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REVIEW

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The roles of the prostaglandin D_2 receptors DP_1 and CRTH2 in promoting allergic responses

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Prostaglandin D_2 (PGD_2) is produced by mast cells, Th2 lymphocytes and dendritic cells and has been detected in high concentrations at sites of allergic inflammation. PGD_2 exerts its inflammatory effects through high affinity interactions with the G protein coupled receptors DP_1 and chemoattractant-homologous receptor expressed on Th2 cells (CRTH2, also known as DP_2). DP_1 and CRTH2 act in concert to promote a number of biological effects associated with the development and maintenance of the allergic response. During the process of allergen sensitization, DP_1 activation may enhance polarization of Th0 cells to Th2 cells by inhibiting production of interleukin 12 by dendritic cells. Upon exposure to allergen in sensitized individuals, activation of DP_1 may contribute to the long lasting blood flow changes in the target organ. CRTH2 is expressed by Th2 lymphocytes, eosinophils and basophils and may mediate the recruitment of these cell types during the late phase allergic response. The role played by CRTH2 in promoting the production of Th2 cytokines and IgE make antagonism of this receptor a particularly attractive approach to the treatment of chronic allergic disease.
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Keywords: prostaglandin D_2; DP_1; CRTH2; mast cells; Th2 lymphocytes; eosinophils; PI3K; allergic rhinitis; asthma

Abbreviations: CRTH2, chemoattractant-homologous receptor expressed on Th2 cells; CysLT, cysteinyl leukotriene receptor; DP_1, D prostanoid receptor 1; IgE, immunoglobulin E; PGD_2, prostaglandin D_2; PI3K, phosphatidylinositol-3-kinase; Th2 cell, T helper type 2 cell; TSLP, thymic stromal lymphopoietin

Introduction

There have been significant improvements in the treatment of allergic diseases over the past 20–30 years. Non-sedating antihistamines have proven to be of great benefit to patients with mild seasonal allergic rhinitis and topical corticosteroids (intranasal for allergic rhinitis and inhaled for asthma) are effective in reducing inflammation and disease severity in the majority of patients with a dramatically improved safety profile compared to systemic steroids. Despite these advances, the incidence of allergy is increasing dramatically and important features of both allergic rhinitis and asthma are resistant to current therapy. In more severe persistent allergic rhinitis antihistamines are less effective, particularly on nasal congestion (Howarth, 1997) and around 5% of asthmatics remain poorly controlled by inhaled steroids (Barnes, 2004). There is therefore a clear need for more effective treatments for allergic disease, particularly if they can be administered safely by the oral route.

The challenge, therefore, is to identify therapeutic approaches that target processes fundamentally important in driving allergic inflammation but with sufficient specificity to have an acceptable side effect profile. Cysteinyl leukotriene receptor ($CysLT_1$) antagonists, most notably montelukast, are the most recently introduced drugs to treat asthma and these drugs specifically block the action of cysteinyl leukotrienes (predominantly LTD_4) on the $CysLT_1$ receptor expressed by bronchial smooth muscle and leukocytes without inhibiting the effects of leukotrienes mediated by $CysLT_2$. Since $CysLT_1$ has a limited expression profile and leukotrienes active on $CysLT_1$ are produced predominantly by mast cells and eosinophils, this approach is highly specific and consequently, $CysLT_1$ antagonists have proven to have an acceptable side-effect profile. However, this specificity of action comes at a cost—while $CysLT_1$ antagonists are effective in inhibiting the bronchoconstrictor element of asthma their anti-inflammatory activity and consequently, clinical efficacy, is modest compared to inhaled corticosteroids (Busse *et al*., 2001). The search therefore continues to identify specific approaches that have improved efficacy and are safe enough for systemic use.

Recent evidence suggests that selective blockade of the action of prostaglandin D_2 (PGD_2) may provide such an opportunity. Production of PGD_2, like that of cysteinyl leukotrienes, is restricted to cells involved in the allergic response (at least in peripheral tissues) and appears to be

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instrumental in orchestrating interactions between mast cells, Th2 lymphocytes, eosinophils and dendritic cells. The inflammatory effects of PGD_2 are mediated by high-affinity interaction with D prostanoid receptor 1 (DP_1) and chemoattractant receptor-homologous molecule expressed on T helper type 2 cell (Th2) cells (CRTH2). The purpose of this review is to summarize the emerging evidence that DP_1 and CRTH2 contribute to allergic inflammation and to highlight the potential therapeutic utility of antagonizing these receptors.

