Role of Prostaglandin D$_2$ and Its Receptors in the Pathophysiology of Asthma

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ABSTRACT
Prostaglandin D$_2$ (PGD$_2$) is one of the most abundant lipid mediators present in the airways of asthmatics. However, little was known of the role it plays in the pathophysiology of asthma, until the identification of DP (DP1, PTGDR) and CRTH2 (DP2), two PGD$_2$-specific transmembrane receptors with different distribution and intracellular signaling. Pharmacological tools, such as receptor-specific agonists and antagonists, and genetically-engineered mice, which lack either DP or CRTH2, have helped understand the complex effects of PGD$_2$ in allergic inflammation of the airways. Furthermore, genetic association studies have shown a positive linkage of the genetic polymorphisms in DP and CRTH2, with asthma phenotypes from specific ethnic backgrounds, further highlighting the importance of PGD$_2$ and its receptors in the pathophysiology of asthma.

KEY WORDS
asthma, CRTH2, DP, eosinophils, lipid mediators, single nucleotide polymorphisms

ROLE OF PROSTAGLANDIN D$_2$ IN ASTHMA
Prostaglandin D$_2$ (PGD$_2$) is a major lipid mediator synthesized from arachidonic acid via the catalytic activities of cyclooxygenases (COX) and PGD$_2$ synthases (PGDS) in mast cells, macrophages, and other cellular sources. In patients suffering from allergic diseases, such as asthma, the de novo production of PGD$_2$ is increased during allergen exposure.$^{1,2}$ In asthmatics, after an allergen challenge, the concentrations of PGD$_2$ are increased in the bronchoalveolar lavage (BAL) fluid,$^{3}$ and the concentrations of 9α, 11β-PGF$_2$α, its main metabolite, are increased in plasma and urine.$^{4}$ An increase in the urinary excretion of 9α, 11β-PGF$_2$α has also been observed after aspirin challenge in patients with aspirin-induced asthma.$^{5}$

It is generally believed that, in asthmatics, PGD$_2$ modulates the physiology of the airways by causing bronchoconstriction,$^{6}$ vasodilation,$^{7}$ increases in capillary permeability$^{8}$ or mucous production.$^{9}$ In addition, the recent identification of new isoforms of PGD$_2$ receptors, the development of isoform-specific receptor agonists and antagonists, and the creation of genetic mouse models, which lack the specific isoform of PGD$_2$ receptors, have helped clarifying the important roles played by PGD$_2$ and its receptors in the pathophysiology of asthma, especially its role in the airway inflammation and bronchial hyperresponsiveness. Besides observations made in animal models, genetic association studies of PGD$_2$-related molecules in humans have also revealed a significant link between PGD$_2$ and asthma. This review updates our understanding of the role played by PGD$_2$ in asthma gathered from animal models and genetic association studies.

PGD$_2$ METABOLISM
The first step in the production of PGD$_2$, is the liberation of arachidonic acid from phospholipids in cellular membranes, via the activities of phospholipases, followed by its conversion to cyclic endoperoxide PGG$_2$ by COXs.$^{10}$ Two isoforms of COXs are present in the airways: COX-1, also known as prostaglandin H synthase 1 (PGHS1) is constitutively expressed and functions as a house keeping gene, while COX-2 (PGHS2) is induced during inflammation. The peroxydase activity of these enzymes transforms PGG$_2$ to PGH$_2$, an
unstable intermediate endoperoxide, which is immediately converted to PGD₂ by PGDS.

These are also 2 isoforms of PGDS.¹¹ Hematopoietic PGDS (H-PGDS) is present in mast cells, macrophages, and dendritic cells, while lipocalin-type PGDS (L-PGDS) is mostly expressed in the central nervous system. Once synthesized, PGD₂ is rapidly metabolized non-enzymatically to 15-deoxy-Δ¹²,14-PGJ₂ or ∆¹²-PGJ₂ depending on the presence of serum albumin.

