Antagonism of the prostaglandin D_2 receptors DP_1 and CRTH2 as an approach to treat allergic diseases

Roy Pettipher, Trevor T. Hansel‡ and Richard Armer**

Abstract | Immunological activation of mast cells is an important trigger in the cascade of inflammatory events leading to the manifestation of allergic diseases. Pharmacological studies using the recently discovered DP_1 and CRTH2 antagonists combined with genetic analysis support the view that these receptors have a pivotal role in mediating aspects of allergic diseases that are resistant to current therapy. This Review focuses on the emerging roles that DP_1 and CRTH2 (also known as DP_2) have in acute and chronic aspects of allergic diseases and proposes that, rather than having opposing actions, these receptors have complementary roles in the initiation and maintenance of the allergy state. We also discuss recent progress in the discovery and development of selective antagonists of these receptors.

Prostaglandins
Acidic lipids derived from the metabolism of arachidonic acid by the action of cyclo-oxygenase enzymes and downstream synthase enzymes. Prostaglandins have a diverse range of activities and have a well recognized role in pain and inflammation. Prostaglandin D_2 (PGD_2) is the main prostanoid produced by mast cells and is the predominant prostaglandin found at sites of allergic inflammation.

**Oxagen Limited, 91 Milton Park, Abingdon, Oxfordshire OX14 4RY, UK.*
‡National Heart and Lung Institute Clinical Studies Unit, Royal Brompton Hospital, Fulham Road, London SW3 6HP, UK.
Correspondence to R.P.
e-mail: r.pettipher@oxagen.co.uk
doi:10.1038/nrd2266

Prostaglandin D_2 (PGD_2) is an acidic lipid mediator that is derived from arachidonic acid by the sequential action of cyclooxygenase(s) (COX) and PGD_2 synthase(s). The COX(s) convert arachidonic acid in a two-step process to first PGG_2 and then PGH_2. These unstable endoperoxide intermediates are converted to PGD_2 by either the haematopoietic or lipocalin PGD_2 synthase (FIG. 1). PGD_2 is produced in the brain where it might be involved in the regulation of sleep[1] and other central nervous system (CNS) activities, which includes pain perception[2]. In peripheral tissues, the richest cellular source of PGD_2 is the mast cell — PGD_2 is produced by immunoglobulin E (IgE)-activated mast cells at levels of around 50 ng per 1×10^6 cells[3,4]. Other leukocyte populations, such as dendritic cells[5] and T helper 2 (T_H2) cells[6], also produce PGD_2, but at levels far lower than those produced by mast cells. Although non-mast-cell sources of PGD_2 might have important functions in some settings, such as in dendritic cell and T_H2 lymphocyte interactions, measurement of PGD_2 and its metabolites has been proposed as selective markers of mast-cell activation in clinical asthma[7–9]. The G-protein-coupled receptors (GPCRs) — CRTH2 (also known as DP_2), DP_1 and TP — that have an important role in mediating the biological effects of PGD_2 are detailed in BOX 1, and the known natural and synthetic ligands for these receptors are detailed in TABLE 1.

It is well established that the presence of an allergen triggers the production of PGD_2 in sensitized individuals. In asthmatics, a bronchial allergen challenge leads to the rapid production of PGD_2, which can be detected in the bronchoalveolar lavage fluid within minutes, reaching biologically active levels at least 150-fold higher than pre-allergen levels[10]. Local antigen challenge also stimulates PGD_2 production in the nasal mucosa of patients with allergic rhinitis[11] and in the skin of patients with atopic dermatitis[12]. Several lines of evidence support the view that mast cells are the principal sources of PGD_2 at sites of allergic inflammation. Cell fractionation studies have shown that PGD_2 is produced predominantly by mast cells[13], and, in chopped human lung parenchyma, mast-cell activation is a requirement for PGD_2 generation[14]. Also, although both mast cells and basophils produce histamine, basophils do not produce appreciable quantities of PGD_2 (REF. 7). This is relevant when discerning the principal cellular source of PGD_2, as PGD_2 is produced during the early response to allergen only, unlike histamine, which is produced during the early and late phases[15]. These data are therefore consistent with the view that mast cells are responsible for the bulk of PGD_2 production in an allergic setting. However, PGD_2 that is produced by mast cells might provide an essential link between the early phase and the late-phase allergic response by initiating cellular processes that lead to the recruitment and activation of T_H2 lymphocytes and eosinophils with associated pathophysiological effects. The mechanisms by which mast-cell-derived PGD_2 orchestrates these effects are the focus of this Review.

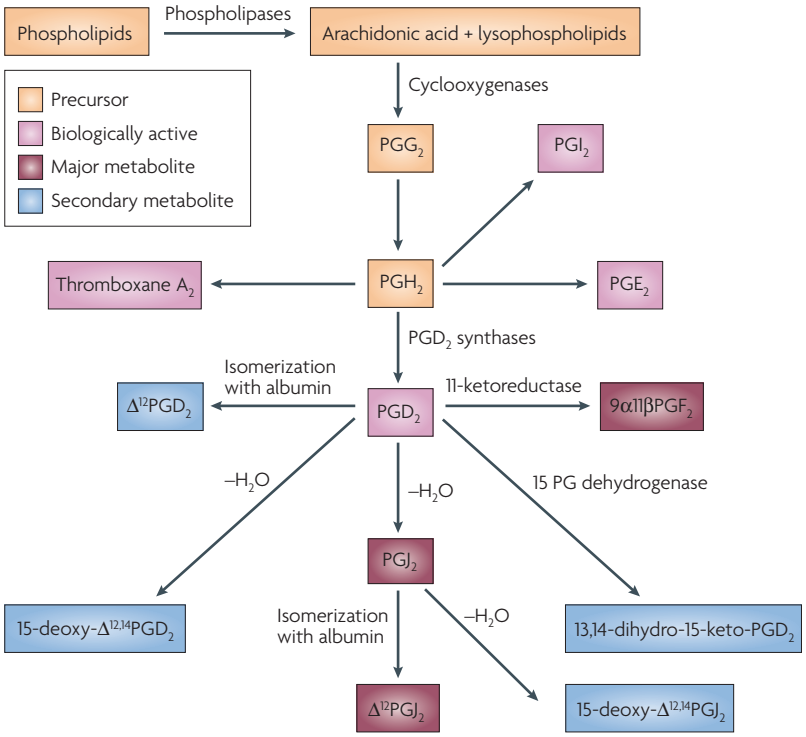

Figure 1 | **Pathways of PGD_2 metabolism.** On cellular activation arachidonic acid is released from membrane phospholipids and converted to the cyclic endoperoxide prostaglandin G_2 (PGG_2) by the action of cyclooxygenase 1 (COX1) or COX2. The peroxidase activity of the same enzymes leads to the formation of PGH_2, which can be converted to a number of biologically active prostaglandins by discrete synthase enzymes that show tissue-specific distribution and might demonstrate preferential coupling to either COX1 or COX2. PGD_2 production is dependent on the action of either the lipocalin PGD_2 synthase or the haematopoietic PGD_2 synthase. Haematopoietic PGD_2 synthase is present in mast cells, T helper 2 (T_H2) cells and other leukocytes, and it is thought to be responsible for the bulk of PGD_2 production during allergic responses. PGD_2 is rapidly metabolized (with a half-life of 1.5 min in blood), and the main products that have been detected *in vivo* are $\Delta^{12}PGJ_2$ and $9\alpha11\beta PGF_2$. The precise pathway of PGD_2 metabolism has not been fully elucidated but it is possible that 13,14-dihydro-15-keto-PGD_2 (DK-PDG_2), $\Delta^{12}PGD_2$ and 15-deoxy-$\Delta^{12,14}PGJ_2$ might be formed locally, which is of interest as these compounds, similar to $\Delta^{12}PGJ_2$, retain significant chemoattractant receptor-homologous molecule expressed on T_H2 cells (CRTH2)-agonist activity.

PGD_2 metabolism

The proposed pathways of PGD_2 metabolism are shown in FIG. 1. It is of particular interest that a number of known and putative PGD_2 metabolites such as 13,14-dihydro-15-keto-PGD_2 (DK-PGD_2)[16], $\Delta^{12}PGD_2$ (REF. 17), $\Delta^{12}PGJ_2$ (REF. 18), 15-deoxy-$\Delta^{12,14}PGD_2$ (REF. 19), 15-deoxy-$\Delta^{12,14}PGJ_2$ (REF. 19) and $9\alpha11\beta PGF_2$ (REF. 20) retain activity on CRTH2, but are less active on DP_1. This implies that the metabolism of PGD_2 might be important in defining the pattern of leukocyte activation in allergic diseases and that some metabolites of PGD_2 (particularly $\Delta^{12}PGJ_2$ (REF. 18)) might have systemic as well as local effects. These findings also illustrate that CRTH2 is a more promiscuous receptor than DP_1 with respect to the diversity of ligands that it interacts with.

11-dehydro-thromboxane B_2 is also a weak CRTH2 agonist[21], an activity that might be relevant to situations in which this thromboxane metabolite is formed in high concentrations, such as at sites of platelet aggregation.

The diverse activities of PGD_2 in the control of local blood flow, bronchial airway calibre and leukocyte function are mediated by high affinity interactions with the GPCRs CRTH2, DP_1 and TP. In addition to the effects that are mediated by the GPCRs discussed in this Review, PGD_2 can be metabolized to $\Delta^{12}PGJ_2$ and 15-deoxy-$\Delta^{12,13}PGJ_2$, which have effects relevant to the resolution of inflammation, including the inhibition of cytokine production and the induction of apoptosis. These effects are mediated by intracellular actions that are mediated by both peroxisome proliferator-activated receptor-γ (PPARγ)-dependent[22] and PPARγ-independent mechanisms, the latter of which involves the inhibition of nuclear factor-κB (NFκB) activation[23]. Generally, concentrations of ligand in the micromolar range have been used to observe the intracellular effects of these PGD_2 metabolites. As these observations are of pharmacological interest, the physiological significance of these findings has been questioned because of the high concentrations that are needed to induce these effects relative to the concentrations of the PGD_2 metabolites that are likely to be formed *in vivo*[24].

Effects of PGD_2 relevant to allergic diseases

Having established that mast-cell-derived PGD_2 is produced in high concentrations in response to an allergen challenge, it is interesting to note that PGD_2 can mimic a number of key features of allergic diseases. Studies in preclinical species have observed the following features when PGD_2 is applied to *in vivo* preparations, or its overproduction is engineered by genetic manipulation:

- Vasodilatation leading to erythema (flare) and potentiation of oedema (wheal).
- Recruitment of eosinophils and T_H2 lymphocytes.
- Modulation of T_H2-cytokine production.
- Bronchoconstriction.

The capacity of PGD_2 to cause or potentiate inflammatory responses has been appreciated for a long time. Injection of PGD_2 into human skin has been shown to produce a long lasting erythema[25,26], to potentiate the effects of other mediators on induration and leukocyte infiltration in human skin[26] and to enhance oedema formation in rat skin[25]. It is most likely that these effects of PGD_2, like those of other vasodilator prostaglandins, are due to an increased blood flow to the inflamed lesion[27] and are, therefore, most likely to be mediated predominantly by the DP_1 receptor. This is based on studies showing that the DP_1 agonist BW245C relaxes vascular smooth muscle preparations and the selective DP_1 antagonist BWA868C blocks the vasodilator effect of PGD_2 (REF. 28). Although these observations make it clear that DP_1 mediates the vascular effects of PGD_2, the capacity of PGD_2 to promote the cellular changes associated with inflammation is not due to an action on DP_1. Studies by Woodward *et al.*[29] in the rabbit eye and guinea pig eye showed that BW245C could mimic the ocular hypotensive effect of PGD_2, but it did not share the ability of PGD_2 to promote inflammatory changes, particularly eosinophil infiltration. This was the first hint that some of the pro-inflammatory effects of PGD_2 occur independently of DP_1 activation. Furthermore, this study also ruled out a role for TP in mediating the

Mast cell
A granular cell that bears Fc receptors for immunoglobulin E (IgE), which, when crosslinked by IgE and antigen, causes degranulation and release of mediators such as histamine, leukotrienes and PGD_2.

Dendritic cells
These professional antigen-presenting cells are increasingly being recognized as having crucial immunoregulatory functions. They are found in various tissues where they take up antigens, process them, migrate to the lymph nodes and present the antigens to T cells.