Production of PGD_2 in allergic inflammation

Prostaglandin D_2 is the major prostanoid produced by mast cells (Lewis *et al*., 1982; Peters *et al*., 1982) and PGD_2 and its metabolites have been proposed as selective markers of mast cell activation *in vivo* (O'Sullivan *et al*., 1996; Bochenek *et al*., 2003; Dahlen and Kumlin, 2004). Cross linking of cell surface immunoglobulin E (IgE) by allergen leads to the rapid production of PGD_2 by mast cells. PGD_2 is derived from liberated arachidonic acid in a two-step process where either COX1 or COX2 catalyses the production of PGH_2, which is then metabolized to PGD_2 by haematopoietic PGD_2 synthase. Using mouse mast cells *in vitro,* it has been shown that the early phase of PGD_2 production is COX1-dependent whereas COX2 is responsible for the more prolonged production of PGD_2 (Reddy *et al*., 1999). *In vivo*, it appears that PGD_2 is produced more rapidly (within minutes) which may reflect the presence of COX2 in these chronically inflamed tissues as has been shown in the airways of aspirin-intolerant asthmatics (Sousa *et al*., 1997). Allergen challenge has been shown to lead to rapid production of PGD_2 in the airways of asthmatics (Murray *et al*., 1986), the nasal mucosa of allergic rhinitis (Naclerio *et al*., 1983) and in the skin of patients with atopic dermatitis (Charlesworth *et al*., 1991).

PGD_2 is also produced in biological meaningful quantities by Th2 cells although the levels are around 1/10th of those produced by mast cells on a per cell basis and is more delayed. In response to anti-CD3/anti-CD28, PGD_2 is produced by human Th2 cells as a consequence of induction of COX2 and haematopoietic PGD_2 synthase (Tanaka *et al*., 2000). Whether dendritic cells produce PGD_2 is more controversial but interestingly, dendritic cells incubated with thymic stromal lymphopoietin (TSLP) create a local environment where Th0 cells polarize to the Th2 phenotype and this process is associated with the upregulation of PGD_2 synthase in Th2 cells (Wang *et al*., 2006). It is likely therefore that Th2 cells may be an important source of biologically active PGD_2 in a chronic allergic setting or in situations where the allergic response occurs independently of mast cell activation.

Pharmacological properties of DP_1 and CRTH2

D prostanoid receptor 1 was the first receptor that was identified for PGD_2. DP_1 is a member of the prostanoid receptor family that includes EP_{1-4}, FP, IP and TP. It is coupled to Gαs and its activation leads to elevation of intracellular levels of cAMP. DP_1 is expressed by vascular smooth muscle and platelets and has well-characterized effects in mediating vasodilatation (Giles *et al*., 1989; Walch *et al*., 1999) and inhibition of platelet aggregation (Whittle *et al*., 1983). Much of our knowledge of the role of DP_1 in biological responses comes from the use of the selective DP_1 agonist BW245C and the selective DP_1 antagonist BWA868C. Dendritic cells express DP_1 and activation of DP_1 may play an important role in modulating the function of these cells, particularly in controlling the production of cytokines such as interleukin 12.

Chemoattractant receptor-homologous molecule expressed on Th2 cells was originally identified as an orphan known as GPR44. Subsequently, it was found that CRTH2 is expressed preferentially by Th2 lymphocytes, eosinophils and basophils (Nagata *et al*., 1999a, b) and it mediated chemotactic responses of Th2 lymphocytes, eosinophils and basophils to PGD_2 (Hirai *et al*., 2001). Although CRTH2 and DP_1 bind the same ligand there is very little homology between the two receptors, CRTH2 being most closely related to other chemotactic receptors such as the leukotriene B_4 receptors BLT1 and BLT2, the complement C5a receptor and the formyl peptide receptors. CRTH2 is coupled to Gαi and its activation leads to elevation of intracellular calcium and reduction in cAMP (Sawyer *et al*., 2002). Downstream activation of CRTH2 results in phosphatidylinositol-3-kinase (PI3K)-dependent phosphorylation of AKT, phosphorylation of GSK-3β and nuclear translocation of NFAT (Xue *et al*., 2007). Phosphatidylinositol-3-kinase-dependent phosphorylation of GSK-3β leads to inactivation of GSK-3β which, in turn, leads to reduction in the phosphorylation of NFAT (Xue *et al*., 2007).

