CRTH2 and D-Type Prostanoid Receptor Antagonists as Novel Therapeutic Agents for Inflammatory Diseases

Rufina Schuligoi Eva Sturm Petra Luschnig Viktoria Konya Sonia Philipose
Miriam Sedej Maria Waldhoer Bernhard A. Peskar Akos Heinemann

Institute of Experimental and Clinical Pharmacology, Medical University of Graz, Graz, Austria

Key Words

CRTH2 · D-type prostanoid receptor · Therapeutic agents

Abstract

Accumulation of type 2 T helper (Th2) lymphocytes and eosinophils is a hallmark of bronchial asthma and other allergic diseases, and it is believed that these cells play a crucial pathogenic role in allergic inflammation. Thus, Th2 cells and eosinophils are currently considered a major therapeutic target in allergic diseases and asthma. However, drugs that selectively target the accumulation and activation of Th2 cells and eosinophils in tissues are unavailable so far. Prostaglandin (PG)_{D2} is a key mediator in various inflammatory diseases including allergy and asthma. It is generated by activated mast cells after allergen exposure and subsequently orchestrates the recruitment of inflammatory cells to the tissue. PG_{D2} induces the chemotaxis of Th2 cells, basophils and eosinophils, stimulates cytokine release from these cells and prolongs their survival, and might hence indirectly promote IgE production. PG_{D2} mediates its biologic functions via 2 distinct G protein-coupled receptors, D-type prostanoid receptor (DP), and the chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2). DP and CRTH2 receptors are currently being considered as highly promising therapeutic targets for combating allergic diseases and asthma.

Here, we revisit the roles of PG_{D2} receptors in the regulation of eosinophil and Th2 cell function and the efforts towards developing candidate compounds for clinical evaluation.

Copyright © 2010 S. Karger AG, Basel

Introduction

Atopy is one of the most prevalent diseases in the developed world. The prevalence of allergic rhinitis, conjunctivitis, bronchial asthma and dermatitis among others is as high as 20% in the populations of Western countries. Atopy is a genetically determined disorder which results in characteristic inflammatory responses to antigens that are innocuous per se. Many forms of atopy such as asthma are associated with a major reduction in quality of life and life expectancy. In addition, some diseases such as intrinsic asthma, aspirin sensitivity, nasal polypsis, adenoid hyperplasia or chronic idiopathic urticaria share several clinical and pathophysiological features of allergy, but with less clear ties to allergens. The pathogenic basis of atopy and related disorders is still only partially understood and no causal therapy, except for specific immunotherapy in selected cases, is available to date. Currently employed symptomatic regimens are

frequently ineffective or, like corticosteroids, might have serious side effects [1, 2]. Therefore, demand persists for additional treatments of allergic diseases, particularly for those patients who need to avoid corticosteroids (e.g. children), and those who are insufficiently treated by standard therapeutic regimens. In the case of bronchial asthma, these patients rely mainly on bronchodilator therapy and are at risk of suffering both from side effects of these drugs and uncontrolled pulmonary inflammation leading to airway remodeling [3]. The discovery of the orphan G protein-coupled receptor GPR44, its expression pattern (basophils, eosinophils and type 2 T helper, Th2, cells) [4] and its ensuing deorphanization as a receptor for the mast cell-derived mediator prostaglandin (PG)_{D2} [5] has fueled additional research in the field. This novel receptor, meanwhile termed 'chemoattractant receptor-homologous molecule expressed on Th2 cells' (CRTH2), has thus provided an innovative and promising target for antiinflammatory drug development.

Role of PGD₂ in Allergic Inflammation

Like other prostanoids, PGD₂ is synthesized by phospholipase A₂, cyclooxygenase and specific terminal synthases upon activation of cells by different stimuli such as allergens, oxidative stress or cytokines. PGD₂ is the major mast cell product that is released during anaphylaxis [6, 7] and substantial evidence has accumulated in the last few years that PGD₂ might be involved in the initiation and perpetuation of allergic inflammation. However, PGD₂ is produced in significant amounts also by dendritic cells, macrophages, eosinophils, Th2 cells and endothelial cells [8–14]. A prominent contribution of PGD₂ to the late-phase allergic reaction is, on the one hand, suggested by enhanced eosinophilic lung inflammation and cytokine release in transgenic mice overexpressing PGD synthase [15]. Moreover, PGD₂ enhances leukotriene (LT) C₄ synthesis by eosinophils during allergic inflammation [16]. Conversely, an inhibitor of hematopoietic PGD synthase attenuates the antigen-induced production of PGD₂ and ameliorates airway inflammation in vivo in mice [17]. Interestingly, the biosynthesis of PGD₂ increases after the intake of cyclooxygenase inhibitors in aspirin-intolerant patients [18–20]. The resulting imbalance between PGD₂ and PGE₂ has been suggested to favor the development of asthma and nasal polyposis [21]. On the other hand, PGD₂ has been suggested to play a beneficial role in the resolution phase of inflammation by controlling Th1-mediated mechanisms [22, 23].

Receptors for PGD₂

The biological effects of PGD₂ are, in principle, mediated by two distinct G protein-coupled receptors, D-type prostanoid receptor (DP) and CRTH2. Moreover, at higher concentrations, PGD₂ is a ligand for the thromboxane (TX) receptor, which mediates the bronchoconstrictor effect of PGD₂ [24, 25].

Chemoattractant Receptor-Homologous Molecule Expressed on Th2 Cells

CRTH2, also known as DP₂, GPR44 or CD294, has initially been found on Th2 cells, eosinophils and basophils [4], and mediates their chemotaxis towards PGD₂ [5, 26]. However, the CRTH2 receptor is activated not only by PGD₂ but also by several PGD₂ metabolites – including 13,14-dihydro-15-keto-PGD₂, PGJ₂, Δ¹²PGJ₂, Δ¹²PGD₂, 9α,11β-PGF₂ and 15-deoxy-PGJ₂ [27–30] –, by PGF₂α [30], a thromboxane metabolite, 11-dehydro-TXB₂ [31], and also by the precursor of all 2-series PG and TX, PGH₂ [32]. In animal models, exogenously administered CRTH2 agonists can induce eosinophil infiltration into the lungs and skin, and aggravate the pathology of allergic responses [33–35]. Moreover, several experimental studies in mice have shown that CRTH2 antagonists can ameliorate allergen-induced cutaneous, pulmonary and upper respiratory inflammation [36–41]. In humans, sequence variants of the gene encoding CRTH2 are associated with asthma and allergic phenotypes [42]. In addition, recent data suggest that CRTH2 is also expressed on monocytes [43] and macrophages, and mediates their migration induced by PGD₂ and endotoxin [8, 44]. Strikingly, PGD₂ levels in bronchoalveolar lavage fluid of patients with chronic obstructive pulmonary disease correlate inversely with lung function [45]. Therefore, using CRTH2 antagonists is currently being considered as a highly promising approach to the treatment of allergic diseases and asthma. Interestingly, a CRTH2 antagonist is currently being evaluated in phase II trials in patients with chronic obstructive pulmonary disease (clinical trial registration No. NCT00690482, NCT00766415).

CRTH2 signals through Gα_i proteins, leading to inhibition of cAMP formation and increases in intracellular Ca²⁺ [5]. In eosinophils, however, we observed that CRTH2 stimulation leads to pertussis-toxin-insensitive activation of phosphatidylinositol 3-kinase (PI3K), phospholipase C, p38 mitogen-activated protein kinase (MAPK) and mitogen-activated protein kinase/extracellular signal-regulated kinase kinase [46]. These pathways mediate eosinophil shape change, actin polymerization

and CD11b upregulation [26, 46]. The migration and survival of Th2 cells in response to PGD₂-induced stimulation of CRTH2 depend on PI3K, while PGD₂-evoked cytokine release involves PI3K, calcineurin and nuclear factor of activated T cells [47]. On the other hand, preactivation of p38 MAPK and glycogen synthase kinase-3 β restrain the CRTH2-mediated migration of eosinophils and the cytokine secretion of Th2 cells, respectively [47, 48]. A recent study revealed that, unexpectedly, CRTH2 is not phosphorylated upon agonist stimulation, a mechanism by which the activity of most G protein-coupled receptors is typically regulated. Instead, the C terminus of CRTH2 inhibits G α_i signaling, which may compensate for the absence of the classical phosphorylation-dependent signal attenuation [49].