Box 1 | **Receptors that mediate the biological effects of prostaglandin D_2**

The molecular biology and pharmacology of prostaglandin D_2 (PGD_2) receptors are discussed elsewhere[94,133]. The following receptors have an important role in mediating the biological effects of PGD_2.

DP_1 (or DP). This receptor is the most studied PGD_2 receptor and its activation leads to G_s-mediated elevation in cyclic AMP. Such activation by DP_1-selective agonists such as BW245C promotes relaxation of both vascular and airway smooth muscle, which leads to vasodilatation[28] and bronchodilatation[40], respectively. DP_1 is also expressed by platelets where its activation is linked to an anti-aggregatory function[134]. Furthermore, DP_1 is expressed by certain leukocyte populations[47–50] including dendritic cells, where it controls various functions including cytokine production.

TP. This G_q-coupled prostanoid receptor binds thromboxane with high affinity, promoting platelet aggregation and constriction of both vascular and airway smooth muscle. PGD_2 activates the TP receptor in human bronchial muscle[37], probably through the formation of the 11-ketoreductase metabolite 9α11βPGF_2 (REFS 38,39). The bronchoconstrictor effects of TP dominate over the bronchodilator effects of DP_1 in the airways.

CRTH2 (or DP_2). Originally identified as an orphan receptor that is expressed by T helper 2 (T_H2) lymphocytes, chemoattractant receptor-homologous molecule expressed on T_H2 cells (CRTH2) is the most recently identified receptor for PGD_2 and mediates its effects by promoting the activation of T_H2 lymphocytes, eosinophils and basophils[16]. Despite binding PGD_2 with a similar affinity as DP_1, CRTH2 is not structurally related to DP_1 and signals through a different mechanism — the effects of CRTH2 are mediated through G_i-dependent elevation in intracellular calcium levels and reduction in intracellular levels of cyclic AMP. CRTH2 and DP_1 signalling pathways are reviewed by Kostenis and Ulven[94].

effect of PGD_2 as the TP agonist 9α11βPGF_2 was also ineffective in promoting eosinophil accumulation in the eye. The selective effect of PGD_2 on eosinophil activation *in vivo* is consistent with earlier work in which infusion of PGD_2 into the dog led to a transient but marked reduction in circulating eosinophils (as a result of activation in the blood) without alterations in the neutrophil count[30]. The ability of PGD_2 to promote the selective accumulation of eosinophils *in vivo* has also been shown in dog airways[31], whereas overexpression of human lipocalin-type PGD_2 synthase in transgenic mice leads to enhanced eosinophil recruitment and exaggerated T_H2-cytokine production in response to an antigen[32].

It has been appreciated for some time that PGD_2 can cause potent activation of isolated eosinophils[33]. However, it was not until recently that the receptor that mediates the chemotactic effect of PGD_2 was identified and characterized. In 2001 it was reported that the receptor mediating the activation of eosinophils in response to PGD_2 was a novel receptor unrelated to DP_1 (REF. 34). Simultaneously, it was discovered that PGD_2 was a ligand for CRTH2 (REF. 16), which was originally described as GPR44, an orphan chemoattractant-like receptor expressed by T_H2 cells. In a series of elegant experiments, Nagata, Hirai and co-workers showed that CRTH2 is selectively expressed by T_H2 lymphocytes, eosinophils and basophils[35,36], and that PGD_2 stimulates chemotaxis of these cell types through a CRTH2-dependent mechanism[16]. Interestingly, in these studies, the key observation was made that PGD_2 is the dominant CRTH2 agonist produced by activated mast cells[16].

PGD_2 is also a potent bronchoconstrictor and this effect is mediated by the TP receptor[37], possibly by the 11-ketoreductase-dependent formation of the TP agonist 9α11βPGF_2 (REFS 38,39). Although exposure to PGD_2 leads to bronchoconstriction, DP_1 receptors are expressed in bronchial smooth muscle and the selective DP_1 agonist BW245C has bronchodilator properties[40]. Overall, however, the predominant effect of PGD_2 in the airways is to cause bronchoconstriction, which indicates that DP_1-mediated bronchodilatation is masked by a more powerful bronchoconstrictor effect that is mediated by TP.

Role of DP_1 and CRTH2 in allergic inflammation

It is now emerging that DP_1 and CRTH2 have crucial, and complementary, roles in mediating aspects of the allergic response based on pharmacological studies with receptor-selective ligands and studies with genetically deficient mice.

DP_1 mediates vasodilatation and polarization of T_H2 cells. PGD_2 is rapidly produced in response to an inhaled allergen in the nose[11] and in the lung[10]. Haematopoietic PGD_2 synthase, DP_1 and CRTH2 all show increased expression in the nasal mucosa of patients with allergic rhinitis[41]. Therefore, it is likely that the activation of DP_1 might contribute to the increased nasal blood flow observed in a response to an allergen as a consequence of the DP_1-mediated vascular effects that are described above. Intravenous administration of PGD_2 to human volunteers leads to intense facial flushing and nasal congestion without having overt effects on the systemic blood pressure or lung function[42]. This demonstrates that PGD_2 has the potential to cause a pattern of vasodilatation that is relevant to the pathology of allergic rhinitis.

Furthermore, insufflation of PGD_2 has a greater effect than histamine on nasal congestion in human volunteers[43]. Treatment with ramatroban (Baynas; Kyorin, Nippon Shinyaku, Bayer Yakuhin), which is now known to be a CRTH2 antagonist (see next section), has no effect on nasal blockage induced by inhalation of PGD_2 (REF. 44), which provides indirect evidence that this effect is DP_1-mediated (as ramatroban inhibits both CRTH2 and TP receptors but not DP_1). The selective DP_1 antagonist S-5751 inhibited early phase increases in nasal pressure in response to antigen in sensitized guinea pigs while the H_1 antagonist terfenadine was without effect[45]. Therefore, at sites of mast-cell-mediated allergic inflammation it is apparent that DP_1 activation has an important role in increasing local blood flow leading to hyperaemia and potentiation of oedema formation, which, in the case of allergic rhinitis, contributes to nasal congestion. Although there are currently no clinical data available on the activity of DP_1 antagonists in allergic diseases it is of interest that niacin-induced skin flushing is reduced by the selective DP_1 antagonist MK-0524 in human volunteers[46], which demonstrates that endogenous production of PGD_2 leads to DP_1-mediated vasodilatation in humans.

Several studies suggest that DP_1 might have additional properties relevant to allergic diseases. In mice genetically deficient in DP_1 receptors, airway inflammation in response to low-dose antigen is diminished. Compared

CRTH2
Chemoattractant receptor-homologous molecule expressed on T helper 2 (T_H2) cells (CRTH2; also known as DP_2) is a cell-surface receptor of the G-protein-coupled receptor family that binds prostaglandin D_2 and mediates its effects on T_H2 lymphocytes, eosinophils and basophils. CRTH2 was originally described as GPR44, an orphan receptor.

DP_1
Another receptor for prostaglandin D_2, encoded by the *PTGDR* gene, that mediates its effects on vascular tissue and might be involved in the polarization of T helper 2 cells.

TP
High affinity receptor for thromboxane A2 that mediates platelet activation, vasoconstriction and bronchoconstriction. The PGD_2 metabolite 9α11βPGF_2 also binds this receptor.

Lymphocytes
White blood cells of lymphoid origin that function as part of the immune system.

Table 1 | **Comparison of the key biological and pharmacological properties of CRTH2, DP_1 and TP**

Receptor	Endogenous ligands	Signalling mechanism	Synthetic agonists	Synthetic antagonists	Location	Biological effects
CRTH2	PGD_2 13,14-dihydro-15-keto-PGD_2 (DK-PGD_2) $\Delta^{12}PGD_2$ $\Delta^{12}PGJ_2$ 15-deoxy-$\Delta^{12,14}PGD_2$ 15-deoxy-$\Delta^{12,14}PGJ_2$ 9α11βPGF$_2$ 11-dehydro-thromboxane B_2	G_i mediated ↑Ca^{2+} ↓cyclic AMP	Indomethacin	Ramatroban	T_H2 lymphocytes, eosinophils, basophils, CNS	Chemotaxis and activation of T_H2 lymphocytes, eosinophils and basophils, CNS effects unknown
DP_1	PGD_2	G_s mediated ↑cyclic AMP	BW245C	BWA868C MK-0524 S-5751	Bronchial smooth muscle, vascular smooth muscle, dendritic cells, platelets, CNS	Bronchodilatation, vasodilatation, suppression of cytokine production by dendritic cells, inhibition of platelet aggregation, probable involvement in CNS effects, for example, sleep and pain cognition
TP	Thromboxane A_2 9α11βPGF$_2$	G_q mediated ↑Ca^{2+}	U44612	Ramatroban GR32191 ICI192605 SQ29558	Bronchial smooth muscle, vascular smooth muscle, platelets	Bronchoconstriction, vasoconstriction, platelet aggregation

The combined action of these receptors mediate the pathophysiological effects of prostaglandin D_2 (PGD_2). Some of these effects, particularly the emerging role of CRTH2 (chemoattractant receptor-homologous molecule expressed on T helper 2 (T_H2) cells; also known as DP_2) and DP_1 in promoting T_H2-cell polarization and recruitment are particularly relevant to the pathogenesis of allergic asthma. CNS, central nervous system.

with wild-type mice, there was a substantial reduction in T_H2-cytokine production, eosinophil infiltration, mucus production and airway hyperresponsiveness in DP_1-null mice[40]. Although the effects of DP_1 deficiency can be overcome with higher doses of antigen, this study provides compelling evidence that DP_1 activation has a crucial role in regulating T_H2-dependent airway inflammation. The effect of DP_1 deficiency is mirrored by the effect of pharmacological blockade in preclinical models. The DP_1 antagonist S-5751 is partially effective in reducing eosinophil and macrophage infiltration into the airways of sensitized guinea pigs exposed to an antigen[45]. At face value it is difficult to reconcile these effects with the known pharmacology of DP_1.

How can these anti-allergic effects of DP_1 deficiency or receptor blockade be consistent with the lack of DP_1-mediated pro-inflammatory effects on T_H2 lymphocytes or eosinophils and the bronchodilator properties mediated by DP_1, which would be expected to be bronchoprotective? Paradoxically, the answer could lie in the anti-inflammatory effects afforded by DP_1-receptor activation. DP_1 is expressed by dendritic cells[47–49] and T_H1 cells[50], and the DP_1 agonist BW245C inhibits cytokine production by these cell types[47–49]. By contrast, DP_1 is not expressed at significant levels by T_H2 cells and does not significantly affect T_H2-cell function[51]. Therefore, it is proposed that DP_1-mediated inhibition of T_H1-inducing cytokines such as interleukin-12 (IL12)[48] will favour T-cell development towards the T_H2 phenotype[49] and, therefore, DP_1 blockade should halt the polarization of T cells. Of course, the corollary of that is that there is a hypothetical possibility that DP_1 blockade might exacerbate T_H1-dependent diseases such as rheumatoid arthritis and related autoimmune disorders. In this context it is worth noting that endogenous PGD_2 has been proposed to have an important role in downregulating the pro-inflammatory response associated with either irritant or T_H1-dependent stimuli. This effect has been observed in preclinical models of non-allergic inflammation, including carrageenan-induced pleurisy[52], trinitrobenzene-sulphonic-acid-induced colitis[53] and T_H1-dependent delayed-type hypersensitivity reactions[54], although additional mechanisms involving PPARγ activation (see above) have been proposed in some cases.

CRTH2 mediates direct activation of T_H2 lymphocytes and eosinophils. Investigations into the role of CRTH2 in allergic responses have been facilitated over the past few years by the discovery that ramatroban is an effective antagonist of this receptor[55]. Ramatroban was originally identified as a TP antagonist[56] but it is now realized that this drug binds to the CRTH2 receptor with moderate affinity (it has approximately tenfold less potent activity on CRTH2 compared with TP) and blocks responses to the selective CRTH2 agonist DK-PGD_2 *in vitro* and *in vivo*[55,57]. Studies with ramatroban suggest that CRTH2 is important in mediating eosinophil accumulation in a number of tissues in response to an allergic challenge including guinea pig nasal mucosa[58], mouse airways[59] and in mouse skin during contact hypersensitivity reactions[60]. These effects of ramatroban on eosinophil recruitment are unlikely to be mediated by TP antagonism as eosinophils do not express TP and selective TP antagonists do not influence eosinophil function[55,57].