It is of interest that many of the metabolites of PGD_2 including 13,14-dihydro-15-keto-PGD_2 (DK-PGD_2) (Hirai *et al*., 2001), $\Delta^{12}PGD_2$ (Gazi *et al*., 2005) $\Delta^{12}PGJ_2$ (Heinemann *et al*., 2003), 15-deoxy-$\Delta^{12,14}PGD_2$ and deoxy-$\Delta^{12,14}PGJ_2$ (Monneret *et al*., 2002) retain activity on CRTH2 but are less active on DP_1. $\Delta^{12}PGD_2$ and $\Delta^{12}PGJ_2$ are both major metabolites of PGD_2 formed rapidly in plasma (Schuligoi *et al*., 2007) and so the effects of PGD_2 at sites of allergic inflammation is likely to be heavily influenced by metabolism and it is tempting to speculate that in such conditions the effects of CRTH2 will dominate over those of DP_1. It has been proposed that $\Delta^{12}PGJ_2$ may act as a circulating hormone and cause blood eosinophilia by stimulating eosinophil release from the bone marrow (Heinemann *et al*., 2003). Some arachidonic acid metabolites formed independently of PGD_2 such as 11-dehydro-thromboxane B_2 and 9α11β-PGF_2 also have CRTH2 agonist activity (Bohm *et al*., 2004; Sandig *et al*., 2006). The isoprostane 15R-PGD_2 is also a potent CRTH2 agonist (Cossette *et al*., 2007). This is of particular interest since if 15R-PGD_2 is formed *in vivo* it would be the first evidence of an endogenous CRTH2 agonist formed independently of the cyclooxygenase pathway of arachidonic acid metabolism.

Chemoattractant receptor-homologous molecule expressed on Th2 cells is a much more promiscuous receptor than DP_1 and a diverse range of natural and synthetic ligands have been found to interact with this receptor. The NSAID indomethacin is a partial agonist (Hirai *et al*., 2002; Sawyer

et al., 2002; Stubbs *et al*., 2002) and the indole acetic acid derivative L888,607 is a CRTH2 agonist devoid of cyclooxygenase activity (Gervais *et al*., 2005). This compound has been proposed as a useful tool to define the role of CRTH2 *in vitro* and *in vivo* (Gervais *et al*., 2005). Indole-3-acetic acid derivatives have been identified that are potent and selective antagonists of CRTH2 (Armer *et al*., 2005). Ramatroban, originally identified as a TP antagonist (McKenniff *et al*., 1991) has been demonstrated to be a competitive CRTH2 antagonist (Sugimoto *et al*., 2003). This observation has led to the identification of ramatroban analogues, which are potent CRTH2 antagonists devoid of TP activity (Ulven and Kostenis, 2005). The only non-acid CRTH2 antagonists so far described are 4-aminotetrahydroquinoline derivatives as exemplified by K117 and K604 (Mimura *et al*., 2005).

The key biological and pharmacological properties of DP_1 and CRTH2 are summarized in Table 1. The molecular pharmacology and signal transduction pathways utilized by DP_1 and CRTH2 is reviewed in more detail by Kostenis and Ulven (2006).

Table 1 Comparison of the key pharmacological and biological properties of DP_1 and CRTH2

CRTH2	*DP_1*
Endogenous ligands	
PGD_2	PGD_2
DK-PGD_2	
15R-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	
Signalling mechanism	
Gαi	Gαs
↓ cAMP	↑ cAMP
↑ Ca^{2+}	
P13K-dependent chemotaxis	
Calcineurin-dependent cytokine production	
Synthetic agonists	
Indomethacin	BW245c
L-888,607	
Synthetic antagonists	
Ramatroban and analogues	BWA868c
Indole-3-acetic acids	MK-0524
4-aminotetrahydroquinoline derivatives K117 and K604	S-5751
Location	
Th2 lymphocytes	Bronchial smooth muscle
Eosinophils	Vascular smooth muscle
Basophils	Dendritic cells
CNS	Platelets
	CNS
Biological effects	
Chemotaxis and activation of Th2 lymphocytes, eosinophils and basophils	Bronchodilatation
CNS effects unknown	Vasodilatation
	Suppression of cytokine production by dendritic cells leading to polarization of Th2 cells
	Inhibition of platelet aggregation
	Likely involvement in CNS effects e.g. sleep and pain cognition

Abbreviations: CRTH2, chemoattractant receptor-homologous molecule expressed on Th2 cells; PGD_2, prostaglandin D_2; Th2 cell, T helper type 2 cell.