D-Type Prostanoid Receptor

DP, also referred to as DP₁, is expressed more widely than CRTH2, including platelets, several types of leukocytes, the vasculature, the central nervous system, retina, nasal mucosa, lungs and intestine [5, 50–54]. Activation of the DP receptor results in cutaneous and pulmonary venous vasodilatation, arterial hypotension, mucin secretion, inhibition of platelet aggregation and lowering of intraocular pressure [54–60]. DP has been described to play both antiinflammatory and proinflammatory roles: on the one hand, DP activation inhibits the function of neutrophils, basophils, dendritic cells, Langerhans cells, Th1 cells and natural killer cells [43, 61–67]. In vivo, inhalation of a selective DP agonist suppresses asthma in a murine model by downmodulation of lung dendritic cell function and induction of regulatory T cells [68]. In addition, PGD₂ but not CRTH2 agonists inhibit the scratching response of mice with atopic dermatitis [69]. Stimulation of DP enhances the barrier function of endothelial cells [70] and confers protection to neurons [71]. On the other hand, PGD₂ and DP receptors contribute to astrogliosis and demyelination in the *twitcher* mouse model [72]. PGD₂ synergizes with tumor necrosis factor via DP receptors to promote the production of monocyte chemoattractant protein-1 and interleukin (IL)-8 in monocytic cells [73]. In vivo, a DP antagonist blocks antigen-induced rhinitis, conjunctivitis and eosinophil infiltration into the lung of the guinea pig [74, 75] and experimental asthma in sheep [76]. DP-deficient mice exhibit reduced pulmonary inflammation in response to allergens [77]. The DP receptor is expressed on bronchiole epithelial cells in antigen-challenged mice [77], as well as nasal epithelial goblet cells in normal human volunteers [52], suggesting that DP might stimulate mucus secretion in allergy. More-

over, an association of polymorphisms of the DP promoter and gene with asthma has been detected in humans [78]. These findings hence point to a proinflammatory role of DP, but are difficult to reconcile with the known functional responses to DP receptor activation.

Despite the fact that it has comparable avidity for PGD₂, the DP receptor shows little similarity with the CRTH2 receptor. In addition to PGD₂, Δ^{12} PGD₂, PGJ₂ and PGH₂ are also potent DP agonists, while other metabolites of PGD₂ such as 13,14-dihydro-15-keto-PGD₂ and 15-deoxy-PGJ₂ are selective for CRTH2 [5, 27, 29, 32, 79]. DP receptor activation leads to G α_s -mediated increases in intracellular cAMP although an agonist-evoked Ca²⁺ flux has also been observed in heterologous expression systems [50, 80]. The effects exerted by PGD₂ on dendritic cells are associated with the phosphorylation of cAMP response element-binding protein, but do not parallel with a deactivation of nuclear factor- κ B and MAPK pathways [43]. So far, further downstream signaling pathways subsequent to DP activation have received very little attention. Interestingly, PGD₂ stimulation of fibroblast repair in an in vitro collagen contraction assay is mediated by DP and Ca²⁺-independent protein kinase C, but it is independent of cAMP and protein kinase A [81].

Regulation of Eosinophil Responses by PGD₂

Eosinophils play important roles in late-phase reactions by releasing bronchoconstrictor mediators such as LTC₄ and other chemoattractants which cause further influx of inflammatory cells into the tissue [82]. Like Th2 cells, eosinophils produce and release proinflammatory cytokines and growth factors including the immunoregulatory type 2 cytokines IL-4, IL-5, IL-10 and IL-13 [83]. Mucosal damage in chronic asthma is associated with cytotoxic and proinflammatory mediators that are released by activated eosinophils. Consequently, eosinophils also play a role in airway remodeling and angiogenesis in chronically inflamed tissue [84]. Importantly, it was shown that asthmatic patients who receive treatment based on eosinophil counts in sputum have significantly fewer severe asthma exacerbations than patients treated according to standard management therapy [85]. Moreover, animal studies also demonstrated that genetically modified mice lacking eosinophils are protected against allergen-induced lung injury and asthma [86, 87]. Therefore, eosinophils are currently considered a major therapeutic target in allergic diseases and asthma [88], but they

might also play pathogenic roles in several other diseases such as eosinophilic esophagitis [89], colitis ulcerosa [90], hypereosinophilic syndrome [91] or renal disease [92].

In many eosinophilic inflammatory diseases, eosinophilia has been attributed to reduced eosinophil apoptosis [93]. The Th2 cytokine IL-5 augments eosinophil recruitment and survival in experimental models of allergic inflammation. However, the limited clinical efficacy of antibodies against IL-5 in ameliorating asthma symptoms [94] suggests that additional mediators are also involved, such as granulocyte-macrophage colony-stimulating factor or a plethora of chemoattractants that can stimulate the locomotion of eosinophils. In particular, these chemoattractants comprise the ligands of the chemokine receptor CCR3 [95] including eotaxin-1, -2 and -3, RANTES and the monocyte chemotactic peptides monocyte chemotactic protein-3 and -4. Moreover, activated complement factors such as C5a, and lipid mediators such as LTB₄ [96], platelet activation factor [97] and 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-ETE) are also potent chemoattractants for eosinophils [98–100].

As mentioned above, PGD₂ and several of its metabolites are potent chemoattractants for eosinophils and this effect mainly relies on CRTH2 activation [5, 28, 32]. Moreover, we observed that PGD₂ and its metabolites are capable of stimulating the release of mature eosinophils from bone marrow [28, 101]. However, PGD₂ not only stimulates eosinophil trafficking but also activates eosinophils with respect to the respiratory burst and release of eosinophil cationic protein, and delays their apoptosis [28, 53]. While most PGD₂ effects in eosinophils are mediated by CRTH2, we could demonstrate that DP receptors are also involved in eosinophil chemotaxis, respiratory burst and mobilization from bone marrow [101, 102]. In contrast, the antiapoptotic effect of PGD₂ in eosinophils is solely mediated by DP receptors [53]. In addition to directly stimulating eosinophil migration, we also demonstrated that PGD₂ very efficiently primes eosinophils for other chemoattractants like eotaxin, 5-oxo-ETE or C5a [28, 48]. The priming effect of PGD₂ is exclusively mediated via CRTH2 receptors. In contrast, eosinophil migration towards PGD₂ is strikingly decreased by other chemoattractants such as eotaxin or 5-oxo-ETE, and PI3K as well as p38 MAPK are involved in these inhibitory effects. Conversely, the priming effect of PGD₂ is not reversed by inhibition of PI3K, p38 MAPK or peroxisome proliferator-activated receptor- γ , which has been described to mediate the priming effect of the PGD₂ metabolite 15-deoxy- $\Delta^{12,14}$ -PGJ₂ [103].

Interestingly, available data also suggest that PGD₂ might inhibit eosinophil degranulation, as investigated by the C5a-induced upregulation of the granule-associated marker CD63 on eosinophils and the release of eosinophil peroxidase [32, 48, 101]. This effect is likewise mediated exclusively by CRTH2 receptors. From these studies we inferred that there is a hierarchy among eosinophil chemoattractants: PGD₂ might be an initial chemoattractant since it maintains its potency also in whole blood [48] and augments the responsiveness of eosinophils to other chemoattractants, but restrains eosinophil degranulation. In contrast, eotaxin seems to be an end point chemoattractant since it has a reduced efficacy in blood, is capable of downmodulating eosinophil responsiveness to other chemoattractants and stimulates eosinophil degranulation [48, 104].