It is also postulated that CRTH2 has an important role in T_H2-lymphocyte recruitment and activation. PGD_2 has the remarkable property of stimulating the production of the cytokines IL4, IL5 and IL13 by human T_H2 cells in the absence of co-stimulation[51]. This effect is inhibited by ramatroban and is mimicked by the selective CRTH2 agonist DK-PGD_2, but not by the DP_1 agonist BW245C[51]. Furthermore, activation of T_H2 lymphocytes in response

Eosinophil
A blood granulocyte that has a physiological role in the destruction of parasites. Eosinophils are strongly implicated in allergic inflammation, and are able to release an array of tissue-destructive mediators.

T_H2 cytokines
Proteins that include interleukin-4 (IL4), IL5 and IL13, which are produced by activated T helper 2 (T_H2) lymphocytes that have a central role in a number of key features of allergic disease, including immunoglobulin E production, eosinophilia, mucus production and airway hyperresponsivenesss.

Chemotaxis
The movement of cells in response to a chemical gradient that is provided by chemotactic agents such as interleukin-8 (IL8) and leukotriene B_4, which attract neutrophils.

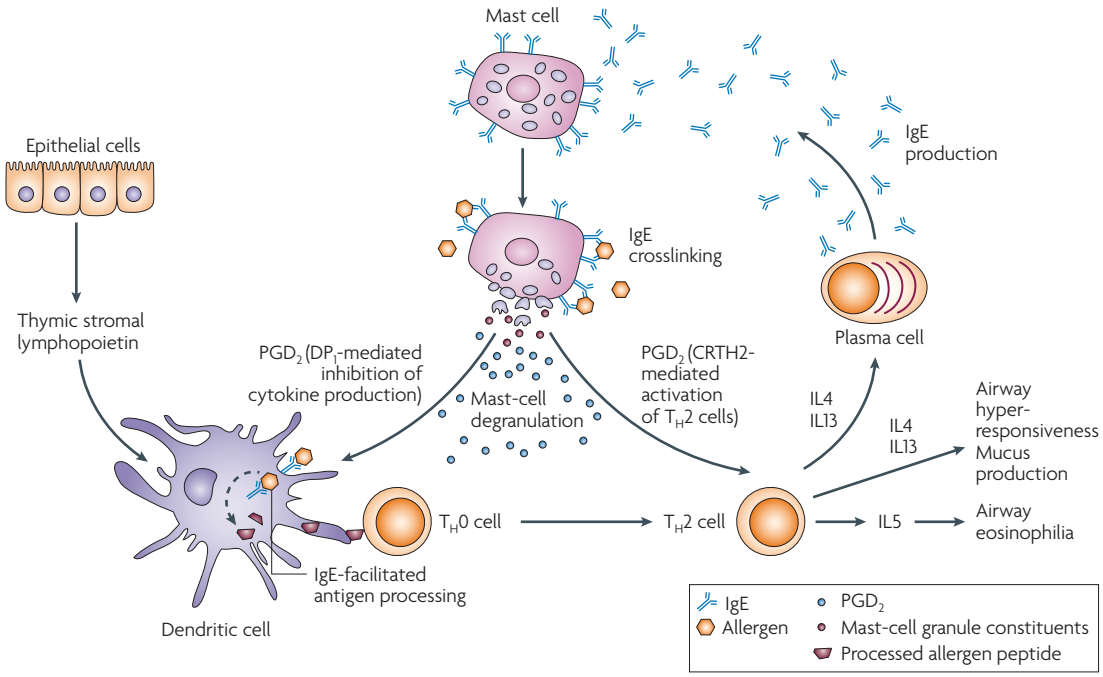

Figure 2 | **Proposed scheme describing the role of mast-cell-derived PGD_2 in the polarization and activation of T_H2 lymphocytes, effects achieved through combined action on DP_1 and CRTH2 receptors.** Antigen-induced crosslinking of cell-surface immunoglobulin E (IgE) on mast cells leads to the rapid and abundant production of prostaglandin D_2 (PGD_2). PGD_2 through interaction with the DP_1 receptor expressed by dendritic cells favours an environment in which T cells are polarized to the T helper 2 (T_H2) phenotype by inhibiting the production of T_H1-promoting cytokines such as interleukin-12 (IL12). Thymic stromal lymphopoietin also acts on dendritic cells to promote T helper 2 (T_H2) polarization, an effect associated with the induction of PGD_2 synthase in T_H2 cells and further PGD_2 production. PGD_2 promotes the recruitment and activation of T_H2 cells through an action on CRTH2 (chemoattractant receptor-homologous molecule expressed on T_H2 cells). T_H2 cells, once activated, produce cytokines that mediate a plethora of pathological effects relevant to allergic diseases including IgE production by plasma cells, which, through interaction with mast cells and IgE-facilitated antigen presentation, reinforces the cycle of allergic exacerbations.

to supernatants from immunologically activated human mast cells is mediated by PGD_2 through an action on CRTH2 (REF. 61). Inhibition of PGD_2 production by diclofenac reduces the production of T_H2-stimulatory activity by mast cells without affecting the responsiveness of T_H2 cells to PGD_2 or other stimuli, and CRTH2 blockade leads to a concordant reduction in the response of T_H2 cells to mast-cell supernatants[61]. *In vivo*, selective CRTH2 agonists enhance allergic responses — injection of PGD_2 leads to the exacerbation of allergic responses in the lungs and skin of mice, an effect mimicked by the selective CRTH2 agonist DK-PGD_2 (REF. 62). Taken together, these data suggest that CRTH2 might have an important role in the recruitment of T_H2 lymphocytes and other leukocytes to sites of allergic inflammation and, perhaps more importantly, in driving T_H2-cytokine production.

Studies of allergic responses in mice in which CRTH2 has been genetically deleted have so far produced apparently conflicting results. Satoh *et al.*[63] have reported that allergic skin inflammation and IgE production were significantly diminished in CRTH2-knockout mice, which is consistent with the pharmacological studies with human cells discussed above. By contrast, and rather surprisingly, Chevalier *et al.*[64] found that allergic airway inflammation, in particular eosinophil accumulation and IL5 production, was enhanced in mice deficient in CRTH2. This apparent discrepancy might be related to the unusual receptor-expression pattern of CRTH2 in mice. Unlike humans in which CRTH2 is expressed selectively by T_H2 cells and not T_H1 cells, in mice CRTH2 is expressed in equal abundance by both T_H1 and T_H2 cells[64,65]. Therefore, it is possible that CRTH2-mediated effects on T_H1 cells might dominate over those on T_H2 cells depending on the setting and variation in experimental protocol, particularly the immunization procedure. This possibility is supported by the observation that that production of IL2 was dramatically reduced in CRTH2-knockout mice[64]. It is also well established that variations in experimental protocols in mice can lead to profound differences in the nature of the allergic response. For example, airway inflammation can be either mast-cell dependent or mast-cell independent depending on the immunization procedure[66]. Therefore, it seems that results from the study of allergic responses in mice should be interpreted with caution, particularly those related to CRTH2.

DP_1 and CRTH2 act together to drive polarization and activation of T_H2 lymphocytes. Based on the proposed roles of DP_1 and CRTH2 described above, it is important

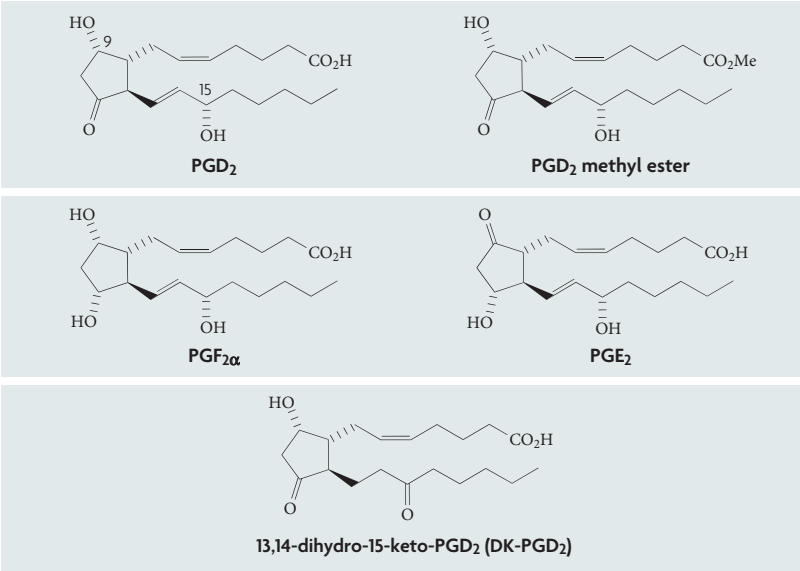

Figure 3 | **PGD_2 and related prostanoids.** The prostaglandin D_2 (PGD_2) methyl ester has a hundredth of the affinity for CRTH2 (chemoattractant receptor-homologous molecule expressed on T_H2 cells) compared to PGD_2 (319 nM and 2.4 nM, respectively), which indicates that the carboxylic-acid group present in PGD_2 is likely to make a key contribution to the binding affinity of PGD_2 at the CRTH2 receptor. $PGF_{2\alpha}$ retains moderate binding affinity for CRTH2 (395 nM), whereas PGE_2 has much lower affinity (4,730 nM), which potentially indicates a preference for the hydroxyl group on the cyclopentyl ring to be at the 9 position for optimal binding. Interestingly, modification to the 15-hydroxy group as in 14-dihydro-15-keto-PGD_2 (DK-PGD_2) does not affect CRTH2 binding (2.9 nM) but does provide high selectivity over DP_1.

to note that DP_1 and CRTH2, rather than having opposing actions, work together to promote T_H2-dependent allergic responses. Activation of DP_1 promotes an environment in which polarization of T_H2 cells can occur, whereas CRTH2 mediates their recruitment and activation to produce cytokines. Recently, it was discovered that CRTH2 is expressed by central memory T_H2 cells in addition to T_H2 effector cells, and that thymic stromal lymphopoietin (TSLP)-activated dendritic cells promote the maintenance and polarization of $CRTH2^+CD4^+$ T_H2 central memory cells[67]. These central memory T_H2 cells express high levels of PGD_2 synthase (also cystatin and Charcot–Leydon crystal protein). In this setting, PGD_2 that is produced by dendritic cells and T_H2 central memory cells might be important in driving polarized T_H2 function through complementary actions of both DP_1 and CRTH2. It is worth noting that TSLP levels in the asthmatic airways correlate with disease severity so this finding could be of clinical relevance[68]. Paradoxically, the DP_1 agonist BW245C has been reported to suppress allergic responses in the skin when administered topically at high concentrations[69]. This effect was associated with the suppression of Langerhans cell migration from the epidermis, the suppression of T_H2-cytokine production and the enhancement of T_H1-cytokine production. Further work is needed to reconcile this effect with other studies that indicate that DP_1 activation promotes T_H2 polarization, but, as in the case of CRTH2, the apparent differences might be related to the receptor-expression pattern differences in mice, the variations in the immunization procedure or the nonspecific actions of BW245C that occur at high doses. A scheme describing the proposed complementary roles of DP_1 and CRTH2 in polarization, recruitment and activation of T_H2 lymphocytes is shown in FIG. 2.

Cystatin
A family of cysteine protease inhibitors.

Charcot–Leydon crystal protein
A cell constituent that is unique to eosinophils and basophils, which possesses lysophospholipase activity.

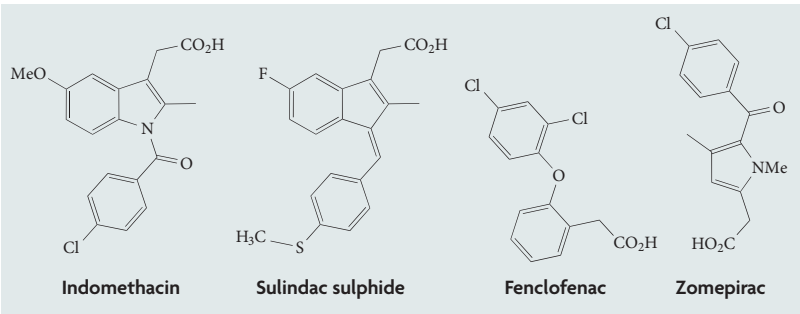

Figure 4 | **NSAIDs used as starting points for the design of selective CRTH2 antagonists.** Several non-steroidal anti-inflammatory drugs (NSAIDs) showed moderate binding affinity for CRTH2 (chemoattractant receptor-homologous molecule expressed on T_H2 cells), such as fenclofenac and sulindac sulphide (4,953 nM and 3,450 nM respectively), but were also active on DP_1. Indomethacin, however, demonstrated selective binding for CRTH2 (binding values vary between 25 nM and 8,000 nM depending on assay conditions) and was shown to be a potent agonist of the receptor (15–50 nM) in functional assays. Zomepirac was subsequently shown to have antagonistic activity *in vitro*.

