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

Lipid mediators including prostaglandin (PG), thromboxane (Tx), leukotriene (LT) and lipoxin (LX) exhibit a wide variety of actions in various cells and tissues to maintain local homeostasis in the body. These substances are released immediately after synthesis and act on the cell surface receptors to elicit their actions. These lipid mediators show strong action on mainly cardiovascular, nerve, reproduction and gastrointestinal systems and inflammatory responses, as shown in Table 1. In addition to above systems, recent extensive investigations indicate that lipid mediators play an important role in immune system.1-6

Among the lipid mediators, PGs are the most common autacoid and there is persuasive evidence that some PGs contribute to the signs and symptoms of inflammation and immune system. Most of PGs are commonly considered as potent proinflammatory mediators, actually involved in the pathogenesis of several inflammatory diseases such as rheumatoid arthritis, periodontitis and other inflammatory diseases.7-10

Apart from these findings, much attention has been paid to allergy, another inflammatory disease related to immune systems, because the incidence of allergic patients had increased dramatically in recent decades.11-15 However the precise role of PGs in allergy is not fully understood. Therefore, we have attempted to elucidate the role of PGs in allergy (allergic inflammation) by employing animal models. This review will provide the reader with an introduction to the role of PGs in allergic inflammation and then will discuss the role of PG, as a target molecule for an anti-allergic drug.
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>
</tr>
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<tbody>
<tr>
<td>PGD₂</td>
<td>DP</td>
<td>c-AMP ↑</td>
<td>Platelet aggregate ↓, Allergic inflammation ↑</td>
</tr>
<tr>
<td></td>
<td>CRTH2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGE₂</td>
<td>EP₁</td>
<td>Ca²⁺ ↑</td>
<td>Smooth muscle ↑, Stress ↑, Ovatian follicle ↑</td>
</tr>
<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 ↑, Ossification ↑, 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>PGI₂</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 c-AMP ↓</td>
<td>Platelet aggregation ↑, Smooth muscle ↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Thrombosis ↑</td>
</tr>
<tr>
<td>LTB₄</td>
<td>BLT₁</td>
<td>IP₃/DG ↑</td>
<td>Chemotaxis ↑</td>
</tr>
<tr>
<td></td>
<td>BLT₂</td>
<td>c-AMP ↓</td>
<td>?</td>
</tr>
<tr>
<td>LTC₄, D₄, E₄</td>
<td>cys LT₁</td>
<td>Ca²⁺ ↑</td>
<td>Airway smooth muscle ↑, Eosinophils ↑, Permeability ↑</td>
</tr>
<tr>
<td></td>
<td>cys LT₂</td>
<td>Ca²⁺ ↑</td>
<td>?</td>
</tr>
</tbody>
</table>

↓, down regulation; ↑, up regulation.

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
in mast cells. Significant quantities of PGD₂ are not produced by the immunological activation of basophils. 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 chemotractant receptor homologous molecule expressed on Th2 cells (CRTH₂).

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. 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. 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. 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
Fig. 2 Effect of indomethacin on antigen-induced cytokine production and leukocytes accumulation in BALF in mice. Each value represents the mean ±SEM of 6–11 mice. NS-Sal; Injection and inhalation of saline instead of allergen, S-OA; Immunization and inhalation of ovalbumin (allergen). **, *** p < 0.01 and 0.001, respectively (vs NS-Sal).
†, †† p < 0.05 and 0.01, respectively (vs S-OA).

Table 2 The role of PGD2 in experimental asthmatic responses in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Eosinophils in BALF</th>
<th>Th2 cytokine in BALF</th>
<th>Chemokine in BALF</th>
<th>AHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP gene deficient</td>
<td>↓↓</td>
<td>↓↓</td>
<td>ND</td>
<td>↓</td>
</tr>
<tr>
<td>L-PGDS gene over expression</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑↑ (eotaxin)</td>
<td>ND</td>
</tr>
<tr>
<td>Inhalation of PGD2</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑↑ (MDC)</td>
<td>↑</td>
</tr>
</tbody>
</table>

†↑↑, Increase when compared to control; ↓↓, Decrease when compared to control; ND, Not done; BALF, Bronchoalveolar lavage fluid; AHR, Airway hyperresponsiveness; PGDS, Prostaglandin D2 synthetase; MDC, Macrophage derived chemokine.

et al. and our studies indicate that PGD2 plays an important role for the accumulation of eosinophils into allergic lesion. Moreover, Honda et al. revealed the mechanism of PGD2 induced eosinophil infiltration. They have described the mediation of macrophage-derived chemokine from airway epithelial cells for PGD2 inducing local eosinophilia.

In addition to DP, recent studies suggest the participation of CRTH2 receptor in allergic inflammation. Some groups have demonstrated that CRTH2 selective agonists induce eosinophilic airway and skin inflammation in animal models. These data support the hypothesis that CRTH2 may play a critical role in allergic inflammation; however, more
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 PGD₂ 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 PGD₂ synthetase and DP gene.45,46 These are important findings to investigate the role of PGD₂ 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 PGD₂ is one of the potent allergic inflammatory mediators and must be a target molecule of anti-allergic agent.