PGD₂ RECEPTORS ON EOSINOPHILS AND OTHER INFLAMMATORY CELLS

Recent studies have identified 2 types of transmembrane receptors specific for PGD₂ expressed on inflammatory cells, such as eosinophils, basophils, and lymphocytes. The first, D prostanoid receptor (DP) is a classic PGD₂ receptor also known as PTGDR or DP₁²,¹³; the second is chemoattractant receptor-homologous molecule expressed on Th2 (CRTH2), also known as DP₂.¹⁴ Both are members of the seven-transmembrane-domain, G-protein-coupled receptor (GPCR) superfamily. DP, which is expressed ubiquitously, belongs to a GPCR cluster that includes other prostanoid receptors (Fig. 1),¹⁵ and is coupled with a Gso protein, which increases the concentration of intracellular cAMP.¹³ In contrast, CRTH2 is genetically closer to chemotactic receptors, such as chemokine receptors and the leukotriene B₄ receptor (Fig. 1), coupled with Gi₀ protein, which causes an increase in calcium and a decrease in concentrations of cAMP. The expression of CRTH2 is limited to eosinophils, basophils, and Th2 lymphocytes.¹⁴

Whether the DP receptor acts as a pro- versus an anti-inflammatory molecule during allergic inflammation remains controversial. PGD₂ or DP-specific agonists inhibit apoptosis and prolong the survival of eosinophils. These compounds also block the production of interleukin-12 (IL-12) in dendritic cells, biasing the development of naive T lymphocytes to cells producing type 2 cytokines.¹⁶,¹⁷ PGD₂, by the suppressing functions of NK cells, including the production of Th1 cytokine via DP, can promote the Th2-mediated
immune response. Conversely, DP-specific agonists inhibit the migration and degranulation of basophils.

In contrast to DP, the activities of CRTH2 observed in vitro strongly suggest that CRTH2 acts as a molecule that exacerbates allergic inflammation. CRTH2 agonists induce a marked increase in cell mobility and degranulation, and increase the expression of adhesion molecules in eosinophils and basophils. PGD2 also increases in a CRTH2-dependent manner the lymphocytic production of Th2 cytokines, including IL-4, -5, and -13.

Besides DP and CRTH2, PGD2 binds to the thromboxan A2 receptor, TP, and one of the PGE2 receptors, EP3. Figure 1 shows the selective agonists and antagonists identified for these receptors. PGD2 and its cyclopentenone-type metabolites, such as 15dPGJ2, also exert an anti-inflammatory activity, partially via a peroxisome proliferator-activated receptor-γ (PPAR-γ). In low concentrations, 15dPGJ2 promotes the eotaxin-induced chemotaxis of eosinophils in a PPAR-γ-dependent manner, while, in higher concentrations, 15dPGJ2 directly acts as a ligand for CRTH2 and activates the eosinophils.

**ROLE OF PGD2 AND ITS RECEPTORS ON ALLERGIC INFLAMMATION OF THE AIRWAYS**

We have previously described the COX-2-dependent synthesis of PGD2 in the lungs of ovalbumin-sensitized and challenged guinea pigs, a COX-2-specific inhibitor, blocked this synthesis, and inhibited the recruitment of eosinophils into the lungs. Conversely, others have reported that increased concentrations of PGD2 exacerbated eosinophilic inflammation of the airways. The intra-tracheal administration of PGD2 intensified the allergen-induced eosinophilic inflammation in mice. The over-expression of lipocalin-type PGDS also increased the concentration of Th2 cytokines, and promoted the accumulation of eosinophils in the lungs. These observations suggest that PGD2 plays important roles in allergic inflammation of the airways.

