and in vitro.66-68 In addition, the TxA2 receptor, TP, exists in immune competent organs including thymus and spleen.69,70 Moreover, recent studies concerning human subject polymorphism in the TP gene are associated with atopic dermatitis.71 Using such background information as pegs, we investigated the role of TxA2 in allergic inflammation by employing TP gene deficient mice.72 Our examination was carried out in two different types of experimental models. An allergic inflammation was caused in the airway and skin by repeated local antigen provocation. Surprisingly, our findings from two experiments indicate a reversal of results in terms of causing an allergic inflammation in the skin and lung. Our data suggest that TxA2 may play a pathological role in the airway inflammation but it acts as a suppressive factor in the skin. These data indicate the existence of different allergic mechanism in the lung and skin regarding the role of TxA2, and also suggest a difficulty in developing a new anti-allergic drug as an anti-allergic drug.

### OTHER EICOSANOIDS

TxA2 is not a PG but an important another cyclooxygenase derived eicosanoid. Many evidences suggest a role of TxA2 in allergic inflammation.63-65 Various reports also indicate the release of TxA2 during allergic reaction and TxA2 causes a potent bronchoconstriction in vivo and in vitro.66-68 In addition, the TxA2 receptor, TP, exists in immune competent organs including thymus and spleen.69,70 Moreover, recent studies concerning human subject polymorphism in the TP gene are associated with atopic dermatitis.71

### CONCLUSION

This review describes the role of PGs in allergic inflammation and the value of PGs as a target molecule for developing a new anti-allergic agent. Several investigators, including us, have shown that some PG and anti-PG agents are effective in the therapy of experimental allergic disease models. Our studies employing PG receptor and TxA2 receptor gene deficient mice suggest that EP3 or IP agonists and DP or TP antagonists would be expected to be a remedy for allergic diseases. IP agonist and TP antagonist, however, indicates the partial efficacy in an allergic...
model. Our data indicate that an EP₃ agonist and DP-antagonist have more possibility as a remedy for allergic airway inflammation. Further studies regarding more effective agent and precise mechanism of PGs in allergic inflammation are needed for discovering a new anti-allergic drug.

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