**PGE₂**

PGE₂ is commonly considered to be a potent proinflammatory mediator and is involved in several inflammatory diseases including rheumatoid arthritis (RA). The activity of PGE₂ is mediated by four receptors, termed E prostanoid receptors (EP₁-₄). Activation of EP₂ and EP₄ increases intracellular cAMP whereas the EP₁ receptor mediates the elevation of intracellular calcium. The different isoforms of the EP₃ 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\textsuperscript{51-55} have reported that PGE2 inhibits the antigen-induced allergic asthmatic responses, but other researchers\textsuperscript{56,57} 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.\textsuperscript{52} 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 PGD\textsubscript{2}.\textsuperscript{51,53} 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.\textsuperscript{53-55} 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.\textsuperscript{56-58} 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.\textsuperscript{58} 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 EP1 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.

**PGL\textsubscript{2}**

PGL\textsubscript{2} is mainly produced by vascular endothelial cells and prevents platelet aggregation caused by a variety of stimuli. Some observations indicated the production of PGL\textsubscript{2} by the local tissues and blood vessels through an acute allergic inflammation and anaphylaxis. In the allergic reaction in lung, produced PGL\textsubscript{2} suppresses the generation of leukotrienes and causes the relaxation of airway smooth muscle.\textsuperscript{59} These evidences suggest the role of PGL\textsubscript{2} in allergic inflammation and bronchial asthma.

Therefore, we carried out the experiments to trace the role of PGL\textsubscript{2} in allergic reaction by employing allergic airway inflammation model in IP gene lacking mice.\textsuperscript{60,61} 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 PGL\textsubscript{2} 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\textsuperscript{+} T cells from nonsensitized mice were examined, the production of IL-4 was not altered but IFN-γ production was signifi-

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Effect of PGE\textsubscript{2} on allergic responses</th>
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<tbody>
<tr>
<td><strong>Anti-allergic actions of PGE\textsubscript{2}</strong></td>
<td></td>
</tr>
<tr>
<td>1. Inhibition of antigen-induced asthmatic responses (Bronchoconstriction, Airway hyperresponsiveness, Eosinophilia, Edema)</td>
<td></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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significant decreased in the T cells from IP gene deficient mice. These data suggest the possibility that PGIL2 suppresses the antigen-induced activation of Th2 cells and stimulates the activity of resting Th1 cells. Furthermore, Jaffar et al. have reported one potential mechanism supporting our findings. They have inferred that the anti-allergic action of PGIL2 is closely related to the production of T cell derived IL-10 which is known to suppress Th2 immunity. Thus, PGIL2 may show an immunomodulating action through a production of IL-10 via IP.

In addition to above-noted sub-chronic airway inflammation, we conducted another experiment to investigate the role of PGIL2 in the chronic airway allergic inflammation, airway wall remodeling in mice by using IP gene deficient mice. Airway wall remodeling model was produced by daily inhalation of antigen for 3 weeks after the systemic immunization in mice. In this model, apparent airway eosinophilia, goblet cell hypertrophy and fibrosis under airway epithelial cell were observed. These changes are depend on Th2 cells, eosinophils and TGF-β1. By employing this experimental model, we confirmed a suppressive role of PGIL2 in chronic airway inflammation, airway wall remodeling in mice. Regarding to chronic inflammatory responses, Straun et al. have reported that the production of connective tissue growth factor and collagen synthesis by TGF-β1 stimulated fibroblast were clearly inhibited by PGIL2. These data suggest that PGIL2 plays a role in the production of collagen directly.

From these data PGIL2 can be considered as a useful source to tap up a remedy for allergic diseases, we examined the effect of Beraprost, a PGIL2 analogue, on the experimental airway allergic inflammation. As shown in Table 5, Beraprost indicated the suppression of airway allergic eosinophilia and Th2 cytokine production, but did not show the inhibition of AHR and IgE production. Moreover, the inhibition pattern of Beraprost is not dose related. To confirm the effect of PGI2 agonists, the effect of two other PGI2 analogues on allergic inflammation were studied, and the results were similar to those of Beraprost. These data indicate the difficulty in developing a new anti-allergic drug from PGI2 analogues and the necessity of additional studies to confirm the suitability of PGI2 analogue as a remedy for allergic diseases.

### OTHER EICOSANOIDS

TxA2 is not a PG but an important another cyclooxygenase derived eicosanoid. Many evidences suggest a role of TxA2 in allergic inflammation. Various reports also indicate the release of TxA2 during allergic reaction and TxA2 causes a potent bronchoconstriction in vitro and in vivo. In addition, the TxA2 receptor, TP, exists in immune competent organs including thymus and spleen. Moreover, recent studies concerning human subject polymorphism in the TP gene are associated with atopic dermatitis. Using such background information as pegs, we investigated the role of TxA2 in allergic inflammation by employing TP gene deficient mice. 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-TxA2 agent as an anti-allergic drug.

### 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 antagonist 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.

ACKNOWLEDGEMENTS

The author would like to thank Professor S. Narumiya (Department of Pharmacology, Faculty of Medicine, Kyoto University) and the members of Department of Pharmacology, Gifu Pharmaceutical University, especially Associate Professor H. Tanaka for their contribution to complete this manuscript.

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