Role of DP_1 and CRTH2 in allergic inflammation

Prostaglandin D_2 can mimic a number of the key features of allergic responses when it is applied to animals or human volunteers. These effects include blood flow changes, recruitment of eosinophils and Th2 lymphocytes and potentiation of Th2 cytokine production. All of these diverse effects can be explained by discrete actions on DP_1 or CRTH2. It has been proposed that PGD_2 and its metabolites through interactions with DP_1, CRTH2 and other intracellular pathways have opposing roles in regulating leukocyte function during inflammatory responses (Kostenis and Ulven, 2006; Sandig *et al*., 2007). There is clear evidence that PGD_2 that might promote resolution of neutrophilic Th1/Th17-mediated inflammatory responses (Gilroy *et al*., 1999; Ajuebor *et al*., 2000; Trivedi *et al*., 2006) but in the case of eosinophilic Th2-mediated allergic responses the predominant effect of CRTH2 activation appears to be pro-inflammatory. The effects of DP_1 activation are more complex but is likely that the 'anti-inflammatory' effect of DP_1-mediated suppression of leukocyte activation, particularly dendritic cells and Th1 cells, can increase allergic responses by promoting Th2 bias, at least during the sensitization phase of an allergic response.

Activation of DP_1 contributes to blood flow changes during allergic responses

It has been recognized for some time that PGD_2 has the potential to mediate the pathological blood flow changes observed in allergic diseases. In the case of allergic rhinitis, engorgement of the vasculature in the nose contributes to congestion, a troublesome symptom, which is largely resistant to the action of H_1 antagonists. The mechanisms of the vascular changes in the mucosa of patients allergic rhinitis is complex but is thought to involve the direct actions of mediators on both the vasculature and neuronal reflexes (Widdicombe, 1990). Blood flow changes contribute to the swelling of the nasal mucosa causing congestion and enhanced leakage of plasma protein, which contributes to nasal secretions. The importance of blood flow changes to the signs and symptoms of rhinitis is evident based on the effectiveness of vasoconstrictors such as pseudoephedrine to reduce 'stuffiness'.

In human volunteers intravenous administration of PGD_2 has been shown to produce nasal congestion associated with intense facial flushing but interestingly, no overt effects on systemic blood pressure or lung function (Heavey *et al*., 1984). Congestion has also been observed after insufflation of PGD_2 in human subjects and it is more effective in this respect than either histamine or bradykinin (Doyle *et al*.,

1990). The ability of PGD_2 to induce nasal air flow resistance is blocked by the vasoconstrictor oxymetazoline highlighting the importance of a vascular event in mediating this response (Howarth *et al*., 1991). Production of PGD_2 in response to allergen in pigs has been proposed to mediate a long-lasting component of airway vasodilatation resistant to antihistamines (Alving *et al*., 1991). This conclusion is based on the ability of PGD_2 to mimic the long-lasting airways vasodilatation induced by allergen and the inhibitory effect of the cyclooxygenase inhibitor diclofenac on allergen-induced blood flow changes. The vasoactive effect of PGD_2 appears to be more marked in the nose than the lower airways and so be more relevant to allergic rhinitis than asthma. The availability of selective DP_1 agonists and antagonists have shown that vasorelaxation of vascular smooth muscle in response to PGD_2 is DP_1-mediated (Giles *et al*., 1989; Walch *et al*., 1999) and the hypotensive effect of PGD_2 is inhibited by BW A868C (Hamid-Bloomfield and Whittle, 1989) which suggests that the vascular effects described above are likely to be mediated by DP_1. Indeed, the selective DP_1 agonist BW245C-induced headache, nasal stuffiness and facial flushing when infused into human volunteers (Al Sinawi *et al*., 1985). Most recently it has been shown that increased nasal airway resistance induced by intranasal instillation of PGD_2 in conscious sheep is completely inhibited by a selective DP_1 antagonist (Sturino *et al*., 2007).

While there are no reports on the effects of selective DP_1 antagonists on vascular engorgement or congestion in clinical allergy it is of interest that the DP_1 antagonist MK-0524 has been reported to reduce facial flushing in human volunteers administered niacin (Cheng *et al*., 2006). Niacin-induced flushing is mediated by the production of endogenous PGD_2 by cells in the skin, probably Langerhans' cells (Maciejewski-Lenoir *et al*., 2006) which then acts on DP_1 to mediate increased blood flow. The relationship between activation of DP_1 and nasal blockage has been explored in sensitized guinea pigs where it was found that the selective DP_1 antagonist S-5751 inhibited the early phase increase in nasal pressure in response to allergen while the H_1 antagonist terfenadine was without effect (Arimura *et al*., 2001).

It seems likely therefore that at sites of mast activation PGD_2 is produced and this mediator contributes to vascular changes leading, in the case of allergic rhinitis, to acute nasal congestion. The contribution of DP_1 to congestion associated with more chronic inflammatory changes is less certain, however, and it is possible that activation of CRTH2 may be important in that setting.