**ROLE OF DP**

In an allergen-induced asthma model of genetically-engineered DP-deficient mice, the phenotypes, such as airway eosinophilia, bronchial hyperresponsiveness, and Th2 cytokine responses were all attenuated. Furthermore, the expression of DP receptors were significantly enhanced in the allergen-stimulated epithelial cells of the airways. The pro-inflammatory role of DP was further supported by the inhibition of the accumulation of eosinophils by a DP-specific antagonist, in sensitized guinea-pig lungs. DP receptor antagonists also partially blocked the PGD2-induced mobilization of eosinophils from the bone marrow in a perfused, guinea pig hind-limb preparation. However, in recent experiments, the intra-tracheal administration of a DP-selective agonist activated the DP receptors on dendritic cells, increased the number of Foxp3+ CD4+ regulatory T cells, and suppressed airway inflammation in a IL-10-dependent manner. The infusion of DP agonist-stimulated dendritic cells similarly suppressed the inflammation, while dendritic cells from DP-deficient mice lacked this activity.

**ROLE OF CRTH2**

We have observed the induction of airway eosinophilia in IL-5- or allergen-exposed rodents by the intra-tracheal administration of PGD2 or CRTH2 agonists, however not by a DP agonist. This chemotactic activity of CRTH2 agonists on eosinophils in vivo was confirmed by other investigators. Furthermore, we and others have shown that ramatroban, a dual receptor blocker for CRTH2 and TP, but not DP- or TP-specific antagonists, suppressed the eosinophilia induced by PGD2 or CRTH2 agonists in the blood and airways. TM30089, a highly CRTH2-selective antagonist, also inhibited airway eosinophilia and goblet cell hyperplasia after allergen challenge. It is, thus, surprising that, after allergen exposure, the eosinophilic inflammation was not attenuated in the airways of CRTH2-deficient mice. Furthermore, the eosinophilia in allergen-exposed, CRTH2-deficient mice with a Balb/c background was as pronounced as that observed in wild-type mice. It is particularly noteworthy that, compared with wild-type mice, CRTH2-deficient mice with a C57BL/6 background developed more severe inflammation of the airways, along with an over-production of IL-3 and IL-5. The observations made with respect to murine CRTH2 must be interpreted cautiously. Whereas, in humans, the expression of CRTH2 on T lymphocytes is limited to Th2 subpopulation and does not include Th1, murine Th1 and Th2 lymphocytes both express CRTH2 mRNA.

We have shown, however, that the exacerbation of airway inflammation induced by the instillation of double-stranded RNA (which mimics the exacerbation of asthma induced by RNA virus infection), does not occur in CRTH2-deficient mice. A similar suppression of the exacerbation of allergic inflammation and bronchial hyperresponsiveness induced by double-stranded RNA was observed with pharmacological blockade of CRTH2, which was conserved among different animal species. These observations point to the discovery of a new role played by the PGD2-CRTH2 axis in the innate immune response to viral infection, and to the potential therapeutic application of CRTH2 antagonists in the prevention of asthmatic exacerbations.
Table 1  Genetic association studies of PGD2-related genes

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Acronym</th>
<th>Gene locus</th>
<th>Mutation(s)</th>
<th>Ethnic origin</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Hematopoietic prostaglandin D synthase</td>
<td>PGDS</td>
<td>4q21-q22</td>
<td>IVS2 + 11 A &gt; C)</td>
<td>Asian (Japanese)</td>
<td>Positive</td>
<td>40</td>
</tr>
<tr>
<td>Prostanoid DP receptor</td>
<td>DP</td>
<td>14q22.1</td>
<td>T-197C, C-2T</td>
<td>Asian (Japanese)</td>
<td>Negative</td>
<td>40</td>
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<tr>
<td></td>
<td>DP1</td>
<td></td>
<td>T-197C, T-549C, C-441T</td>
<td>European-American</td>
<td>Positive</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>PTGDR</td>
<td></td>
<td>T-197C, T-549C, C-441T</td>
<td>African-American</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A-731G, G-338A, C6541T, C6651T</td>
<td>European (UK and Danish) American</td>
<td>Positive</td>
<td>45</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>T-197C, T-549C, C-441T</td>
<td>Asian (Chinese-Han)</td>
<td>Negative</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T-197C, T-549C, C-441T</td>
<td>European; Spanish-American</td>
<td>Positive</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>G1544C, G1651A, T11336C, G12375T</td>
<td>Asian (Japanese)</td>
<td>Negative</td>
<td>49</td>
</tr>
</tbody>
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Table 2  Haplotypes frequencies of polymorphisms in the promoter lesion of DP