CRTH2 expression levels are likely to be elevated in eosinophils from patients with allergic diseases, as suggested by observations in atopic dermatitis [105].

Role of PGD₂ Receptors in Regulation of Th2 Lymphocyte Function

Ligation of the T cell receptor by the antigen-major histocompatibility complex class II induces the clonal expansion of naïve Th lymphocytes and their differentiation into at least four subtypes of effector cells: Th1, Th2, Th17 and regulatory T (T_{reg}) cells. Th cells can be divided into two subsets, Th1 and Th2, based on their cytokine expression profile. Th1 lymphocytes are characterized by the production of interferon (IFN)- γ and IL-2, whereas Th2 lymphocytes produce IL-4, IL-5, IL-6, IL-10 and IL-13 [106]. The cytokines produced by the Th1 lymphocytes, in particular IFN- γ , activate macrophages and are essentially responsible for cell-mediated immunity against intracellular pathogens [106]. In contrast, Th2 cytokines are responsible for humoral immune responses as well as the growth and differentiation of mast cells and eosinophils, and are involved in antibody-mediated responses to extracellular parasites [107]. Recently identified Th17 cells mainly express IL-17 and are crucial in certain autoimmune diseases [108]. T_{reg} cells are known to suppress various immune responses including autoimmune responsiveness [109]. An imbalance in the relative proportion and function of Th cell populations, as determined by the complex interaction of the genetic background with environmental factors such as exposure to certain infectious agents, has been implicated in the development of abnormal immune re-

sponses and the onset of autoimmune and allergic conditions [110].

Asthma and allergy are inflammatory diseases caused by dysregulation of immune responses in the mucosa or the skin, and it is believed that exaggerated Th2-driven responses play a crucial pathogenic role. Several studies have documented increased numbers of activated CD4-positive T cells showing a Th2 profile in the lungs of asthmatic subjects [111–113]. Thus, CD4-positive T cells producing Th2 cytokines play a prominent role in the lungs, particularly because IL-4 and IL-13 enhance IgE production, IL-4, IL-9 and IL-10 enhance mast cell growth, IL-5 enhances eosinophil accumulation, and IL-9 and IL-13 directly enhance mucus hypersecretion and airway hyperreactivity [114, 115]. Activated Th2 cells are the main cells expressing CD40 ligand (CD40L), and binding of CD40L to CD40 molecules on B cells and dendritic cells promotes their IgE production and antigen-presenting capability, respectively [116]. Correspondingly, clinically useful drugs such as corticosteroids potently suppress cytokine expression in Th2 cells [113, 117]. Although it is clear that Th2-driven immune responses critically regulate the development of allergy and asthma, the underlying causes for Th2 overactivity are still unclear and, to date, no specific therapeutic regimens are available to restrain Th2 overactivity.

A large number of cell surface molecules have been reported to be differentially expressed between Th1 and Th2 cells. For example, the chemokine receptors CXCR3 and CCR5 are selectively expressed on in-vitro-derived Th1 lymphocytes, whereas CCR4, CCR8 and CCR3 are expressed on Th2 lymphocytes [118–121]. Most importantly, CRTH2 has been identified as a receptor that is selectively expressed on Th2 cells [5] and it has been proposed that CRTH2 is the most selective marker for human Th2 cells in peripheral blood [122]. In contrast, the DP receptor is expressed in both Th1 and Th2 subsets, and reinforces an inhibitory effect on cytokine production, e.g. IFN- γ and IL-4, respectively [65]. As mentioned above, CRTH2 mediates the migration of Th2 cells towards PGD₂ [5]. Furthermore, activation of the CRTH2 receptor induces the production of proinflammatory cytokines in Th2 cells [65, 123], upregulates the expression of CD40L on Th2 cells [65], and delays their apoptosis [124]. Interestingly, Th2 cells can themselves produce PGD₂, which may constitute an autocrine mechanism to recruit further Th2 cells to the inflamed tissue [11]. In vitro studies have revealed that CRTH2 expression increases during Th2 polarization [125] and the number of CRTH2-positive cells in peripheral blood is elevated in

patients with atopic dermatitis or HIV infection [122, 126]. These findings suggest that CRTH2 might be a highly promising therapeutic target as the blockade of CRTH2 by means of small-molecule antagonists is likely to attenuate Th2 activity with respect to their accumulation at sites of inflammation, cytokine release and survival in tissue.

Further Inflammatory Cells Expressing CRTH2

Basophils were among the first cells that were shown to express CRTH2 and to migrate towards PGD₂ [5, 127]. Moreover, CRTH2 activation was found to enhance basophil expression of the adhesion molecule CD11b and histamine release, while activation of the DP receptor was found to have opposing effects in basophils [62, 128]. CRTH2 is also detectable in human monocytes and murine macrophages and mediates their migration towards PGD₂ and endotoxin, respectively [8, 43]. Similarly, PGD₂ is chemotactic for murine bone marrow-derived mast cells and this response is ablated by a CRTH2 antagonist [129]. Correspondingly, elevated numbers of CRTH2-positive macrophages and mast cells are present in nasal mucosa of allergic patients [44].

Antagonists of PGD₂ Receptors as Novel Therapeutic Agents

CRTH2 Receptor Antagonists

Our laboratory and others have simultaneously discovered that the cyclooxygenase inhibitor indomethacin is a potent CRTH2 agonist [46, 130], which has thus provided a useful pharmacophore for small-molecule antagonists to CRTH2 [131–134]. Consecutively, it was shown that ramatroban, formerly used as a TX receptor antagonist, was also a potent CRTH2 antagonist in cell lines transfected with CRTH2 and in human eosinophils [31, 131]. Mathiesen et al. described three novel ramatroban analogs that differ chemically from ramatroban by either (i) a single additional methyl group, or (ii) an acetic acid instead of a propionic acid side chain, or (iii) a combination of both [133]. Each of the three compounds acted as a potent CRTH2 antagonist, yet the nature of their antagonism differed markedly, either decreasing the affinity of PGD₂ to CRTH2, or depressing the ligand-binding capacity causing functional insurmountability. One of these compounds (now referred to as TM30089 or Cay10471) proved to be one of the most potent CRTH2

antagonists described so far. Currently, three chemical classes of CRTH2 antagonists have been reported: (i) indole-acetic acids and ramatroban derivatives [127, 135–139]; (ii) phenylacetic acids [140–142], and (iii) tetrahydroquinolines [143, 144]. Although several phase II trials have been conducted with CRTH2 antagonists, only one study has been presented so far: when compared with placebo, oral treatment with a CRTH2 antagonist for 28 days of moderate persistent asthmatics free of inhaled steroids showed significant beneficial effects with respect to forced expiratory volume, peak flow, circulating IgE levels, nighttime asthma symptoms and quality of life [145].

DP Receptor Antagonists

The hydantoin derivative, BWA868c, was the first selective DP antagonist available [25]. Subsequently, a class of bicycloheptane-derivative DP antagonists, including S-5751, has been developed that block the actions of PGD₂ in experimental models of inflammation [74, 75]. In addition to being a starting point for the synthesis of CRTH2 antagonists, indomethacin was also a useful pharmacophore for a number of DP antagonists including laropiprant [146–148]. Although recent phase II studies in asthmatics and patients with allergic rhinitis failed to confirm the promising results obtained from animal models [149], DP antagonisms might be useful in other forms of inflammatory disease. In addition, laropiprant is currently being marketed in combination with niacin as Tre-daptive® to prevent niacin-induced flush that is partially mediated by PGD₂ [150].