DP_1 suppresses dendritic cell function: possible relevance to polarization of Th2 cells

DP_1 is expressed by dendritic cells and BW245C is able to suppress the ability of these cells to produce cytokines including interleukin 12 (Faveeuw *et al*., 2003; Hammad *et al*., 2003; Gosset *et al*., 2005). It is now well recognized that dendritic cells, in addition to presenting antigen to reactive T cells, also play a central role in controlling the polarization of T cells to either the Th1 or Th2 phenotype (Lambrecht, 2005). Dendritic cells isolated from the human respiratory mucosa preferentially induce polarization to the Th2 phenotype while peripheral blood-derived dendritic cells promote a Th1 pattern of differentiation (Faith *et al*., 2005). The effect of respiratory mucosal dendritic cells to promote Th2 polarization is associated with low production of IL-12 while high production of this cytokine promotes Th1 differentiation. Dendritic cells from mucosal origins tend to cause an increase in Th2 bias and this effect can be influenced by the local production of soluble factors. Thymic stromal lymphopoietin produced by epithelial cells stimulates dendritic cells to produce an environment which favours polarization and maintenance of $CD4^+CRTH2^+$ central memory Th2 cells (Wang *et al*., 2006). This effect is associated with the induction of PGD_2 synthase and may be relevant to the pathogenesis of asthma since thymic stromal lymphopoietin levels in the asthmatic lung correlate with disease severity (Ying *et al*., 2005). Activated mast cells induce Th2-promoting dendritic cells (Kitawaki *et al*., 2006), an effect mimicked by PGD_2 acting through inhibition of interleukin 12 production (Theiner *et al*., 2006). It is likely then at sites of mast cell activation the production of PGD_2 contributes to the maintenance of Th2 dominance with associated production of IgE which in turn leads to immunological mast cell activation so creating an escalating cycle of increased severity and chronicity. Histamine produced by mast cells is believed to act in a similar manner (Caron *et al*., 2001; Mazzoni *et al*., 2001).

The ability of DP_1 to control Th2 polarization is a plausible explanation for the reduction in allergic responses afforded by DP_1 deficiency (Matsuoka *et al*., 2000). Compared to wild-type mice, there was a reduction in Th2 cytokine production, eosinophil infiltration, mucus production and airway responsiveness in DP_1-null mice. Although enhanced mucus production may be due to a direct action of DP_1 (Wright *et al*., 2000) and DP_1-mediated inhibition of eosinophil apoptosis (Gervais *et al*., 2001) may have contributed to airway eosinophilia, the mouse knockout data are consistent with a role for DP_1 in controlling the polarization of Th2 cells and development of the allergic phenotype. However, this view is controversial since it has been shown that local injection of the DP_1 agonist BW245C reduces allergic responses in the skin (Angeli *et al*., 2004) and lungs (Hammad *et al*., 2007) of mice. In the case of the lung, the effect of BW245C was due to a specific action on DP_1 as its effects were ablated in DP_1-null mice (Hammad *et al*., 2007). Furthermore, it was proposed that activation of DP_1 by an endogenous ligand plays an important role in suppressing allergic inflammation since selective deficiency in haematopoietic PGD_2 synthase enhanced airway inflammation and DP_1-deficient dendritic cells demonstrated an enhanced ability to promote Th2 responses in the lung (Hammad *et al*., 2007). Since lung CD11c+ dendritic cells are essential for the maintenance of Th2-dependent airway inflammation (van Rijt *et al*., 2005), DP_1 activation can lead to suppression of dendritic cell functions that are critical to stimulation of Th2 cells within the allergic airways. It appears that DP_1-mediated suppression of dendritic cell function can have disparate effects on Th2-mediated allergic inflammation. On the one hand, DP_1-mediated inhibition of IL-12 promotes

Th2 polarization during the sensitization phase while suppression of dendritic cell function during the effector phase reduces activation of antigen-specific Th2 cells. Consequently, the effect of DP_1 that dominates may vary depending on whether PGD_2 is produced during the sensitization or effector phase of the allergic response and on the location (target organ vs lymph node). The role played by DP_1 in controlling the Th2 polarization during allergen sensitization may explain why allergic airway responses were reduced in DP_1-null mice but it is possible DP_1 blockade may exacerbate an established allergic response. The effect of DP_1 blockade on airway responses to allergen in sensitized animals has not yet been studied extensively but one study has shown that the selective DP_1 antagonist S-5751 reduced, rather than enhanced, airway inflammation in allergic guinea pigs (Arimura *et al*., 2001). Given the complexity of the effects of DP_1 activation it is uncertain whether DP_1 antagonists will be effective in asthma and other allergic disorders.