Clinical relevance of TP, DP_1 and CRTH2

TP-mediated bronchoconstriction. In patients with asthma, inhalation of PGD_2 or its metabolite $9\alpha11\beta PGF_2$ leads to bronchoconstriction[70], and this effect is inhibited both by ramatroban[71,72] and by the more selective TP antagonists GR32191 (REFS 73,74) and ICI192605 (REF. 75). At doses that effectively block the bronchoconstrictor effect of PGD_2, TP antagonists have been tested for their ability to modify lung function in patients with asthma. GR32191 caused a modest inhibition of early phase bronchoconstriction in response to an allergen in some subjects[73], whereas both GR32191 and ramatroban were ineffective against exercise-induced decline in lung function[71,76]. Chronic treatment of patients with asthma with GR32191 did not lead to an improvement in lung function or reduce symptoms[77], which effectively rules out an important role for TP-mediated bronchoconstrictor effects of PGD_2 and other prostanoids in asthma.

Inflammatory effects of DP_1 and CRTH2. Although an important role for TP-mediated bronchoconstrictor effects has been ruled out, there is emerging interest in the inflammatory effects of PGD_2 that are mediated by DP_1 and CRTH2. There is considerable preclinical evidence that both DP_1 and CRTH2, acting together, have a key role in the initiation and maintenance of allergic diseases, but, at present, there are no data available on the clinical effect of selective antagonists in patients with asthma or related disorders. The only clinical data available on DP_1 antagonists are for MK-0524, which has been shown to inhibit PGD_2-mediated vasodilatation in human volunteers[46], but this compound has not yet been tested in patients with allergic diseases. The data currently available on the clinical effects of CRTH2 antagonists are restricted

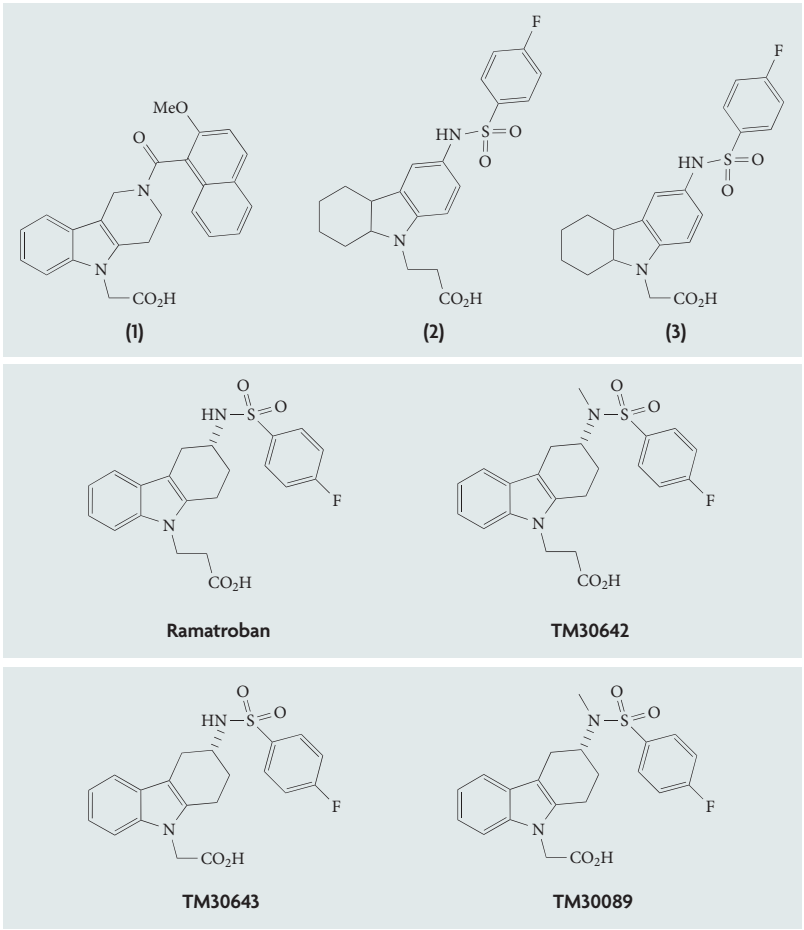

Figure 5 | **Ramatroban analogues with CRTH2 antagonist activity.** Moving the pendant amide nitrogen present in ramatroban into the core ring structure led to compound 1, which demonstrated potent (3 nM) antagonistic activity *in vitro*. By transposing the fused benzene and cyclohexyl rings a series of compounds were identified with moderate binding activity and selectivity as exemplified by compound 2 (CRTH2 (chemoattractant receptor-homologous molecule expressed on T_H2 cells) binding K_i = 250 nM, TP binding K_i = 1,500 nM). Further modification of compound 2 by shortening the propionic acid group to an acetic acid group gave compound 3, a more potent and selective analogue (CRTH2 binding K_i = 30 nM, TP binding K_i = >20,000 nM) demonstrating once again the strong preference for the acetic-acid group compared to propionic-acid groups in potent CRTH2 antagonists. By simply *N*-methylating the sulphonamide NH (compound TM30642, CRTH2 binding K_i = 1.9 nM, TP binding K_i = 3,000 nM) or by truncating the propionic-acid group to an acetic-acid group (compound TM30643, CRTH2 binding K_i = 0.51 nM, TP binding K_i = 540 nM) or a combination of both modifications (compound TM30089, CRTH2 binding K_i = 0.6 nM, TP binding K_i = >10,000 nM), three potent CRTH2 binding compounds with high selectivity over TP binding were identified with improved selectivity over ramatroban (CRTH2 binding K_i = 4.3 nM, TP binding K_i = 4.5 nM).

to studies with ramatroban, which is non-selective and has only moderate potency. Indeed, although ramatroban is clearly an effective TP antagonist at clinical doses it is not clear whether these doses are high enough to inhibit CRTH2-mediated effects. Despite these shortcomings, ramatroban is approved in Japan under the trade name Baynas for the treatment of perennial rhinitis, and published clinical studies with this compound provide tentative support for a role for CRTH2 in certain aspects of clinical allergy. In patients with perennial allergic rhinitis, treatment with ramatroban over 4 weeks inhibited chronic nasal swelling but did not increase blood flow to the nasal mucosa (which is likely to be DP_1-mediated, see above)[78]. This effect was associated with a partial reduction in rhinitis symptoms. Treatment of asthmatics with ramatroban for 2 weeks inhibited airway hyperresponsiveness to methacholine[79]. This effect was attributed to TP antagonism but it is possible that blockade of CRTH2 might have contributed to this response.

Effect of COX inhibition on PGD_2 production and asthma symptoms. If, as proposed, mast-cell-derived PGD_2 has an important role in allergy why are COX inhibitors generally ineffective in the treatment of asthma and related disorders? Bronchial allergen challenge studies have shown that COX inhibitors — non-steroidal anti-inflammatory drugs (NSAIDs) — do not inhibit the early phase or late-phase airway response in asthmatics[80]. As PGD_2 is produced by mast cells in a COX1- and COX2-dependent process it is expected that COX inhibitors would be effective in inhibiting PGD_2 production and this would inhibit both the early and late-phase response to some extent. However, NSAIDs also inhibit the production of PGE_2, which inhibits mast-cell activation[81] and has bronchodilator properties. The bronchoprotective effect of inhaled PGE_2 has been demonstrated in patients with asthma after a bronchial allergen challenge[82]. Consequently, it is likely that the bronchoprotective effects of PGE_2 mask the pro-inflammatory effects of PGD_2, and that selective inhibition of PGD_2 production or action is required to observe a therapeutic benefit in patients with asthma.

The importance of the protective effect of PGE_2 is evident in aspirin-intolerant asthma. In asthmatics intolerant to aspirin, inhibition of COX1-dependent production of PGE_2 leads to mast-cell activation and leukotriene-mediated bronchospasm[83,84]. Paradoxically, PGD_2 might contribute to aspects of aspirin-intolerant asthma, as aspirin challenge increases PGD_2 production, as measured by levels of its metabolite 9α11βPGF_2, in these patients[7,8]. It is unclear why aspirin enhances rather than inhibits PGD_2 production in aspirin-intolerant asthmatics, but it might be related to the high induction of COX2 that occurs in the airways of aspirin-intolerant asthmatics[85], which is less sensitive than COX1 to inhibition by aspirin. On the basis of these observations, it is likely that the selective inhibition of PGD_2 production or action is likely to have a profoundly different pharmacological profile to the non-selective inhibition of the formation of all prostanoids (including PGE_2). This is analogous to the situation in the cardiovascular system in which the balance between thromboxane and prostacyclin production is crucial in controlling platelet activation, blood pressure and atherosclerosis — 'selective' inhibition of thromboxane production with low-dose aspirin is cardioprotective, whereas inhibition of COX2-mediated formation of prostacyclin leaves the effects of thromboxane unopposed and increases the risk of myocardial infarction and stroke[86]. Inhibition of both thromboxane and prostacyclin production with mixed COX1 and COX2 inhibitors (traditional NSAIDs) is generally considered to be neutral with respect to cardiovascular risk.

Langerhans cell
Professional antigen-presenting dendritic cells that are localized in the skin epidermis.

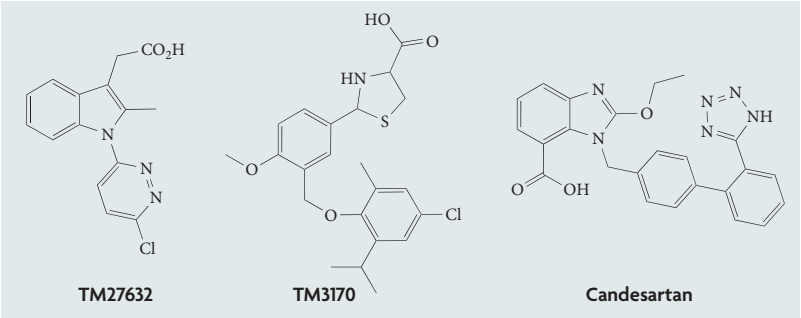

Figure 6 | **Physicogenetically identified CRTH2 antagonists.** A physicogenetically based method that classifies seven transmembrane receptors with respect to the physicochemical properties of the key amino-acid residues located in a common core ligand-binding site to CRTH2 (chemoattractant receptor-homologous molecule expressed on T_H2 cells) identified angiotensin II receptor type 1 (AGTR1) and AGTR2 receptors as likely to share similar binding properties to CRTH2. Screening of a focused compound library led to the identification of TM27632 and TM3170 as CRTH2 ligands with low micromolar affinity, whereas selected screening of AGTR1 and AGTR2 ligands identified candesartan as the most potent (2,100 nM) ligand for CRTH2.

Genetic variants of DP_1 and CRTH2 as risk factors for the development of asthma. Although the biological validation for the supporting roles of both DP_1 and CRTH2 in pathogenesis of allergic diseases is encouraging, experience in this therapeutic area has provided a salutary lesson that apparently compelling preclinical biology does not always translate into clinical efficacy. Several approaches have looked promising on the basis of animal data but have not proved to be of clinical benefit to patients.

One therapeutic approach that that has received a lot of attention is anti-IL5. IL5 has a well-recognized role as the main terminal differentiation factor during eosinopoiesis in the bone marrow[87]. Many preclinical studies have been performed in mice, guinea pigs and monkeys with monoclonal antibodies directed against IL5 (reviewed by Leckie[88]). Allergen challenge studies in Cynomolgus monkeys have shown that anti-IL5 abolishes eosinophilia and airway hyperresponsiveness[89]. However, although the preclinical effects of anti-IL5 are compelling, the clinical experience to date has been disappointing[90,91].

It is clear that the efficacy of any particular agent in animal models, even non-human primates, does not translate directly to humans. However, both DP_1 and CRTH2 differ from most targets studied in asthma so far in that their role in human disease is supported by clinical genetic studies. Genetic variants of *PTGDR* (the gene encoding DP_1) and *CRTH2* predispose individuals towards the development of asthma. In the case of *CRTH2* an association with severe asthma has been found in African–American and Chinese populations[92]. The associated polymorphisms in the 3′-untranslated region of *CRTH2* lead to increased mRNA stability, which suggests that gain-of-function variants in *CRTH2* are causally linked with asthma[92]. Such individuals are likely to demonstrate an exaggerated chemotactic response to PGD_2, leading to enhanced recruitment and activation of T_H2 lymphocytes and other leukocytes involved in allergic responses. By contrast, variants of *PTGDR* have been detected in patients with asthma that have reduced transcriptional activity[93]. It has been demonstrated in a small cohort of asthmatics that these putative loss-of-function variants have a protective role against the development of asthma[92].