Bivalent CRTH2/DP Receptor Antagonists

Considering the multiple roles that DP and CRTH2 receptors play in allergic disease, a hybrid antagonist may exert clinically relevant, beneficial effects that are not achieved when just one PGD₂ receptor is blocked. For in-

stance, DP antagonism would prevent IL-12 generation by dendritic cells and the ensuing polarization of Th2 cells, while CRTH2 antagonism could prevent the recruitment of eosinophils, basophils and Th2 cells to inflammatory sites [151]. Dual CRTH2/DP antagonists derived from phenylacetic acid have recently been presented [152].

Conclusion

For a long time, PGD₂ has been disregarded as a by-product of mast cells with unclear biological relevance. The discovery of its novel receptor, CRTH2, has fueled extensive research into the biology and pharmacology of PGD₂ and has attracted enormous attention from the pharmaceutical industry. Currently, PGD₂ receptor antagonists are being developed for the treatment of allergic diseases and asthma, but they might also be useful in other diseases that involve eosinophils, or even in chronic obstructive pulmonary disease. Small-molecule antagonists of CRTH2 and DP have already been demonstrated to be efficacious in a variety of animal models and their clinical usefulness in human disease will be unveiled in the near future.

Acknowledgments

V.K., S.P. and M.S. were funded by the PhD Program Molecular Medicine of the Medical University of Graz. This study was supported by the Jubiläumsfonds of the Austrian National Bank (grant No. 11967 and 13487), the Austrian Science Fund FWF (grant No. P19424-B05 and P22521-B18) and the Franz Lanyar Foundation (grant No. 315, 316 and 343). A.H. has received research support and consultancy fees from AstraZeneca and Almirall.

References

- 1 Dahl R: Systemic side effects of inhaled corticosteroids in patients with asthma. *Respir Med* 2006;100:1307–1317.
- 2 McDonough AK, Curtis JR, Saag KG: The epidemiology of glucocorticoid-associated adverse events. *Curr Opin Rheumatol* 2008; 20:131–137.
- 3 Wraight JM, Smith AD, Cowan JO, Flannery EM, Herbison GP, Taylor DR: Adverse effects of short-acting beta-agonists: potential impact when anti-inflammatory therapy is inadequate. *Respirology* 2004;9:215–221.
- 4 Nagata K, Hirai H, Tanaka K, Ogawa K, Aso T, Sugamura K, Nakamura M, Takano S: 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* 1999;459:195–199.
- 5 Hirai H, Tanaka K, Yoshie O, Ogawa K, Kenmotsu K, Takamori Y, Ichimasa M, Sugamura K, Nakamura M, Takano S, Nagata K: Prostaglandin D₂ selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. *J Exp Med* 2001;193:255–261.
- 6 Anhut H, Peskar BA, Bernauer W: Release of 15-keto-13,14-dihydro-thromboxane B₂ and prostaglandin D₂ during anaphylaxis as measured by radioimmunoassay. *Naunyn Schmiedeberg Arch Pharmacol* 1978;305: 247–252.
- 7 Schleimer RP, Fox CC, Naclerio RM, Plaut M, Creticos PS, Togias AG, Warner JA, Kagey-Sobotka A, Lichtenstein LM: Role of human basophils and mast cells in the pathogenesis of allergic diseases. *J Allergy Clin Immunol* 1985;76:369–374.