<table>
<thead>
<tr>
<th>Ethnic origin</th>
<th>European-American</th>
<th>African-American</th>
<th>Hispanic</th>
<th>Asian</th>
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<td>Control n</td>
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<td>79</td>
<td>254</td>
<td></td>
</tr>
<tr>
<td>Haplotype</td>
<td>Oguma et al. 43</td>
<td>Sanz et al. 44</td>
<td>Combined</td>
<td></td>
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<tr>
<td>CCT</td>
<td>36</td>
<td>39</td>
<td>37</td>
<td></td>
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<tr>
<td>TTT</td>
<td>24</td>
<td>27</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>TCT</td>
<td>32</td>
<td>22</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>CCC</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Asthmatic n</td>
<td>518</td>
<td>118</td>
<td>636</td>
<td></td>
</tr>
<tr>
<td>Haplotype</td>
<td>Oguma et al. 43</td>
<td>Tsai et al. 47</td>
<td>Combined</td>
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</tr>
<tr>
<td>CCT</td>
<td>39</td>
<td>43</td>
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<tr>
<td>TTT</td>
<td>27</td>
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<tr>
<td>TCT</td>
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<tr>
<td>CCC</td>
<td>12 #</td>
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</tbody>
</table>

* p < 0.05; # p = 0.08; NA = not applicable.

GENETIC ASSOCIATION STUDIES OF PGD2-RELATED MOLECULES WITH ASTHMA

Several noteworthy associations have been reported between the genotypes of PGD2-related molecules, such as PGDS, DP, and CRTH2, and the phenotype of asthma (Table 1). The hematopoietic PGDS gene is located on chromosome 4q22.3. In Japanese children, a single nucleotide polymorphism (SNP) in the second intron of this gene predicted a risk of asthma.40

The human DP gene is located on chromosome 14q21, where multiple whole-genome linkage analyses have suggested the presence of an asthma-related region.41,42 Sequencing of the DP gene revealed three variants (T-549C, C-441T, and T-197C) in the 5'-flanking region, as well as three variants (C+367A, G+894A, and G+1044A) in the coding region. We found that, in both Europeans and African-Americans, carriers of the C allele of T-549C, or of the T allele of C-441T, were at higher risk of asthma.43 Furthermore, there was a significant association between promoter haplotypes of three SNPs in the 5'-flanking region and susceptibility to asthma. The carriers of the haplotype with lower promoter activity were less susceptible to asthma. Our observations were reproduced in three different Caucasian populations, in Spain, the UK, and the Netherlands.44,45 however not...
in Japanese, Han Chinese, or Hispanics, suggesting the existence of marked differences among ethnic groups (Table 2). 46

The haplotype frequency measured in two studies of Caucasian populations has been consistent (Table 2). The haplotype frequency observed in African-Americans and Puerto-Ricans was the same as that found in Caucasians, though significantly different from that measured in Asians and Mexicans (Table 2). The absence of a Hispanic control population limits the validity of that study. 46,47

The human CRTH2 gene is located on chromosome11q13. In a case-control study of 2 SNPs, G1544C and G1651A, in the 3'-untranslated region of the CRTH2, the allele of 1651G and the haplotype of 1544G-1651G were both significantly associated with asthma in African-Americans and in Chinese. A functional analysis of experiments made in vitro suggested that the haplotype of 1544G-1651G was associated with a higher stability of CRTH2 mRNA than the 1544C-1651A haplotype. The association between CRTH2 gene polymorphism and asthma was, however, not confirmed in a Japanese population. 48

CONCLUSIONS

PGD2 is abundantly present in the airways of asthmatics. However, its role in the pathophysiology of asthma remains mysterious because of its multi-dimensional activity, both pro- and anti-inflammatory. While the identification of isoforms of the PGD2 receptors, DP and CRTH2, has solved some of the enigmas of the PGD2 story, several issues need to be clarified before the pharmacological modification of PGD2 signaling can be applied in therapeutics.

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