Although the genetic studies highlighting that an 'antagonist surrogate phenotype' protects against asthma are encouraging, the importance of DP_1 and CRTH2 in the pathogenesis of allergic diseases will only be known with certainty when carefully controlled clinical trials with potent and selective antagonists have been completed. The following section describes the progress in identifying such antagonists.

Identifying selective CRTH2 antagonists

The chemical literature on DP_1 antagonists has been reviewed elsewhere[94–96]. This review of the chemical literature will focus on more recently described CRTH2 antagonists, as these compounds target the receptor that mediates the direct pro-inflammatory effects of PGD_2 on the recruitment and activation of T_H2 lymphocytes, eosinophils and basophils. Representative compounds from the literature and patents have been selected to illustrate the classes of CRTH2 antagonists currently known.

Identifying potential chemical start points — ligand approaches. Simple modifications to PGD_2 and screening of known and related prostanoids have allowed some basic ligand-binding structure–activity relationships (SARs) to be generated[97] (FIG. 3).

Of more interest to the drug discovery chemist was the discovery that non-prostanoid structures present in known NSAIDs have affinity for CRTH2 (REF. 97) (FIG. 4). Several NSAIDs showed moderate binding affinity for CRTH2, such as fenclofenac and sulindac sulphide, but were also active on DP_1. Indomethacin, however, demonstrated selective binding for CRTH2 (binding values vary between 25 nM and 8,000 nM depending on assay conditions[97–100]) and, interestingly, was shown to be a potent CRTH2 agonist in functional assays[97,98]. The results with these NSAIDs were replicated when the mouse CRTH2 receptor was studied[99,100]. Interestingly, in these studies, zomepirac (FIG. 4), which retains the same *p*-chlorobenzoyl group present in indomethacin, was shown to bind mouse CRTH2 with moderate affinity (3,300 nM) but weakly antagonized the effects of indomethacin[100].

The first identification of a potent CRTH2 antagonist was reported by Bayer when it was found that ramatroban (FIG. 5) possessed CRTH2 antagonistic activity[55]. Ramatroban bound CRTH2 with an IC_{50} of 100 nM and inhibited PGD_2-mediated human eosinophil migration (IC_{50} of 170 nM). As ramatroban is marketed as an anti-allergic drug in Japan, these findings provided encouragement that a small-molecule oral CRTH2 antagonist was a realistic and tractable therapeutic goal.

Identifying potential chemical start points — utilizing receptor information. A physicogenetic approach to seven transmembrane-spanning receptors has been used by researchers at 7TM Pharma to classify these receptors

IC_{50}
The half maximal inhibitory concentration. Represents the concentration of an inhibitor that is required for 50% inhibition of a biological or molecular process. pIC_{50} referes to the negative logarithm of this value.

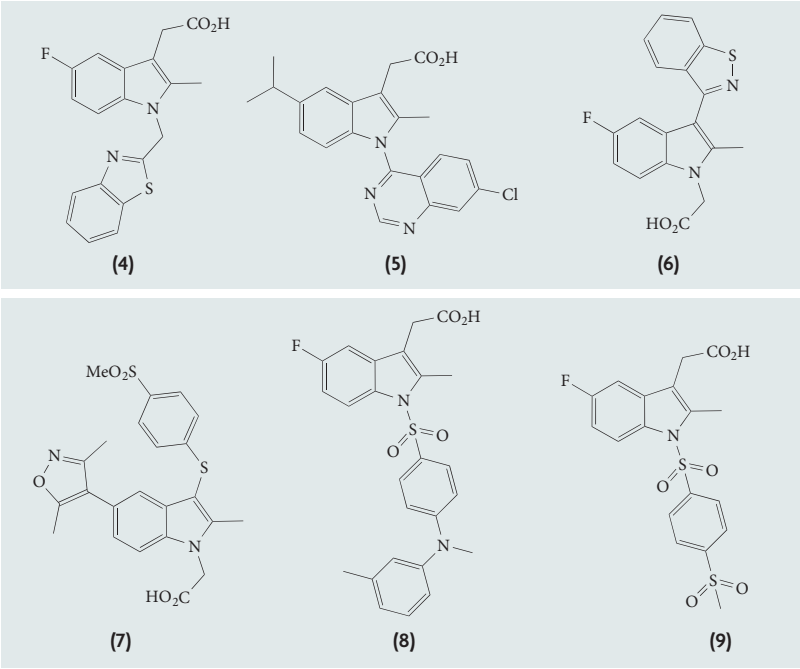

Figure 7 | **Indole-acetic-acid based compounds with CRTH2 antagonist activity.** Indole 4 was the first-identified CRTH2 (chemoattractant receptor-homologous molecule expressed on T_H2 cells) antagonist within this structural class with 40-fold functional selectivity for CRTH2 over DP_1. *N*-aryl substituted analogues such as compound 5 demonstrated moderate functional antagonist activity in an *in vitro* calcium mobilization assay (pA_2 = 6.8). Subsequent transposition of the acetic-acid and aryl groups led to more potent compounds such as 6, which bound CRTH2 with a pIC_{50} of 8.15 and introduction of a hetero-spacer group (S, SO_2, O) between the aromatic group and the indole at position 3 generated highly potent compounds such as 7, which bound CRTH2 with a pIC_{50} of 9.4. The corresponding transposed indole 3-acetic-acid compounds with the same spacer groups from the indole at position 1 have also been reported. Interestingly, some of the extended sulphonamide analogues such as compound 8 retain binding affinity for both CRTH2 (K_i = 222 nM) and DP_1 (K_i = 86 nM), whereas truncated compounds such as 9 are CRTH2 selective.

with respect to the physicochemical properties of the key amino-acid residues located in a common core ligand-binding site defined from the crystal structure of rhodopsin[101]. When this approach was applied to CRTH2, the closest related receptors identified were chemokine-like receptor 1 (CMKLR1), angiotensin II receptor type 1 and 2 (AGTR1, AGTR2), in contrast to standard phylogenetic methods of analysis, which highlighted the cysteinyl leukotriene receptors (CysLTs), BLTs (leukotriene B4 receptors, BLT1 and BLT2), and formyl-peptide-receptor-like receptors (FPRLs) as the closest relatives. By focusing on the physicogenetically related AGTR1 and AGTR2 receptors, the existing knowledge around ligands and site-directed mutagenesis data associated with these receptors, a pharmacophore model specific for CRTH2 was derived and used in an *in silico* screening exercise. Virtual screening of a diverse 1.2 million compound library identified 600 compounds, which were tested at a concentration of 10 μM for CRTH2 binding activity. TM27632 and TM3170 (FIG. 6) were identified as chemically distinct, low micromolar active hits, and further compounds with binding potencies as low as 20–30 nM were also found. Interestingly, the screening of 40 AGTR1 and AGTR2 ligands gave a 25% hit rate, with candesartan (FIG. 6) being the most potent hit (2,100 nM), but because the first approach did not rely on any specific AGTR-ligand scaffold or structural input the hits identified from the 600 compounds screened were selective CRTH2 ligands, which did not require substantial optimization to remove any unwanted AGT-receptor activity[101].

Site-directed mutagenesis of the highly homologous mouse CRTH2 has been studied and provides key information on how prostanoid and non-prostanoid ligands bind[102]. PGD_2 and indomethacin interact with distinct but overlapping sets of residues within the CRTH2 binding pockets, and CRTH2 resembles chemoattractant receptors more closely than other prostanoid receptors in terms of ligand-binding parameters. Amino-acid residues were selected for mutation on the basis of CRTH2-receptor modelling and those key residues common to the well-studied and related formyl peptide receptor (FPR) and C5a chemotactic receptors. It was found that by sequentially replacing His106 (TM III), Lys209 (TM V), Glu268 (TM VI) and Arg178 (extracellular loop II) with alanine PGD_2 binding was reduced, but the His106Ala and Glu268Ala mutants bound indomethacin and ramatroban with similar affinity to the wild-type receptor. The Arg178Ala mutant and Lys209Arg modification reduced binding of PGD_2, indomethacin and ramatroban by fourfold to tenfold, indicating that these residues are important for the binding of both prostanoid and non-prostanoid ligands. Interestingly, the Tyr261Phe mutant retained PGD_2 binding but reduced binding of both indomethacin and ramatroban indicating, again, the existence of overlapping but distinct binding pockets for these two classes of ligand. Ligand-docking studies with PGD_2 and indomethacin suggested that Lys209 might interact with the carboxylate group of each ligand and that for prostanoid ligands the 9-hydroxyl group on the cyclopentyl ring forms a hydrogen bond to Glu268.

Structural classes of CRTH2 antagonists

Indole acetic acids. Once indomethacin was identified as a potent ligand for CRTH2 with agonistic activity, it provided a template from which to try and derive potent and selective CRTH2 antagonists devoid of COX activity. The first indication that this was possible came from a Pfizer screening patent that identified the compound indole 4 (FIG. 7) as an antagonist with 40-fold selectivity over DP_1 (REF. 103). Researchers at AstraZeneca have subsequently patented a range of indole-acetic-acid derivatives[104–110] (FIG. 7).

Oxagen have patented 1-acetic-acid derivatives with either methylene or sulphonyl spacers between the indole and aromatic groups from the indole-3 position[111,112], and have also disclosed the corresponding transposed indole-3-acetic-acid compounds with the same spacer groups from the indole-1 position[113,114]. More detailed information on the SAR of truncated sulphonamides has been disclosed with compound 9 (FIG. 7), which demonstrated highly selective CRTH2-binding K_i of 68 nM and functional antagonist activity on human eosinophil shape change (IC_{50} = 74 nM) and human T_H2-cell chemotaxis (IC_{50} = 67 nM)[115]. Indole 9

Site-directed mutagenesis
An *in vitro* technique that introduces mutations (base-pair changes) at a specific site in the DNA sequence, giving rise to amino-acid charges.

pA_2
pA_2 refers to the negative logarithm of the concentration of antagonist that gives a concentration ratio of 2 when agonist concentration–response curves, conducted in the presence of the antagoinst, are plotted using Schild analysis. It is a measure of the activity of an antagonist.

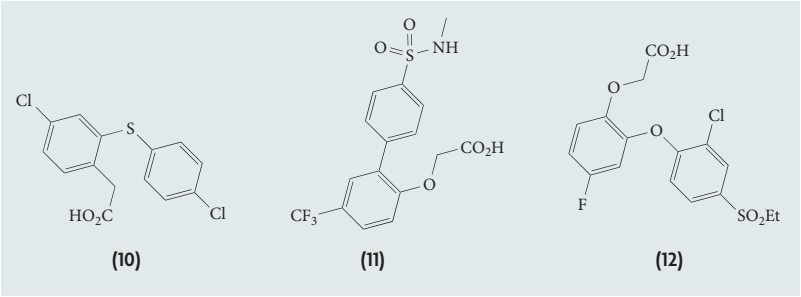

Figure 8 | **Aryl acetic acid CRTH2 antagonists.** The biaryl thioether 10 was one of the first-identified CRTH2 (chemoattractant receptor-homologous molecule expressed on T_H2 cells) antagonists within this structural class with 40-fold functional selectivity for CRTH2 over DP_1. Biaryl-acetic-acid derivatives such as compound 11 with a CRTH2 binding pIC_{50} of 8.2 and biaryl-ether-acetic-acid derivatives such as compound 12 with a CRTH2 binding pIC_{50} of 9.0 have been patented.

was also shown to be metabolically stable *in vitro* and devoid of any COX, cytochrome P450, cytotoxicity or human ERG (ether-a-go-go related gene) liabilities. Pharmacokinetic studies in rats with compound 9 was shown to be 56% bioavailable with an oral half-life of 5.5 hours[115].

Ramatroban analogues. Although ramatroban is a non-selective CRTH2 antagonist with only moderate potency, it also provided an attractive starting point from which to derive selective CRTH2 antagonists as, unlike indomethacin, it already had antagonist functionality. Shionogi disclosed a range of ramatroban analogues in the patent literature[116] and, subsequently, Actelion have patented analogues in which the pendant amide nitrogen in ramatroban has been moved into the core ring structure, with compound 1 (FIG. 5) having 3 nM antagonist CRTH2 activity in an *in vitro* calcium mobilization assay[117].