- 8 Tajima T, Murata T, Aritake K, Urade Y, Hirai H, Nakamura M, Ozaki H, Hori M: Lipopolysaccharide induces macrophage migration via prostaglandin D₂ and prostaglandin E₂. *J Pharmacol Exp Ther* 2008;326:493–501.
- 9 Söderström M, Wigren J, Surapureddi S, Glass CK, Hammarström S: Novel prostaglandin D₂-derived activators of peroxisome proliferator-activated receptor-gamma are formed in macrophage cell cultures. *Biochim Biophys Acta* 2003;1631:35–41.
- 10 Urade Y, Ujihara M, Horiguchi Y, Ikai K, Hayaishi O: The major source of endogenous prostaglandin D₂ 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* 1989;143:2982–2989.
- 11 Vinall SL, Townsend ER, Pettipher R: A paracrine role for chemoattractant receptor-homologous molecule expressed on T helper type 2 cells (CRTH2) in mediating chemotactic activation of CRTH2+CD4+ T helper type 2 lymphocytes. *Immunology* 2007;121:577–584.
- 12 Hyo S, Kawata R, Kadoyama K, Eguchi N, Kubota T, Takenaka H, Urade Y: Expression of prostaglandin D₂ synthase in activated eosinophils in nasal polyps. *Arch Otolaryngol Head Neck Surg* 2007;133:693–700.
- 13 Camacho M, Lopez-Belmonte J, Vila L: Rate of vasoconstrictor prostanoids released by endothelial cells depends on cyclooxygenase-2 expression and prostaglandin I synthase activity. *Circ Res* 1998;83:353–365.
- 14 Soler M, Camacho M, Escudero JR, Iniguez MA, Vila L: Human vascular smooth muscle cells but not endothelial cells express prostaglandin E synthase. *Circ Res* 2000;87:504–507.
- 15 Fujitani Y, Kanaoka Y, Aritake K, Uodome N, Okazaki-Hatake K, Urade Y: Pronounced eosinophilic lung inflammation and Th2 cytokine release in human lipocalin-type prostaglandin D synthase transgenic mice. *J Immunol* 2002;168:443–449.
- 16 Mesquita-Santos FP, Vieira-de-Abreu A, Calheiros AS, Figueiredo IH, Castro-Faria-Neto HC, Weller PF, Bozza PT, Diaz BL, Bandeira-Melo C: Cutting edge: prostaglandin D₂ enhances leukotriene C₄ synthesis by eosinophils during allergic inflammation: synergistic in vivo role of endogenous eotaxin. *J Immunol* 2006;176:1326–1330.
- 17 Aritake K, Kado Y, Inoue T, Miyano M, Urade Y: Structural and functional characterization of HQL-79, an orally active, selective inhibitor for human hematopoietic prostaglandin D synthase. *J Biol Chem* 2006;281:15277–15286.
- 18 Szczeklik A, Sladek K, Dworski R, Nizankowska E, Soja J, Sheller J, Oates J: Bronchial aspirin challenge causes specific eicosanoid response in aspirin-sensitive asthmatics. *Am J Respir Crit Care Med* 1996;154:1608–1614.
- 19 O'Sullivan S, Dahlén B, Dahlén SE, Kumlin M: Increased urinary excretion of the prostaglandin D₂ metabolite 9 α ,11 β -prostaglandin F₂ after aspirin challenge supports mast cell activation in aspirin-induced airway obstruction. *J Allergy Clin Immunol* 1996;98:421–432.
- 20 Higashi N, Mita H, Ono E, Fukutomi Y, Yamaguchi H, Kajiura K, Tanimoto H, Sekiya K, Akiyama K, Taniguchi M: Profile of eicosanoid generation in aspirin-intolerant asthma and anaphylaxis assessed by new biomarkers. *J Allergy Clin Immunol* 2010, Epub ahead of print.
- 21 Pierzchalska M, Szabo Z, Sanak M, Soja J, Szczeklik A: Deficient prostaglandin E₂ production by bronchial fibroblasts of asthmatic patients, with special reference to aspirin-induced asthma. *J Allergy Clin Immunol* 2003;111:1041–1048.
- 22 Ajuebor MN, Singh A, Wallace JL: Cyclooxygenase-2-derived prostaglandin D₂ is an early anti-inflammatory signal in experimental colitis. *Am J Physiol Gastrointest Liver Physiol* 2000;279:G238–G244.
- 23 Trivedi SG, Newson J, Rajakariar R, Jacques TS, Hannon R, Kanaoka Y, Eguchi N, Colville-Nash P, Gilroy DW: Essential role for hematopoietic prostaglandin D₂ synthase in the control of delayed-type hypersensitivity. *Proc Natl Acad Sci USA* 2006;103:5179–5184.
- 24 Coleman RA, Sheldrick RL: Prostanoid-induced contraction of human bronchial smooth muscle is mediated by TP receptors. *Br J Pharmacol* 1989;96:688–692.
- 25 Giles H, Leff P, Bolofo ML, Kelly MG, Robertson AD: The classification of prostaglandin DP receptors in platelets and vasculature using BW A868C, a novel, selective and potent competitive antagonist. *Br J Pharmacol* 1989;96:291–300.
- 26 Monneret G, Gravel S, Diamond M, Rokach J, Powell WS: Prostaglandin D₂ is a potent chemoattractant for human eosinophils that acts via a novel DP receptor. *Blood* 2001;98:1942–1948.
- 27 Monneret G, Li H, Vasilescu J, Rokach J, Powell WS: 15-deoxy- $\Delta^{12,14}$ -prostaglandins D₂ and J₂ are potent activators of human eosinophils. *J Immunol* 2002;168:3563–3569.
- 28 Heinemann A, Schuligoi R, Sabroe I, Hartnell A, Peskar BA: Δ^{12} -prostaglandin J₂, a plasma metabolite of prostaglandin D₂, causes eosinophil mobilization from the bone marrow and primes eosinophils for chemotaxis. *J Immunol* 2003;170:4752–4758.
- 29 Gazi L, Gyles S, Rose J, Lees S, Allan C, Xue L, Jassal R, Speight G, Gamble V, Pettipher R: Δ^{12} -prostaglandin D₂ is a potent and selective CRTH2 receptor agonist and causes activation of human eosinophils and Th2 lymphocytes. *Prostaglandins Other Lipid Mediat* 2005;75:153–167.
- 30 Sandig H, Andrew D, Barnes AA, Sabroe I, Pease J: 9 α ,11 β -PGF₂ and its stereoisomer PGF₂ α are novel agonists of the chemoattractant receptor, CRTH2. *FEBS Lett* 2006;580:373–379.
- 31 Böhm E, Sturm GJ, Weiglhofer I, Sandig H, Shichijo M, McNamee A, Pease JE, Kollroser M, Peskar BA, Heinemann A: 11-dehydrothromboxane B₂, 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* 2004;279:7663–7670.
- 32 Schuligoi R, Sedej M, Waldhoer M, Vukoja A, Sturm EM, Lippe IT, Peskar BA, Heinemann A: Prostaglandin H₂ induces the migration of human eosinophils through the chemoattractant receptor homologous molecule of Th2 cells, CRTH2. *J Leukoc Biol* 2009;85:136–145.
- 33 Almishri W, Cossette C, Rokach J, Martin JG, Hamid Q, Powell W: Effects of prostaglandin D₂, 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂, and selective DP₁ and DP₂ receptor agonists on pulmonary infiltration of eosinophils in brown Norway rats. *J Pharmacol Exp Ther* 2005;313:64–69.
- 34 Shiraishi Y, Asano K, Nakajima T, Oguma T, Suzuki Y, Shiomi T, Sayama K, Niimi K, Wakaki M, Kagyo J, Ikeda E, Hirai H, Yamaguchi K, Ishizaka A: Prostaglandin D₂-induced eosinophilic airway inflammation is mediated by CRTH2 receptor. *J Pharmacol Exp Ther* 2005;312:954–960.
- 35 Spik I, Brenuchon C, Angeli V, Staumont D, Fleury S, Capron M, Trottein F, Dombrowicz D: Activation of the prostaglandin D₂ receptor DP₂/CRTH2 increases allergic inflammation in mouse. *J Immunol* 2005;174:3703–3708.
- 36 Uller L, Mathiesen JM, Alenmyr L, Korsgren M, Ulven T, Högberg T, Andersson G, Persson CG, Kostenis E: Antagonism of the prostaglandin D₂ receptor CRTH2 attenuates asthma pathology in mouse eosinophilic airway inflammation. *Respir Res* 2007;8:16.
- 37 Lukacs NW, Berlin AA, Franz-Bacon K, Saksik R, Sprague LJ, Ly TW, Hardiman G, Boehme SA, Bacon KB: CRTH2 antagonism significantly ameliorates airway hyperactivity and downregulates inflammation-induced genes in a mouse model of airway inflammation. *Am J Physiol Lung Cell Mol Physiol* 2008;295:L767–L779.
- 38 Oiwa M, Satoh T, Watanabe M, Niwa H, Hirai H, Nakamura M, Yokozeki H: CRTH2-dependent, STAT6-independent induction of cedar pollen dermatitis. *Clin Exp Allergy* 2008;38:1357–1366.
- 39 Shiraishi Y, Asano K, Niimi K, Fukunaga K, Wakaki M, Kagyo J, Takihara T, Ueda S, Nakajima T, Oguma T, Suzuki Y, Shiomi T, Sayama K, Kagawa S, Ikeda E, Hirai H, Nagata K, Nakamura M, Miyasho T, Ishizaka A: Cyclooxygenase-2/prostaglandin D₂/CRTH2 pathway mediates double-stranded RNA-induced enhancement of allergic airway inflammation. *J Immunol* 2008;180:541–549.