CRTH2 mediates activation of Th2 lymphocytes, eosinophils and basophils

Chemotaxis of Th2 cells, eosinophils and basophils in response to PGD_2 is blocked by an anti-CRTH2 antibody whereas Th1 cells, which do not express CRTH2, do not migrate in response to PGD_2 (Hirai *et al*., 2001). Independently it has been shown that PGD_2 promotes chemotaxis of eosinophils through a receptor unrelated to DP_1 and this was designated DP_2 (Monneret *et al*., 2001). The ability of PGD_2 to promote eosinophil accumulation in the airways of preclinical species is mimicked by selective CRTH2 agonists but not DP_1 agonists (Almishri *et al*., 2005; Shiraishi *et al*., 2005) and inhibited by the CRTH2 antagonist ramatroban (Shiraishi *et al*., 2005). Injection of PGD_2 enhances allergic response in mouse skin and airways and these effects are mimicked by 13,14-dihydro-15-keto-PGD_2 but not BW245C (Spik *et al*., 2005). In addition to promoting increased migration of leukocytes to sites of allergic inflammation activation by PGD_2 can lead to production of the Th2 cytokines including interleukin 4, 5 and 13. Overexpression of the human lipocalin-type PGD_2 synthase in mice leads to enhanced Th2 cytokine production in response to allergen (Fujitani *et al*., 2002). PGD_2 and 13,14-dihydro-15-keto-PGD_2 have been shown to enhance cytokine production by Th2 in response to anti-CD3/anti-CD28 (Tanaka *et al*., 2004). In fact, PGD_2 has unusual ability to induce production of interleukin 4, 5 and 13 in the absence of allergen or costimulation, an effect that is CRTH2-dependent (Xue *et al*., 2005).

When atopic individuals for example, allergic asthmatics are exposed to allergen there is an early-phase response followed in some cases by a late-phase response (Figure 1). Classically, it is thought that the early phase is mediated by IgE-dependent activation of mast cells and the late-phase response is a consequence of activation of antigen-specific T cells by antigen-presenting cells. This view is supported by a number of findings including the observation that cyclosporine blocks the late phase airway response to allergen but not the early phase (Sihra *et al*., 1997). Interestingly, though, the anti-IgE antibody omalizumab blocked both the early phase and late-phase airway response (Fahy *et al*., 1997) suggesting that IgE contributes to the late-phase response as well as the early phase. This effect could be mediated through activation of mast cells or through IgE-facilitated antigen presentation by dendritic cells or both. There is considerable evidence that IgE-facilitated antigen presentation is an important pathological process in allergic disease (van Neerven *et al*., 2006). However, there is also some evidence that mast cell activation is important in promoting Th2 accumulation independently of antigen presentation by dendritic cells. Studies in IgE transgenic mice have shown that immunological activation of mast cells can lead to recruitment of Th2 cells into the airways in the absence of antigen-specific T-cell activation (Maezawa *et al*., 2003, 2004). Since it is also known that PGD_2 is the major CRTH2 agonist produced by mast cells (Hirai *et al*., 2001) we have conducted experiments to define the roles of PGD_2 and CRTH2 in mediating mast cell-dependent activation of Th2 cells (Gyles *et al*., 2006). It was found that activation of mast cells with IgE/anti-IgE led to the production of a factor, which promoted increased migration of Th2 lymphocytes. The production of this factor was inhibited by the cyclooxygenase inhibitor diclofenac and its pattern of production was similar to that of PGD_2. The effect of the mast cell supernatants on migration of Th2 lymphocytes were blocked by ramatroban (Gyles *et al*., 2006) as was the effect of mast cell supernatants on activation of eosinophils (unpublished observations). Taken together, these data suggest that mast cell-dependent activation of Th2 cells and eosinophils is mediated by PGD_2 acting on CRTH2.