7TM Pharma have also demonstrated that only minor changes to the structure of ramatroban can lead to highly selective CRTH2 antagonists without TP activity[118] (FIG. 5). Subsequently, it was shown that the functional activities of these three antagonists were different — ramatroban and TM30642 demonstrated competitive antagonism of PGD_2, whereas both TM30643 and TM30089 showed non-competitive antagonism, possibly due to CRTH2 blockade mediated by an orthosteric site of the receptor[119].

More recently, researchers at Athersys have used a 'reverse scaffold' approach to isosteric ramatroban analogues[120] (FIG. 5).

Aryl acetic acids. Aryl acetic acids were first identified as CRTH2 ligands from fenclofenac (FIG. 4) and as antagonist templates in the same Pfizer screening patent that identified the first indole acetic acid CRTH2 antagonists. In that patent the compound biaryl thioether 10 was identified as a CRTH2 antagonist with 40-fold functional selectivity for CRTH2 over DP_1 (REF. 103) (FIG. 8). Subsequently, several companies have filed patents on the aryl-acetic-acid template as CRTH2 antagonists, for example, AstraZeneca have patented biaryl-acetic-acid derivatives[121–124] such as compound 11 (FIG. 8) with a CRTH2-binding pIC_{50} of 8.2 (REF. 122) and biaryl-ether-acetic-acid derivatives[125,126] such as compound 12 (FIG. 8) with a CRTH2-binding pIC_{50} of 9.0 (REF. 125).

Non-acidic CRTH2 antagonists. The non-acidic CRTH2 antagonists identified so far are dominated by the tetrahydroquinoline structural class. It is most likely that this class of compounds was derived from screening campaigns within pharmaceutical companies as there has been a flurry of patent publications all outlining very similar structures within a short time frame. The class itself is important because they highlight that potent and selective CRTH2 antagonists can be identified that lack what was assumed to be an essential carboxylic acid within their structure. Although the lack of a chemical handle from which to derive salts might prove to be a hindrance in terms of solubility and pharmaceutical development of this class of compound they do offer the ability to cross the blood–brain barrier and treat CNS disorders without resorting to a carboxylic-acid prodrug approach.

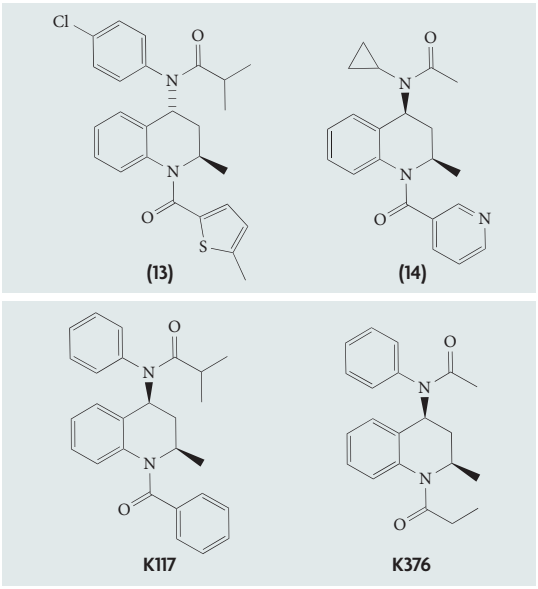

Figure 9 | **Non-acidic CRTH2 antagonists.** Tetrahydroquinoline 13 was one of the first non-acidic CRTH2 (chemoattractant receptor-homologous molecule expressed on T_H2 cells) antagonists identified. Subsequently compound 14 has been demonstrated to penetrate into the cerebrospinal fluid after oral dosing and possess efficacy in both inflammatory and neuropathic pain models. K117 demonstrated an IC_{50} of 7.8 nM against prostaglandin-D_2-induced human eosinophil chemotaxis and was selective for CRTH2 in ligand-binding experiments when compared with several prostanoid receptors and prostanoid-generating enzymes, including cyclooxygenases. Interestingly, the close analogue K376 was shown to be a CRTH2 agonist indicating the requirement for an aromatic-group-based amide from the tetrahydroquinoline nitrogen atom to maintain CRTH2-antagonist activity.

The first patent applications in the tetrahydroquinoline class were made by Millennium (now believed to be licensed to Sanofi–Aventis) followed closely by Warner–Lambert (now Pfizer). Millennium disclosed a range of analogues including compound 13 (REF. 127) (FIG. 9) and followed this with a further application claiming more extended amide compounds[128]. Warner–Lambert (Pfizer) have patented similar structures[129,130] (FIG. 9) and, interestingly, have filed use patents demonstrating penetration into cerebral spinal fluid after an oral dosing of compound 14 (FIG. 9) and efficacy in models of inflammatory and neuropathic pain at oral doses of 25 mg per kg[131].

Researchers at Kyowa Hakko Kogyo have also published some preliminary SAR information within this series[132] (FIG. 9).

Conclusions

It is now becoming clear that PGD_2 is an important mediator of allergic responses. The high concentrations produced in response to an allergic stimulation combined with its highly potent activity result in PGD_2 having a dominant role in mediating mast-cell-dependent activation of T_H2 lymphocytes, an effect mediated by CRTH2. Therefore, PGD_2 produced by mast cells might provide an essential link between the early phase and late-phase allergic responses and such antagonism of PGD_2 provides an attractive target for therapeutic intervention. There are a number of potent and selective CRTH2 antagonist series identified with drug-like properties and results of ongoing clinical trials in asthma and allergic rhinitis are awaited with interest.

1. Urade, Y. & Hayaishi, O. Prostaglandin D_2 and sleep regulation. *Biochim. Biophys. Acta* **1436**, 606–615 (1999).
2. Eguchi, N. *et al.* Lack of tactile pain (allodynia) in lipocalin-type prostaglandin D synthase-deficient mice. *Proc. Natl Acad. Sci. USA* **96**, 726–730 (1999).
3. Lewis, R. A. *et al.* Prostaglandin D_2 generation after activation of rat and human mast cells with anti-IgE. *J. Immunol.* **129**, 1627–1631 (1982).
4. Peters, S. P. *et al.* The role of prostaglandin D_2 in IgE-mediated reactions in man. *Trans. Assoc. Am. Physicians* **95**, 221–228 (1982).
5. Urade, Y., Ujihara, M., Horiguchi, Y., Ikai, K. & Hayaishi, O. The major source of endogenous prostaglandin D_2 production is likely antigen-presenting cells. Localization of glutathione-requiring prostaglandin D synthetase in histiocytes, dendritic, and Kupffer cells in various rat tissues. *J. Immunol.* **143**, 2982–2989 (1989).
6. Tanaka, K. *et al.* Differential production of prostaglandin D_2 by human helper T cell subsets. *J. Immunol.* **164**, 2277–2280 (2000).
7. O'Sullivan, S., Dahlen, B., Dahlen, S. E. & Kumlin, M. Increased urinary excretion of the prostaglandin D_2 metabolite 9α, 11β-prostaglandin F2 after aspirin challenge supports mast cell activation in aspirin-induced airway obstruction. *J. Allergy Clin. Immunol.* **98**, 421–432 (1996).
8. Bochenek, G., Nagraba, K., Nizankowska, E. & Szczeklik, A. A controlled study of 9α, 11β-PGF2 (a prostaglandin D_2 metabolite) in plasma and urine of patients with bronchial asthma and healthy controls after aspirin challenge. *J. Allergy Clin. Immunol.* **111**, 743–749 (2003).
9. Dahlen, S. E. & Kumlin, M. Monitoring mast cell activation by prostaglandin D_2 *in vivo*. *Thorax* **59**, 453–455 (2004).
An editorial on the utility of using the PGD_2 metabolite $9\alpha11\beta PGF_2$ as an *in vivo* marker of mast-cell activation in humans. $9\alpha11\beta PGF_2$ is a more sensitive marker of mast-cell activation than tryptase or histamine metabolites.
10. Murray, J. J. *et al.* Release of prostaglandin D_2 into human airways during acute antigen challenge. *N. Engl. J. Med.* **315**, 800–804 (1986).
One of the first studies to show that PGD_2 is produced rapidly and in high concentrations in response to an allergen in subjects with allergies.
11. Naclerio, R. M. *et al.* Mediator release after nasal airway challenge with allergen. *Am. Rev. Respir. Dis.* **128**, 597–602 (1983).
12. Charlesworth, E. N., Kagey-Sobotka, A., Schleimer, R. P., Norman, P. S. & Lichtenstein, L. M. Prednisone inhibits the appearance of inflammatory mediators and the influx of eosinophils and basophils associated with the cutaneous late-phase response to allergen. *J. Immunol.* **146**, 671–676 (1991).
13. Benyon, R. C., Robinson, C. & Church, M. K. Differential release of histamine and eicosanoids from human skin mast cells activated by IgE-dependent and non-immunological stimuli. *Br. J. Pharmacol.* **97**, 898–904 (1989).
14. Schulman, E. S., Newball, H. H., Demers, L. M., Fitzpatrick, F. A. & Adkinson, N. F. Jr. Anaphylactic release of thromboxane A_2, prostaglandin D_2, and prostacyclin from human lung parenchyma. *Am. Rev. Respir. Dis.* **124**, 402–406 (1981).
15. Naclerio, R. M. *et al.* Inflammatory mediators in late antigen-induced rhinitis. *N. Engl. J. Med.* **313**, 65–70 (1985).
16. Hirai, H. *et al.* Prostaglandin D_2 selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. *J. Exp. Med.* **193**, 255–261 (2001).
A landmark paper demonstrating that CRTH2 mediates the effect of PGD_2 on T_H2 lymphocytes, eosinophils and basophils and that PGD_2 is the predominant CRTH2 agonist produced by mast cells.
17. Gazi, L. *et al.* Δ^{12}-prostaglandin D_2 is a potent and selective CRTH2 receptor agonist and causes activation of human eosinophils and Th2 lymphocytes. *Prostaglandins Other Lipid Mediat.* **75**, 153–167 (2005).
18. Heinemann, A., Schuligoi, R., Sabroe, I., Hartnell, A. & Peskar, B. A. Δ^{12}-prostaglandin J_2, a plasma metabolite of prostaglandin D_2, causes eosinophil mobilization from the bone marrow and primes eosinophils for chemotaxis. *J. Immunol.* **170**, 4752–4758 (2003).
19. Monneret, G., Li, H., Vasilescu, J., Rokach, J. & Powell, W. S. 15-Deoxy-Δ^{12}, 14-prostaglandins D_2 and J_2 are potent activators of human eosinophils. *J. Immunol.* **168**, 3563–3569 (2002).
20. Sandig, H., Andrew, D., Barnes, A. A., Sabroe, I. & Pease, J. 9α, 11β-PGF_2 and its stereoisomer PGF2α are novel agonists of the chemoattractant receptor, CRTH2. *FEBS Lett.* **580**, 373–379 (2006).
21. Bohm, E. *et al.* 11-dehydro-thromboxane B_2, a stable thromboxane metabolite, is a full agonist of chemoattractant receptor-homologous molecule expressed on TH2 cells (CRTH2) in human eosinophils and basophils. *J. Biol. Chem.* **279**, 7663–7670 (2004).
22. Forman, B. M. *et al.* 15-deoxy-Δ^{12}, 14-prostaglandin J_2 is a ligand for the adipocyte determination factor PPARγ. *Cell* **83**, 803–812 (1995).
23. Rossi, A. *et al.* Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IκB kinase. *Nature* **403**, 103–108 (2000).
24. Ide, T., Egan, K., Bell-Parikh, L. C. & Fitzgerald, G. A. Activation of nuclear receptors by prostaglandins. *Thromb. Res.* **110**, 311–315 (2003).
25. Flower, R. J., Harvey, E. A. & Kingston, W. P. Inflammatory effects of prostaglandin D_2 in rat and human skin. *Br. J. Pharmacol.* **56**, 229–233 (1976).
26. Soter, N. A., Lewis, R. A., Corey, E. J. & Austen, K. F. Local effects of synthetic leukotrienes (LTC_4, LTD_4, LTE_4, and LTB_4) in human skin. *J. Invest. Dermatol.* **80**, 115–119 (1983).
27. Williams, T. J. & Peck, M. J. Role of prostaglandin-mediated vasodilatation in inflammation. *Nature* **270**, 530–532 (1977).
28. Giles, H., Leff, P., Bolofo, M. L., Kelly, M. G. & Robertson, A. D. The classification of prostaglandin DP-receptors in platelets and vasculature using BW A868C, a novel, selective and potent competitive antagonist. *Br. J. Pharmacol.* **96**, 291–300 (1989).
29. Woodward, D. F. *et al.* Studies on the ocular pharmacology of prostaglandin D_2. *Invest. Ophthalmol. Vis. Sci.* **31**, 138–146 (1990).
The first study to provide evidence that the effects of PGD_2 on eosinophil accumulation *in vivo* are not DP_1-mediated.
30. Marsden, K. A., Rao, P. S., Cavanagh, D. & Spaziani, E. The effect of prostaglandin D_2 (PGD_2) on circulating eosinophils. *Prostaglandins Leukot. Med.* **15**, 387–397 (1984).
31. Emery, D. L., Djokic, T. D., Graf, P. D. & Nadel, J. A. Prostaglandin D_2 causes accumulation of eosinophils in the lumen of the dog trachea. *J. Appl. Physiol* **67**, 959–962 (1989).
32. Fujitani, Y. *et al.* Pronounced eosinophilic lung inflammation and Th2 cytokine release in human lipocalin-type prostaglandin D synthase transgenic mice. *J. Immunol.* **168**, 443–449 (2002).
This study demonstrates that the overproduction of PGD_2 enhances allergic airway inflammation.
33. Raible, D. G., Schulman, E. S., DiMuzio, J., Cardillo, R. & Post, T. J. Mast cell mediators prostaglandin-D_2 and histamine activate human eosinophils. *J. Immunol.* **148**, 3536–3542 (1992).
34. Monneret, G., Gravel, S., Diamond, M., Rokach, J. & Powell, W. S. Prostaglandin D_2 is a potent chemoattractant for human eosinophils that acts via a novel DP receptor. *Blood* **98**, 1942–1948 (2001).
The first definitive study demonstrating that the effects of PGD_2 on eosinophil activation are mediated by a novel receptor.
35. Nagata, K. *et al.* Selective expression of a novel surface molecule by human Th2 cells *in vivo*. *J. Immunol.* **162**, 1278–1286 (1999).
36. Nagata, K. *et al.* CRTH2, an orphan receptor of T-helper-2-cells, is expressed on basophils and eosinophils and responds to mast cell-derived factor(s). *FEBS Lett.* **459**, 195–199 (1999).
37. Coleman, R. A. & Sheldrick, R. L. L. Prostanoid-induced contraction of human bronchial smooth muscle is mediated by TP-receptors. *Br. J. Pharmacol.* **96**, 688–692 (1989).
38. Liston, T. E. & Roberts, L. J. Transformation of prostaglandin D_2 to 9α, 11 β-(15S)-trihydroxyprosta-(5Z, 13E)-dien-1-oic acid (9α, 11β-prostaglandin F_2): a unique biologically active prostaglandin produced enzymatically *in vivo* in humans. *Proc. Natl Acad. Sci. USA* **82**, 6030–6034 (1985).
39. Beasley, C. R. *et al.* 9α, 11β-prostaglandin F_2, a novel metabolite of prostaglandin D_2 is a potent contractile agonist of human and guinea pig airways. *J. Clin. Invest.* **79**, 978–983 (1987).
40. Matsuoka, T. *et al.* Prostaglandin D_2 as a mediator of allergic asthma. *Science* **287**, 2013–2017 (2000).
This study examines the phenotype of the DP_1-knockout mouse and demonstrates that DP_1-receptor activation contributes to T_H2-cytokine production, eosinophil infiltration, mucus production and airway hyperresponsiveness in response to allergic challenge.
41. Nantel, F. *et al.* Expression of prostaglandin D synthase and the prostaglandin D_2 receptors DP and CRTH2 in human nasal mucosa. *Prostaglandins Other Lipid Mediat.* **73**, 87–101 (2004).