- 40 Nomiya R, Okano M, Fujiwara T, Maeda M, Kimura Y, Kino K, Yokoyama M, Hirai H, Nagata K, Hara T, Nishizaki K, Nakamura M: CRTH2 plays an essential role in the pathophysiology of Cry j 1-induced pollinosis in mice. *J Immunol* 2008;180:5680–5688.
- 41 Boehme SA, Chen EP, Franz-Bacon K, Sasik R, Sprague LJ, Ly TW, Hardiman G, Bacon KB: Antagonism of CRTH2 ameliorates chronic epicutaneous sensitization-induced inflammation by multiple mechanisms. *Int Immunol* 2009;21:1–17.
- 42 Cameron L, Depner M, Kormann M, Klopp N, Illig T, von Mutius E, Kabesch M: Genetic variation in CRTH2 influences development of allergic phenotypes. *Allergy* 2009;64:1478–1485.
- 43 Gosset P, Bureau F, Angeli V, Pichavant M, Faveeuw C, Tonnel AB, Trottein F: Prostaglandin D₂ affects the maturation of human monocyte-derived dendritic cells: consequence on the polarization of naïve Th cells. *J Immunol* 2003;170:4943–4952.
- 44 Shirasaki H, Kikuchi M, Kanaizumi E, Himi T: Accumulation of CRTH2-positive leukocytes in human allergic nasal mucosa. *Ann Allergy Asthma Immunol* 2009;102:110–115.
- 45 Csanky E, Ruhl R, Scholtz B, Vasko A, Takacs L, Hempel WM: Lipid metabolite levels of prostaglandin D₂ and eicosapentaenoic acid recovered from bronchoalveolar lavage fluid correlate with lung function of chronic obstructive pulmonary disease patients and controls. *Electrophoresis* 2009;30:1228–1234.
- 46 Stubbs VE, Schratl P, Hartnell A, Williams TJ, Peskar BA, Heinemann A, Sabroe I: Indomethacin causes prostaglandin D₂-like and eotaxin-like selective responses in eosinophils and basophils. *J Biol Chem* 2002;277:26012–26020.
- 47 Xue L, Gyles SL, Barrow A, Pettipher R: Inhibition of PI3K and calcineurin suppresses chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2)-dependent responses of Th2 lymphocytes to prostaglandin D₂. *Biochem Pharmacol* 2007;73:843–853.
- 48 Schratl P, Sturm EM, Royer JF, Sturm GJ, Lippe IT, Peskar BA, Heinemann A: Hierarchy of eosinophil chemoattractants: role of p38 mitogen-activated protein kinase. *Eur J Immunol* 2006;36:2401–2409.
- 49 Schröder R, Merten N, Mathiesen JM, Martini L, Kruljac-Letic A, Krop F, Blaukat A, Fang Y, Tran E, Ulven T, Drewke C, Whistler J, Pardo L, Gomeza J, Kostenis E: The C-terminal tail of CRTH2 is a key molecular determinant that constrains G α_i and downstream signaling cascade activation. *J Biol Chem* 2009;284:1324–1336.
- 50 Boie Y, Sawyer N, Slipetz DM, Metters KM, Abramovitz M: Molecular cloning and characterization of the human prostanoid DP receptor. *J Biol Chem* 1995;270:18910–18916.
- 51 Gerashchenko D, Beuckmann CT, Kanaoka Y, Eguchi N, Gordon WC, Urade Y, Bazan NG, Hayaishi O: Dominant expression of rat prostanoid DP receptor mRNA in leptomeninges, inner segments of photoreceptor cells, iris epithelium, and ciliary processes. *J Neurochem* 1998;71:937–945.
- 52 Nantel F, Fong C, Lamontagne S, Wright DH, Giaid A, Desrosiers M, Metters KM, O'Neill GP, Gervais FG: Expression of prostaglandin D synthase and the prostaglandin D₂ receptors DP and CRTH2 in human nasal mucosa. *Prostaglandins Other Lipid Mediat* 2004;73:87–101.
- 53 Gervais FG, Cruz RP, Chateau-neuf A, Gale S, Sawyer N, Nantel F, Metters KM, O'Neill GP: Selective modulation of chemokinesis, degranulation, and apoptosis in eosinophils through the PGD₂ receptors CRTH2 and DP. *J Allergy Clin Immunol* 2001;108:982–988.
- 54 Wright DH, Ford-Hutchinson AW, Chadee K, Metters KM: The human prostanoid DP receptor stimulates mucin secretion in LS174T cells. *Br J Pharmacol* 2000;131:1537–1545.
- 55 Whittle BJ, Moncada S, Mullane K, Vane JR: Platelet and cardiovascular activity of the hydantoin BW245C, a potent prostaglandin analogue. *Prostaglandins* 1983;25:205–223.
- 56 Gray SJ, Giles H, Posner J: The effect of a prostaglandin DP receptor partial agonist (192C86) on platelet aggregation and the cardiovascular system in healthy volunteers. *Br J Clin Pharmacol* 1992;34:344–351.
- 57 Walch L, Labat C, Gascard JP, de Montpreville V, Brink C, Norel X: Prostanoid receptors involved in the relaxation of human pulmonary vessels. *Br J Pharmacol* 1999;126:859–866.
- 58 Woodward DF, Spada CS, Hawley SB, Williams LS, Protzman CE, Nieves AL: Further studies on ocular responses to DP receptor stimulation. *Eur J Pharmacol* 1993;230:327–333.
- 59 Moreland RB, Nehra A, Kim NN, Min KS, Albadawi H, Watkins MT, Goldstein I, Traish AM: Expression of functional prostaglandin D (DP) receptors in human corpus cavernosum smooth muscle. *Int J Impot Res* 2002;14:446–452.
- 60 van Hecken A, Depré M, de Lepeleire I, Thach C, Oeyen M, van Effen J, Laethem T, Mazina K, Crumley T, Wenning L, Gottesdiener KM, Deutsch P, Clement P, Lai E, de Hoon JN: The effect of MK-0524, a prostaglandin D₂ receptor antagonist, on prostaglandin D₂-induced nasal airway obstruction in healthy volunteers. *Eur J Clin Pharmacol* 2007;63:135–141.
- 61 Wheeldon A, Vardey CJ: Characterization of the inhibitory prostanoid receptors on human neutrophils. *Br J Pharmacol* 1993;108:1051–1054.
- 62 Yoshimura-Uchiyama C, Iikura M, Yamaguchi M, Nagase H, Ishii A, Matsushima K, Yamamoto K, Shichijo M, Bacon KB, Hirai K: Differential modulation of human basophil functions through prostaglandin D₂ receptors DP and chemoattractant receptor-homologous molecule expressed on Th2 cells/DP₂. *Clin Exp Allergy* 2004;34:1283–1290.
- 63 Hammad H, de Heer HJ, Soullie T, Hoogsteden HC, Trottein F, Lambrecht BN: Prostaglandin D₂ inhibits airway dendritic cell migration and function in steady-state conditions by selective activation of the D prostanoid receptor 1. *J Immunol* 2003;171:3936–3940.
- 64 Angeli V, Faveeuw C, Roye O, Fontaine J, Teissier E, Capron A, Wolowczuk I, Capron M, Trottein F: Role of the parasite-derived prostaglandin D₂ in the inhibition of epidermal Langerhans cell migration during schistosomiasis infection. *J Exp Med* 2001;193:1135–1147.
- 65 Tanaka K, Hirai H, Takano S, Nakamura M, Nagata K: Effects of prostaglandin D₂ on helper T cell functions. *Biochem Biophys Res Commun* 2004;316:1009–1014.
- 66 Chen Y, Perussia B, Campbell KS: Prostaglandin D₂ suppresses human NK cell function via signaling through D prostanoid receptor. *J Immunol* 2007;179:2766–2773.
- 67 Torres D, Paget C, Fontaine J, Mallevaey T, Matsuoka T, Maruyama T, Narumiya S, Capron M, Gosset P, Faveeuw C, Trottein F: Prostaglandin D₂ inhibits the production of IFN- γ by invariant NK T cells: consequences in the control of B16 melanoma. *J Immunol* 2008;180:783–792.
- 68 Hammad H, Kool M, Soullie T, Narumiya S, Trottein F, Hoogsteden HC, Lambrecht BN: Activation of the D prostanoid 1 receptor suppresses asthma by modulation of lung dendritic cell function and induction of regulatory T cells. *J Exp Med* 2007;204:357–367.
- 69 Arai I, Takano N, Hashimoto Y, Futaki N, Sugimoto M, Takahashi N, Inoue T, Nakaike S: Prostanoid DP₁ receptor agonist inhibits the pruritic activity in NC/Nga mice with atopic dermatitis. *Eur J Pharmacol* 2004;505:229–235.
- 70 Murata T, Lin MI, Aritake K, Matsumoto S, Narumiya S, Ozaki H, Urade Y, Hori M, Sessa WC: Role of prostaglandin D₂ receptor DP as a suppressor of tumor hyperpermeability and angiogenesis in vivo. *Proc Natl Acad Sci USA* 2008;105:20009–20014.
- 71 Liang X, Wu L, Hand T, Andreasson K: Prostaglandin D₂ mediates neuronal protection via the DP₁ receptor. *J Neurochem* 2005;92:477–486.
- 72 Mohri I, Taniike M, Taniguchi H, Kanekiyo T, Aritake K, Inui T, Fukumoto N, Eguchi N, Kushi A, Sasaki H, Kanaoka Y, Ozono K, Narumiya S, Suzuki K, Urade Y: Prostaglandin D₂-mediated microglia/astrocyte interaction enhances astrogliosis and demyelination in twitcher. *J Neurosci* 2006;26:4383–4393.
- 73 Hirano Y, Shichijo M, Deguchi M, Nagira M, Suzuki N, Nishitani Y, Hattori M, Arimura A: Synergistic effect of PGD₂ via prostanoid DP receptor on TNF- α -induced produc-