CRTH2 also plays an important role in the paracrine activation of Th2 cells (Vinall *et al*., 2007). Supernatants collected from Th2 cells activated with anti-CD3/anti-CD28 stimulated the migration of naive Th2 cells through a

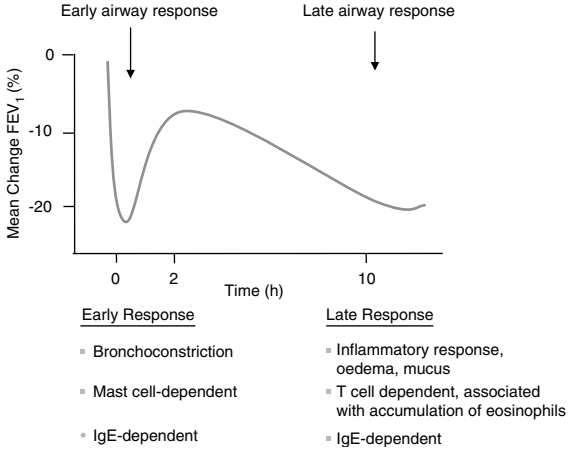

Figure 1 Development of an early- and late-phase airway response in response to allergen in a sensitized individual. The early phase fall in lung function is due to mast cell-dependent bronchospasm, mediated largely by leukotrienes. The late-phase response is mediated by activated Th2 cells and suppressed by drugs that inhibit T-cell function such as steroids and cyclosporine. It is proposed that CRTH2 is involved in both mast cell-mediated activation of Th2 cells and the amplification of Th2 recruitment that occurs as a result of IgE-facilitated antigen presentation by airway dendritic cells.

CRTH2-dependent mechanism. Interestingly, there is evidence that Th2 cells produce some CRTH2 agonist activity independently of the cyclooxygenase pathway since this effect was only partially dependent on PGD_2 (Vinall *et al.*, 2007). These data suggest that CRTH2 is important in both the early mast cell-dependent recruitment of Th2 cells and in the amplification of Th2 cell accumulation resulting from activation of antigen-specific T cells.

In vivo *effects of CRTH2 blockade or deficiency*

The *in vitro* effects described above may explain why ramatroban is effective in inhibiting eosinophil accumulation in a number of tissues in response to an allergic challenge, including guinea-pig nasal mucosa (Narita *et al.*, 1996), mouse airways (Nagai *et al.*, 1995) and mouse skin (Takeshita *et al.*, 2004). Recently, it has been shown that a novel ramatroban analogue lacking TP activity is effective in reducing airway eosinophilia and mucus cell hyperplasia in allergic mice (Uller *et al.*, 2007). CRTH2 antagonists that are structurally unrelated to ramatroban are also effective in reducing airway inflammation in preclinical models. A number of different chemical series have been described (Pettipher *et al.*, 2007) and indole acetic acids, in particular, have been identified as highly potent and selective CRTH2 antagonists (Armer *et al.*, 2005). The effect of a compound from an indole acetic acid series has been studied in a guinea-pig model of allergic airway inflammation. When sensitised, guinea pigs were challenged with an aerosol of antigen there was a significant increase in the numbers of leukocytes in the bronchoalveolar lavage fluid. The majority of these leukocytes were eosinophils although increased numbers of monocytes and neutrophils were also detected. Pretreatment with a selective CRTH2 antagonist caused profound inhibition of the recruitment of total leukocytes, including eosinophils (Figure 2).

Studies on knockout mice have apparently produced conflicting results with respect to involvement of CRTH2 in allergic responses. The lack of consistency may be related to differences in immunization procedure and the fact that in the mouse CRTH2 may be involved in the regulation of Th1 function. This is discussed in more detail elsewhere (Pettipher *et al.*, 2007). In situations where CRTH2 does contribute to the development of allergic responses, some interesting observations have been made. In a model of allergic airways inflammation deficiency in CRTH2 is associated with inhibition of interleukin 4 and interleukin

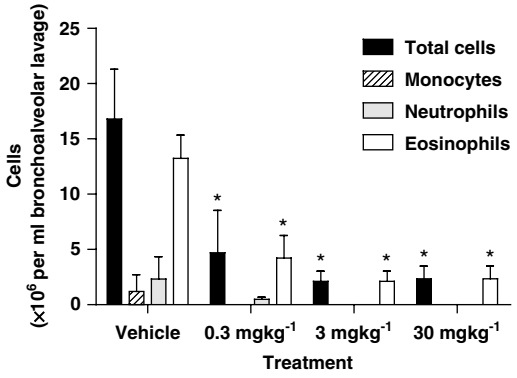

Figure 2 Effect of a selective CRTH2 antagonist on airway inflammation 24 h after challenge with antigen in sensitized guinea pigs. The drug was dosed by oral gavage 1 h before and 12 h after allergen exposure. Data are presented as the mean ± s.e.mean ($n = 10$ animals per group, $^*P < 0.01$ compared to vehicle control).