42. Heavey, D. J. *et al*. Effects of intravenous infusions of prostaglandin D_2 in man. *Prostaglandins* **28**, 755–767 (1984).
43. Doyle, W. J., Boehm, S. & Skoner, D. P. Physiologic responses to intranasal dose–response challenges with histamine, methacholine, bradykinin, and prostaglandin in adult volunteers with and without nasal allergy. *J. Allergy Clin. Immunol.* **86**, 924–935 (1990).
44. Johnston, S. L., Smith, S., Harrison, J., Ritter, W. & Howarth, P. H. The effect of BAY u 3405, a thromboxane receptor antagonist, on prostaglandin D_2-induced nasal blockage. *J. Allergy Clin. Immunol.* **91**, 903–909 (1993).
45. Arimura, A. *et al*. Prevention of allergic inflammation by a novel prostaglandin receptor antagonist, S-5751. *J. Pharmacol. Exp. Ther.* **298**, 411–419 (2001).
46. Cheng, K. *et al*. Antagonism of the prostaglandin D_2 receptor 1 suppresses nicotinic acid-induced vasodilation in mice and humans. *Proc. Natl Acad. Sci. USA* **103**, 6682–6687 (2006).
47. Hammad, H. *et al*. Prostaglandin D_2 inhibits airway dendritic cell migration and function in steady state conditions by selective activation of the D prostanoid receptor 1. *J. Immunol.* **171**, 3936–3940 (2003).
48. Faveeuw, C. *et al*. Prostaglandin D_2 inhibits the production of interleukin-12 in murine dendritic cells through multiple signaling pathways. *Eur. J. Immunol.* **33**, 889–898 (2003).
49. Gosset, P. *et al*. Prostaglandin D_2 affects the differentiation and functions of human dendritic cells: impact on the T cell response. *Eur. J. Immunol.* **35**, 1491–1500 (2005).
50. Tanaka, K., Hirai, H., Takano, S., Nakamura, M. & Nagata, K. Effects of prostaglandin D_2 on helper T cell functions. *Biochem. Biophys. Res. Commun.* **316**, 1009–1014 (2004).
51. Xue, L. *et al*. Prostaglandin D_2 causes preferential induction of proinflammatory Th2 cytokine production through an action on chemoattractant receptor-like molecule expressed on Th2 cells. *J. Immunol.* **175**, 6531–6536 (2005).
52. Gilroy, D. W. *et al*. Inducible cyclooxygenase may have anti-inflammatory properties. *Nature Med.* **5**, 698–701 (1999).
53. Ajuebor, M. N., Singh, A. & Wallace, J. L. Cyclooxygenase-2-derived prostaglandin $D_{(2)}$ is an early anti-inflammatory signal in experimental colitis. *Am. J. Physiol. Gastrointest. Liver Physiol.* **279**, G238–G244 (2000).
54. Trivedi, S. G. *et al*. Essential role for hematopoietic prostaglandin D_2 synthase in the control of delayed type hypersensitivity. *Proc. Natl Acad. Sci. USA* **103**, 5179–5184 (2006).
55. Sugimoto, H. *et al*. An orally bioavailable small molecule antagonist of CRTH2, ramatroban (BAY u3405), inhibits prostaglandin D_2-induced eosinophil migration *in vitro*. *J. Pharmacol. Exp. Ther.* **305**, 347–352 (2003).
The first demonstration that ramatroban is an effective CRTH2 antagonist that inhibits PGD_2-mediated eosinophil migration.
56. McKenniff, M. G., Norman, P., Cuthbert, N. J. & Gardiner, P. J. BAY u3405, a potent and selective thromboxane A_2 receptor antagonist on airway smooth muscle *in vitro*. *Br. J. Pharmacol.* **104**, 585–590 (1991).
57. Shichijo, M. *et al*. Chemoattractant receptor-homologous molecule expressed on Th2 cells activation *in vivo* increases blood leukocyte counts and its blockade abrogates 13,14-dihydro-15-keto-prostaglandin D_2-induced eosinophilia in rats. *J. Pharmacol. Exp. Ther.* **307**, 518–525 (2003).
58. Narita, S., Asakura, K. & Kataura, A. Effects of thromboxane A_2 receptor antagonist (Bay u 3405) on nasal symptoms after antigen challenge in sensitized guinea pigs. *Int. Arch. Allergy Immunol.* **109**, 161–166 (1996).
59. Nagai, H. *et al*. The effect of a thromboxane A_2 receptor antagonist BAY-u-3405 on experimental allergic reactions. *Prostaglandins* **50**, 75–87 (1995).
60. Takeshita, K. *et al*. CRTH2 is a prominent effector in contact hypersensitivity-induced neutrophil inflammation. *Int. Immunol.* **16**, 947–959 (2004).
61. Gyles S. L. *et al*. A dominant role for chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) in mediating chemotaxis of $CRTH2^+CD4^+$ Th2 lymphocytes in response to mast cell supernatants. *Immunology* **119**, 362–368 (2006).
This study provides evidence that mast-cell-dependent activation of mast cells is mediated by PGD_2 acting on CRTH2.
62. Spik, I. *et al*. Activation of the prostaglandin D_2 receptor DP2/CRTH2 increases allergic inflammation in mouse. *J. Immunol.* **174**, 3703–3708 (2005).
63. Satoh, T. *et al*. Prostaglandin D_2 plays an essential role in chronic allergic inflammation of the skin via CRTH2 receptor. *J. Immunol.* **177**, 2621–2629 (2006).
A study demonstrating that allergic responses are diminished in CRTH2-knockout mice.
64. Chevalier, E. *et al*. Chemoattractant receptor-homologous molecule expressed on Th2 cells plays a restricting role on IL-5 production and eosinophil recruitment. *J. Immunol.* **175**, 2056–2060 (2005).
65. Abe, H. *et al*. Molecular cloning, chromosome mapping and characterization of the mouse *CRTH2* gene, a putative member of the leukocyte chemoattractant receptor family. *Gene* **227**, 71–77 (1999).
66. Williams, C. M. & Galli, S. J. Mast cells can amplify airway reactivity and features of chronic inflammation in an asthma model in mice. *J. Exp. Med.* **192**, 455–462 (2000).
67. Wang, Y. H. *et al*. Maintenance and polarization of human Th2 central memory T cells by thymic stromal lymphopoietin-activated dendritic cells. *Immunity* **24**, 827–838 (2006).
68. Ying, S. *et al*. Thymic stromal lymphopoietin expression is increased in asthmatic airways and correlates with expression of Th2-attracting chemokines and disease severity. *J. Immunol.* **174**, 8183–8190 (2005).
69. Angeli, V. *et al*. Activation of the D prostanoid receptor 1 regulates immune and skin allergic responses. *J. Immunol.* **172**, 3822–3829 (2004).
70. Hardy, C. C., Robinson, C., Tattersfield, A. E. & Holgate, S. T. The bronchoconstrictor effect of inhaled prostaglandin D_2 in normal and asthmatic men. *N. Engl. J. Med.* **311**, 209–213 (1984).
71. Magnussen, H., Boerger, S., Templin, K. & Baunack, A. R. Effects of a thromboxane-receptor antagonist, BAY u 3405, on prostaglandin D_2- and exercise-induced bronchoconstriction. *J. Allergy Clin. Immunol.* **89**, 1119–1126 (1992).
72. Johnston, S. L. *et al*. The effects of an oral thromboxane TP receptor antagonist BAY u 3405, on prostaglandin D_2- and histamine-induced bronchoconstriction in asthma, and relationship to plasma drug concentrations. *Br. J. Clin. Pharmacol.* **34**, 402–408 (1992).
73. Beasley, R. C. *et al*. Effect of a thromboxane receptor antagonist on PGD_2- and allergen-induced bronchoconstriction. *J. Appl. Physiol* **66**, 1685–1693 (1989).
74. Rajakulasingam, K. *et al*. Effect of thromboxane A_2-receptor antagonist on bradykinin-induced bronchoconstriction in asthma. *J. Appl. Physiol* **80**, 1973–1977 (1996).
75. al Jarad, N., Hui, K. P. & Barnes, N. Effects of a thromboxane receptor antagonist on prostaglandin D_2 and histamine induced bronchoconstriction in man. *Br. J. Clin. Pharmacol.* **37**, 97–100 (1994).
76. Finnerty, J. P., Twentyman, O. P., Harris, A., Palmer, J. B. & Holgate, S. T. Effect of GR32191, a potent thromboxane receptor antagonist, on exercise induced bronchoconstriction in asthma. *Thorax* **46**, 190–192 (1991).
77. Coleman, R. A. GR32191 and the role of thromboxane A_2 in asthma--preclinical and clinical findings. *Agents Actions Suppl.* **34**, 211–220 (1991).
This provides evidence that the TP-mediated bronchoconstrictor actions of PGD_2 and thromboxane A_2 do not make a significant contribultion to the manifestation of clincal asthma.
78. Terada, N. *et al*. The effect of ramatroban (BAY u 3405), a thromboxane A_2 receptor antagonist, on nasal cavity volume and minimum cross-sectional area and nasal mucosal hemodynamics after nasal mucosal allergen challenge in patients with perennial allergic rhinitis. *Acta Otolaryngol. Suppl.* **537**, 32–37 (1998).
79. Aizawa, H., Shigyo, M., Nogami, H., Hirose, T. & Hara, N. BAY u3405, a thromboxane A_2 antagonist, reduces bronchial hyperresponsiveness in asthmatics. *Chest* **109**, 338–342 (1996).
80. Kirby, J. G., Hargreave, F. E., Cockcroft, D. W. & O'Byrne, P. M. Effect of indomethacin on allergen-induced asthmatic responses. *J. Appl. Physiol* **66**, 578–583 (1989).
81. Raud, J., Dahlen, S. E., Sydbom, A., Lindbom, L. & Hedqvist, P. Enhancement of acute allergic inflammation by indomethacin is reversed by prostaglandin E_2: apparent correlation with *in vivo* modulation of mediator release. *Proc. Natl Acad. Sci. USA* **85**, 2315–2319 (1988).
82. Pavord, I. D., Wong, C. S., Williams, J. & Tattersfield, A. E. Effect of inhaled prostaglandin E_2 on allergen-induced asthma. *Am. Rev. Respir. Dis.* **148**, 87–90 (1993).
83. Gyllfors, P. *et al*. Biochemical and clinical evidence that aspirin-intolerant asthmatic subjects tolerate the cyclooxygenase 2-selective analgetic drug celecoxib. *J. Allergy Clin. Immunol.* **111**, 1116–1121 (2003).
84. Dahlen, S. E. *et al*. Improvement of aspirin-intolerant asthma by montelukast, a leukotriene antagonist: a randomized, double-blind, placebo-controlled trial. *Am. J. Respir. Crit. Care Med.* **165**, 9–14 (2002).
85. Sousa, A. *et al*. Enhanced expression of cyclo-oxygenase isoenzyme 2 (COX-2) in asthmatic airways and its cellular distribution in aspirin-sensitive asthma. *Thorax* **52**, 940–945 (1997).
86. Grosser, T., Fries, S. & Fitzgerald, G. A. Biological basis for the cardiovascular consequences of COX-2 inhibition: therapeutic challenges and opportunities. *J. Clin. Invest.* **116**, 4–15 (2006).
87. Hamelmann, E. & Gelfand, E. W. IL-5-induced airway eosinophilia — the key to asthma? *Immunol. Rev.* **179**, 182–191 (2001).
88. Leckie, M. J. Anti-interleukin-5 monoclonal antibodies: preclinical and clinical evidence in asthma models. *Am. J. Respir. Med.* **2**, 245–259 (2003).
89. Mauser, P. J. *et al*. Effects of an antibody to interleukin-5 in a monkey model of asthma. *Am. J. Respir. Crit. Care Med.* **152**, 467–472 (1995).
90. Leckie, M. J. *et al*. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* **356**, 2144–2148 (2000).
91. Kips, J. C. *et al*. Effect of SCH55700, a humanized anti-human interleukin-5 antibody, in severe persistent asthma: a pilot study. *Am. J. Respir. Crit. Care Med.* **167**, 1655–1659 (2003).
92. Huang, J. L. *et al*. Sequence variants of the gene encoding chemoattractant receptor expressed on Th2 cells (*CRTH2*) are associated with asthma and differentially influence mRNA stability. *Hum. Mol. Genet.* **13**, 2691–2697 (2004).
93. Oguma, T. *et al*. Role of prostanoid DP receptor variants in susceptibility to asthma. *N. Engl. J. Med.* **351**, 1752–1763 (2004).
94. Kostensis, E. & Ulven, T. Emerging roles of DP and CRTH2 in allergic inflammation. *Trends Mol. Med.* **12**, 148–158 (2006).
95. Mitsumori, S. Recent progress in work on PGD_2 antagonists for drugs targeting allergic diseases. *Curr. Pharm. Design* **10**, 3533–3538 (2004).
96. Ly, T. W. & Bacon, K. B. Small-molecule CRTH2 antagonists for the treatment of allergic inflammation: an overview. *Expert Opin. Investig. Drugs* **14**, 769–773 (2005).
97. Sawyer, N. *et al*. Molecular pharmacology of the human prostaglandin D_2 receptor, CRTH2. *Br. J. Pharmacol.* **137**, 1163–1172 (2002).
A comprehensive study of the ligand-binding profile of CRTH2 including the activity of a number of NSAIDs.
98. Hirai, H. *et al*. Agonistic effect of indomethacin on a prostaglandin D_2 receptor, CRTH2. *J. Immunol.* **168**, 981–985 (2002).
A demonstration that indomethacin has CRTH2 agonist activity. This was the first indication that CRTH2 could be targeted by drug-like molecules.
99. Hata, A, N., Zent, R., Breyer, M. D. & Breyer, R. M. Expression and molecular pharmacology of the mouse CRTH2 receptor. *J. Pharmacol. Exp. Ther.* **306**, 463–470 (2003).
100. Hata, A, N., Lybrand, T. P., Marnett, L. J. & Breyer, R. M. Structural determinants of arylacetic acid NSAIDs necessary for binding and activation of the PGD_2 receptor CRTH2. *Mol. Pharmacol.* **67**, 640–647 (2005).
101. Frimurer, T. M. *et al*. A physicogenetic method to assign ligand-binding relationships between 7TM receptors. *Bioorg. Med. Chem. Lett.* **15**, 3707–3712 (2005).
102. Hata, A. N., Lybrand, T. P. & Breyer, R. M. Identification of determinants of ligand binding affinity and selectivity in the prostaglandin D_2 receptor CRTH2. *J. Biol. Chem.* **280**, 32442–32451 (2005).