- tion of MCP-1 and IL-8 in human monocyte THP-1 cells. *Eur J Pharmacol* 2007;560:81–88.
- 74 Arimura A, Yasui K, Kishino J, Asanuma F, Hasegawa H, Kakudo S, Ohtani M, Arita H: Prevention of allergic inflammation by a novel prostaglandin receptor antagonist, S-5751. *J Pharmacol Exp Ther* 2001;298:411–419.
- 75 Yasui K, Asanuma F, Hirano Y, Shichijo M, Deguchi M, Arimura A: Contribution of prostaglandin D₂ via prostanoid DP receptor to nasal hyperresponsiveness in guinea pigs repeatedly exposed to antigen. *Eur J Pharmacol* 2008;578:286–291.
- 76 Shichijo M, Arimura A, Hirano Y, Yasui K, Suzuki N, Deguchi M, Abraham WM: A prostaglandin D₂ receptor antagonist modifies experimental asthma in sheep. *Clin Exp Allergy* 2009;39:1404–1414.
- 77 Matsuoka T, Hirata M, Tanaka H, Takahashi Y, Murata T, Kabashima K, Sugimoto Y, Kobayashi T, Ushikubi F, Aze Y, Eguchi N, Urade Y, Yoshida N, Kimura K, Mizoguchi A, Honda Y, Nagai H, Narumiya S: Prostaglandin D₂ as a mediator of allergic asthma. *Science* 2000;287:2013–2017.
- 78 Zhu G, Vestbo J, Lenney W, Silverman M, Whyte M, Helms P, Anderson WH, Pillai SG: Association of PTGDR gene polymorphisms with asthma in two Caucasian populations. *Genes Immun* 2007;8:398–403.
- 79 Schuligoi R, Schmidt R, Geisslinger G, Kollrosier M, Peskar BA, Heinemann A: PGD₂ metabolism in plasma: kinetics and relationship with bioactivity on DP₁ and CRTH2 receptors. *Biochem Pharmacol* 2007;74:107–117.
- 80 Hirata M, Kakizuka A, Aizawa M, Ushikubi F, Narumiya S: Molecular characterization of a mouse prostaglandin D receptor and functional expression of the cloned gene. *Proc Natl Acad Sci USA* 1994;91:11192–11196.
- 81 Kohyama T, Wyatt TA, Liu X, Wen FQ, Kobayashi T, Fang Q, Kim HJ, Rennard SI: PGD₂ modulates fibroblast-mediated native collagen gel contraction. *Am J Respir Cell Mol Biol* 2002;27:375–381.
- 82 O'Byrne PM: Leukotrienes in the pathogenesis of asthma. *Chest* 1997;111:27S–34S.
- 83 Woerly G, Roger N, Loiseau S, Capron M: Expression of Th1 and Th2 immunoregulatory cytokines by human eosinophils. *Int Arch Allergy Immunol* 1999;118:95–97.
- 84 Aceves SS, Broide DH: Airway fibrosis and angiogenesis due to eosinophil trafficking in chronic asthma. *Curr Mol Med* 2008;8:350–358.
- 85 Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, Wardlaw AJ, Pavord ID: Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002;360:1715–1721.
- 86 Humbles AA, Lloyd CM, McMillan SJ, Friend DS, Xanthou G, McKenna EE, Ghiran S, Gerard NP, Yu C, Orkin SH, Gerard C: A critical role for eosinophils in allergic airways remodeling. *Science* 2004;305:1776–1779.
- 87 Lee JJ, Dimina D, Macias MP, Ochur SI, McGarry MP, O'Neill KR, Protheroe C, Pero R, Nguyen T, Cormier SA, Lenkiewicz E, Colbert D, Rinaldi L, Ackerman SJ, Irvin CG, Lee NA: Defining a link with asthma in mice congenitally deficient in eosinophils. *Science* 2004;305:1773–1776.
- 88 Foster PS, Rosenberg HF, Asquith KL, Kumar RK: Targeting eosinophils in asthma. *Curr Mol Med* 2008;8:585–590.
- 89 Straumann A, Simon HU: Eosinophilic esophagitis: escalating epidemiology? *J Allergy Clin Immunol* 2005;115:418–419.
- 90 Wedemeyer J, Vosskuhl K: Role of gastrointestinal eosinophils in inflammatory bowel disease and intestinal tumours. *Best Pract Res Clin Gastroenterol* 2008;22:537–549.
- 91 Simon D, Simon HU: Eosinophilic disorders. *J Allergy Clin Immunol* 2007;119:1291–1300.
- 92 Fukui M, Tanaka M, Hamaguchi M, Senmaru T, Sakabe K, Shiraishi E, Harusato I, Yamazaki M, Hasegawa G, Nakamura N: Eosinophil count is positively correlated with albumin excretion rate in men with type 2 diabetes. *Clin J Am Soc Nephrol* 2009;4:1761–1765.
- 93 Simon HU: Molecules involved in the regulation of eosinophil apoptosis. *Chem Immunol Allergy* 2006;91:49–58.
- 94 Leckie MJ: Anti-interleukin-5 monoclonal antibodies: preclinical and clinical evidence in asthma models. *Am J Respir Med* 2003;2:245–259.
- 95 Garcia-Zepeda EA, Combadiere C, Rothenberg ME, Sarafi MN, Lavigne F, Hamid Q, Murphy PM, Luster AD: Human monocyte chemoattractant protein (MCP)-4 is a novel CC chemokine with activities on monocytes, eosinophils, and basophils induced in allergic and nonallergic inflammation that signals through the CC chemokine receptors (CCR)-2 and -3. *J Immunol* 1996;157:5613–5626.
- 96 Nagy L, Lee TH, Goetzl EJ, Pickett WC, Kay AB: Complement receptor enhancement and chemotaxis of human neutrophils and eosinophils by leukotrienes and other lipoxygenase products. *Clin Exp Immunol* 1982;47:541–547.
- 97 Wardlaw AJ, Moqbel R, Cromwell O, Kay AB: Platelet-activating factor: a potent chemotactic and chemokinetic factor for human eosinophils. *J Clin Invest* 1986;78:1701–1706.
- 98 Powell WS, Chung D, Gravel S: 5-oxo-6,8,11,14-eicosatetraenoic acid is a potent stimulator of human eosinophil migration. *J Immunol* 1995;154:4123–4132.
- 99 Sturm GJ, Schuligoi R, Sturm EM, Royer JF, Lang-Loidolt D, Stammberger H, Amann R, Peskar BA, Heinemann A: 5-oxo-6,8,11,14-eicosatetraenoic acid is a potent chemoattractant for human basophils. *J Allergy Clin Immunol* 2005;116:1014–1019.
- 100 Sturm EM, Schratl P, Schuligoi R, Konya V, Sturm GJ, Lippe IT, Peskar BA, Heinemann A: Prostaglandin E₂ inhibits eosinophil trafficking through E-prostanoid 2 receptors. *J Immunol* 2008;181:7273–7283.
- 101 Schratl P, Royer JF, Kostenis E, Ulven T, Sturm EM, Waldhoer M, Hoefler G, Schuligoi R, Lippe IT, Peskar BA, Heinemann A: The role of the prostaglandin D₂ receptor, DP, in eosinophil trafficking. *J Immunol* 2007;179:4792–4799.
- 102 Royer JF, Schratl P, Lorenz S, Kostenis E, Ulven T, Schuligoi R, Peskar BA, Heinemann A: A novel antagonist of CRTH2 blocks eosinophil release from bone marrow, chemotaxis and respiratory burst. *Allergy* 2007;62:1401–1409.
- 103 Kobayashi Y, Ueki S, Mahemuti G, Chiba T, Oyama H, Saito N, Kanda A, Kayaba H, Chihara J: Physiological levels of 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ prime eotaxin-induced chemotaxis on human eosinophils through peroxisome proliferator-activated receptor-gamma ligation. *J Immunol* 2005;175:5744–5750.
- 104 Fujisawa T, Kato Y, Nagase H, Atsuta J, Terada A, Iguchi K, Kamiya H, Morita Y, Kitaura M, Kawasaki H, Yoshie O, Hirai K: Chemokines induce eosinophil degranulation through CCR-3. *J Allergy Clin Immunol* 2000;106:507–513.
- 105 Yahara H, Satoh T, Miyagishi C, Yokozeki H: Increased expression of CRTH2 on eosinophils in allergic skin diseases. *J Eur Acad Dermatol Venerol* 2010;24:75–76.
- 106 Annunziato F, Galli G, Cosmi L, Romagnani P, Manetti R, Maggi E, Romagnani S: Molecules associated with human Th1 or Th2 cells. *Eur Cytokine Netw* 1998;9:12–16.
- 107 Romagnani S: Cytokines and chemoattractants in allergic inflammation. *Mol Immunol* 2002;38:881–885.
- 108 Peck A, Mellins ED: Precarious balance: Th17 cells in host defense. *Infect Immun* 2010;78:32–38.
- 109 Robinson DS: Regulatory T cells and asthma. *Clin Exp Allergy* 2009;39:1314–1323.
- 110 Wills-Karp M, Santeliz J, Karp CL: The germless theory of allergic disease: revisiting the hygiene hypothesis. *Nat Rev Immunol* 2001;1:69–75.
- 111 Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, Corrigan C, Durham SR, Kay AB: Predominant Th2-like bronchoalveolar T lymphocyte population in atopic asthma. *N Engl J Med* 1992;326:298–304.
- 112 Robinson D, Hamid Q, Bentley A, Ying S, Kay AB, Durham SR: Activation of CD4⁺ T cells, increased Th2-type cytokine mRNA expression, and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation challenge in patients with atopic asthma. *J Allergy Clin Immunol* 1993;92:313–324.