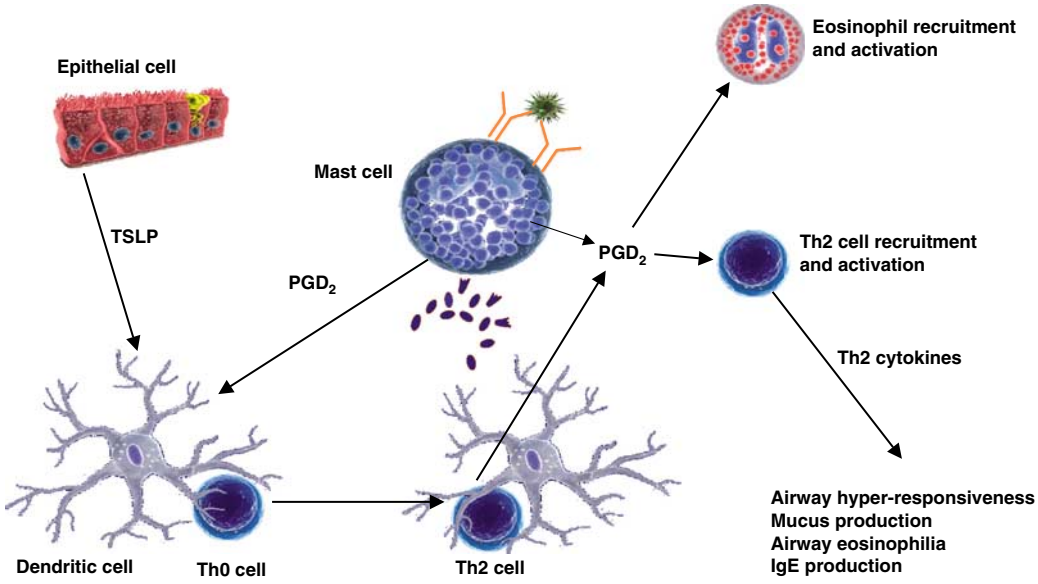

Figure 3 Proposed scheme whereby the combined action of DP_1 and CRTH2 leads to the polarization and activation of Th2 lymphocytes. PGD_2 derived from mast cells acts on dendritic cells to inhibit production of interleukin 12 by an action on DP_1 and thereby creates an environment where T cells are polarized to the Th2 phenotype. Thymic stromal lymphopoietin also acts on dendritic cells to promote Th2 polarization, an effect associated with induction of PGD_2 synthase. CRTH2 contributes both to mast cell-dependent activation of Th2 cells and eosinophils and to paracrine activation of Th2 cells as might occur during IgE-facilitated antigen presentation.

13 production, lower levels of mucus and suppression of airway hyper-responsiveness (Gonzalo *et al.*, 2005). Furthermore, Satoh *et al*. (2006) have reported that in CRTH2 knockout mice there is a reduction in allergic skin inflammation and production of IgE, an effect most likely to be secondary to inhibition of Th2 cytokine production. Since IgE plays an important role in mast cell activation and in facilitating antigen presentation this observation has important implications for understanding how to prevent the progression of chronic allergic disease, the so-called 'atopic march', a phrase used to describe the sequential progression of atopy in childhood to progressively more severe allergic disease in adulthood (Hahn and Bacharier, 2005).

The clinical information on CRTH2 antagonists is limited to data on ramatroban, which is marketed in Japan under the trade name Baynas for the treatment of perennial rhinitis. Although ramatroban is non-selective and possesses only moderate potency, its clinical effects in perennial allergic rhinitis are likely to be due to CRTH2 blockade. In patients with perennial allergic rhinitis, treatment with ramatroban for 4 weeks inhibited chronic nasal swelling and reduced other signs and symptoms (Terada *et al.*, 1998).

Conclusion

A scheme summarizing the roles of DP_1 and CRTH2 in the allergic response is shown in Figure 3.

Although DP_1 and CRTH2 are structurally unrelated and have distinct signalling pathways, they share a common ligand, PGD_2, and through complementary activities contribute to the development and maintenance of allergic inflammation. In addition to mediating vascular changes associated with the acute allergic response, DP_1 may also promote Th2 polarization during allergen sensitization. In contrast, CRTH2 is involved in the recruitment and activation of Th2 lymphocytes and eosinophils that occurs when a sensitized individual encounters allergen. Of particular note is the central role played by CRTH2 in promoting the production of Th2 cytokines and IgE, highlighting the potential utility of CRTH2 antagonists in treating chronic aspects of allergic disease.

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Conflict of interest

The author is named as an inventor on patents relating to the use of CRTH2 antagonists in allergic and other diseases.

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