103. Bauer, P. H. *et al*. Methods for the identification of compounds useful for the treatment of disease states mediated by prostaglandin D_2. European Patent EP1170594 (2002).
104. Baxter, A., Steele, J. & Teague, S. Use of indole-3-acetic acids in the treatment of asthma, COPD and other diseases. World Patent WO03066046 (2003).
105. Baxter, A., Steele, J. & Teague, S. Use of indole-3-acetic acids in the treatment of asthma, COPD and other diseases. World Patent WO03066047 (2003).
106. Birkinshaw, T., Bonnert, R., Cook, A., Rasul, R., Sanganee, H. & Teague, S. Novel substituted indoles. World Patent WO03101981 (2003).
107. Bonnert, R. *et al*. Novel substituted indoles. World Patent WO03101961 (2003).
108. Bonnert, R., Dickinson, M., Rasul, R., Sanganee, H. & Teague, S. Indole-3-sulphur derivatives. World Patent WO2004007451 (2004).
109. Bonnert, R. V., Cook, A. R., Luker, T. J., Mohammed, R. T. & Thom, S. Substituted indole derivatives for pharmaceutical composition for treating respiratory diseases. World Patent WO2005019171 (2005).
110. Bonnert, R. & Rasul, R. Novel substituted 3-sulfur indoles. World Patent WO2004106302 (2004).
111. Middlemiss, D., Ashton, M. R., Boyd, E. A. & Brookfield, F. A. Use of CRTH2 antagonist compounds in therapy. World Patent WO2005044260 (2005).
112. Middlemiss, D., Ashton, M. R., Boyd, E. A., Brookfield, F. A. & Armer, R. E. Compounds having CRTH2 antagonist activity. World Patent WO2005040114 (2005).
113. Middlemiss, D., Ashton, M. R., Boyd, E. A. & Brookfield, F. A. Substituted indol-3-yl acetic acid derivatives. UK Patent GB2407318 (2005).
114. Middlemiss, D., Ashton, M. R., Boyd, E. A., Brookfield, F. A. & Armer, R. E. Compounds with CRTH2 antagonist activity. World Patent WO2005040112 (2005).
115. Armer, R. E. *et al*. Indole-3-acetic acid antagonists of the PGD_2 receptor CRTH2. *J. Med. Chem.* **48**, 6174–6177 (2005).
116. Arimura, A., Kishino, J. & Tanimoto, N. PGD_2 receptor antagonist. World Patent WO03097042 (2003).
117. Fretz, H., Fecher, A., Hilpert, K. & Riederer, M. Tetrahydropyridoindole derivatives. World Patent WO2005095397 (2005).
118. Ulven, T. & Kostensis, E. Minor structural modifications convert the dual TP/CRTH2 antagonist ramatroban into a highly selective and potent CRTH2 antagonist. *J. Med. Chem.*, **48**, 897–900 (2005).
119. Mathiesen, J. M. *et al*. On the mechanism of interaction of potent surmountable and insurmountable antagonists with the prostaglandin D_2 receptor CRTH2. *Mol. Pharmacol.* **69**, 1441–1453 (2006).
120. Robarge, M. J. *et al*. Isosteric ramatroban analogs: selective and potent CRTH-2 antagonists *Biorg. Med. Chem. Letts.*, **15**, 1749–1753 (2005).
121. Pairaudeau, G., Rasul, R. & Thom, S. Novel compounds. World Patent WO2004089884 (2004).
122. Bonnert, R. *et al*. Novel compounds. World Patent WO2004089885 (2004).
123. Luker, T. J., Birkinshaw, T. N. & Mohammed, R. T. Biphenyloxyacetic acid derivatives for the treatment of respiratory disaese. World Patent WO2006021759 (2006).
124. Bonnert, R. V., Luker, T. J., Pairaudeau, G. & Thom, S. Novel compounds. World Patent WO2006037982 (2006).
125. Bonnert, R. V., Patel, A. & Thom, S. Phenoxiacetic acid derivatives. World Patent WO2005018529 (2005).
126. Bonnert, R. V., Thom, S., Patel, A. & Luker, T. J. Substituted acids for the treatment of respiratory diseases. World Patent WO2006005909 (2006).
127. Ghosh, S., Elder, A. M., Carson, K. G., Sprott, K. & Harrison, S. PGD_2 receptor antagonists for the treatment of inflammatory diseases. World Patent WO2004032848 (2004).
128. Ghosh, S. *et al*. PGD_2 receptor antagonists for the treatment of inflammatory diseases. World Patent WO2005100321 (2005).
129. Awad, M. *et al*. Tetrahydroquinoline derivatives as CRTH2 antagonists. World Patent WO2004035543 (2004).
130. Kuhn, C., Feru, F., Bazin, M., Awad, M. & Goldstein, S. W. Quinoline derivatives as CRTH2 antagonists. European Patent EP1435356 (2004).
131. Corradini, L., Field, M. J., Kinloch, R. A. & Williams-Jones, B. I. Method of treating neuropathic pain using a CRTH2 receptor antagonist. World Patent WO2005102338 (2005).
132. Hideki, M. *et al*. Inhibitory Effect of the 4-aminotetrahydroquinoline derivatives, selective chemoattractant receptor-homologous molecule expressed on T helper 2 cell antagonists, on eosinophil migration induced by prostaglandin D_2. *J. Pharmacol. Exp. Ther.* **314**, 244–251 (2005).
133. Hata, A. N. & Breyer, R. M. Pharmacology and signaling of prostaglandin receptors: multiple roles in inflammation and immune modulation. *Pharmacol. Ther.* **103**, 147–166 (2004).
134. Whittle, B. J., Hamid, S., Lidbury, P. & Rosam, A. C. Specificity between the anti-aggregatory actions of prostacyclin, prostaglandin E_1 and D_2 on platelets. *Adv. Exp. Med. Biol.* **192**, 109–125 (1985).

Acknowledgements
The authors would like to thank M. Hunter and M. Payton for their comments on this manuscript and for their encouragement in conducting research referred to in this Review.

Competing interests statement
The authors declare competing financial interests: see web version for details.

DATABASES
The following terms in this article are linked online to:
Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
GPR44 | *PTGDR*

FURTHER INFORMATION
National Heart and Lung Institute:
http://www1.imperial.ac.uk/medicine/about/divisions/nhli
Oxagen Limited: http://www.oxagen.co.uk
Access to this links box is available online.