- 113 Corrigan CJ, Hamid Q, North J, Barkans J, Moqbel R, Durham S, Gemou-Engesaeth V, Kay AB: Peripheral blood CD4 but not CD8 T lymphocytes in patients with exacerbation of asthma transcribe and translate messenger RNA encoding cytokines which prolong eosinophil survival in the context of a Th2-type pattern: effect of glucocorticoid therapy. *Am J Respir Cell Mol Biol* 1995;12:567–578.
- 114 Umetsu DT, DeKruyff RH: Th1 and Th2 CD4+ cells in human allergic diseases. *J Allergy Clin Immunol* 1997;100:1–6.
- 115 Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, Donaldson DD: Interleukin-13: central mediator of allergic asthma. *Science* 1998;282:2258–2261.
- 116 Banchereau J, Bazan F, Blanchard D, Briere F, Galizzi JP, van Kooten C, Liu YJ, Rousset F, Saeland S: The CD40 antigen and its ligand. *Annu Rev Immunol* 1994;12:881–922.
- 117 Umland SP, Nahrebne DK, Razac S, Beavis A, Pennline KJ, Egan RW, Billah MM: The inhibitory effects of topically active glucocorticoids on IL-4, IL-5, and interferon-gamma production by cultured primary CD4+ T cells. *J Allergy Clin Immunol* 1997;100:511–519.
- 118 Colantonio L, Recalde H, Sinigaglia F, D'Ambrosio D: Modulation of chemokine receptor expression and chemotactic responsiveness during differentiation of human naive T cells into Th1 or Th2 cells. *Eur J Immunol* 2002;32:1264–1273.
- 119 Bonocchi R, Bianchi G, Bordignon PP, D'Ambrosio D, Lang R, Borsatti A, Sozzani S, Allavena P, Gray PA, Mantovani A, Sinigaglia F: Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. *J Exp Med* 1998;187:129–134.
- 120 Sallusto F, Lenig D, Mackay CR, Lanzavecchia A: Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. *J Exp Med* 1998;187:875–883.
- 121 Syrbe U, Siveke J, Hamann A: Th1/Th2 subsets: distinct differences in homing and chemokine receptor expression? *Springer Semin Immunopathol* 1999;21:263–285.
- 122 Cosmi L, Annunziato F, Galli MIG, Maggi RME, Nagata K, Romagnani S: CRTH2 is the most reliable marker for the detection of circulating human type 2 Th and type 2 T cytotoxic cells in health and disease. *Eur J Immunol* 2000;30:2972–2979.
- 123 Xue L, Gyles SL, Wettest FR, Gazi L, Townsend E, Hunter MG, Pettipher R: Prostaglandin D₂ causes preferential induction of proinflammatory Th2 cytokine production through an action on chemoattractant receptor-like molecule expressed on Th2 cells. *J Immunol* 2005;175:6531–6536.
- 124 Xue L, Barrow A, Pettipher R: Novel function of CRTH2 in preventing apoptosis of human Th2 cells through activation of the phosphatidylinositol 3-kinase pathway. *J Immunol* 2009;182:7580–7586.
- 125 de Fanis U, Mori F, Kurnat RJ, Lee WK, Bova M, Adkinson NF, Casolaro V: GATA3 up-regulation associated with surface expression of CD294/CRTH2: a unique feature of human Th cells. *Blood* 2007;109:4343–4350.
- 126 Iwasaki M, Nagata K, Takano S, Takahashi K, Ishii N, Ikezawa Z: Association of a new-type prostaglandin D₂ receptor CRTH2 with circulating T helper 2 cells in patients with atopic dermatitis. *J Invest Dermatol* 2002;119:609–616.
- 127 Royer JF, Schratl P, Carrillo JJ, Jupp R, Barker J, Weyman-Jones C, Beri R, Sargent C, Schmidt JA, Lang-Loidolt D, Heinemann A: A novel antagonist of prostaglandin D₂ blocks the locomotion of eosinophils and basophils. *Eur J Clin Invest* 2008;38:663–671.
- 128 Monneret G, Boumiza R, Gravel S, Cossette C, Bienvenu J, Rokach J, Powell WS: Effects of prostaglandin D₂ and 5-lipoxygenase products on the expression of CD203c and CD11b by basophils. *J Pharmacol Exp Ther* 2005;312:627–634.
- 129 Boehme SA, Franz-Bacon K, Chen EP, Ly TW, Kawakami Y, Bacon KB: Murine bone marrow-derived mast cells express chemoattractant receptor-homologous molecule expressed on T helper class 2 cells (CRTH2). *Int Immunol* 2009;21:621–632.
- 130 Hirai H, Tanaka K, Takano S, Ichimasa M, Nakamura M, Nagata K: Cutting edge: agonistic effect of indomethacin on a prostaglandin D₂ receptor, CRTH2. *J Immunol* 2002;168:981–985.
- 131 Sugimoto H, Shichijo M, Iino T, Manabe Y, Watanabe A, Shimazaki M, Gantner F, Bacon KB: An orally bioavailable small molecule antagonist of CRTH2, ramatroban (BAY u3405), inhibits prostaglandin D₂-induced eosinophil migration in vitro. *J Pharmacol Exp Ther* 2003;305:347–352.
- 132 Mathiesen JM, Ulven T, Martini L, Gerlach LO, Heinemann A, Kostenis E: Identification of indole derivatives exclusively interfering with a G protein-independent signaling pathway of the prostaglandin D₂ receptor CRTH2. *Mol Pharmacol* 2005;68:393–402.
- 133 Mathiesen JM, Christopoulos A, Ulven T, Royer J, Campillo M, Heinemann A, Pardo L, Kostenis E: On the mechanism of interaction of potent, surmountable and insurmountable antagonists with the prostaglandin D₂ receptor CRTH2. *Mol Pharmacol* 2006;69:1441–1453.
- 134 Liu F, Gonzalo JA, Manning S, O'Connell LE, Fedyk ER, Burke KE, Elder AM, Pulido JC, Cao W, Tayber O, Qiu Y, Ghosh S, Ocain TD, Hodge MR, Suzuki-Yagawa Y: Pharmacological characterization of guinea pig chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2). *Prostaglandins Other Lipid Mediat* 2005;76:133–147.
- 135 Armer RE, Ashton MR, Boyd EA, Brennan CJ, Brookfield FA, Gazi L, Gyles SL, Hay PA, Hunter MG, Middlemiss D, Whittaker M, Xue L, Pettipher R: Indole-3-acetic acid antagonists of the prostaglandin D₂ receptor CRTH2. *J Med Chem* 2005;48:6174–6177.
- 136 Ulven T, Kostenis E: Minor structural modifications convert the dual TP/CRTH2 antagonist ramatroban into a highly selective and potent CRTH2 antagonist. *J Med Chem* 2005;48:897–900.
- 137 Birkinshaw TN, Teague SJ, Beech C, Bonnert RV, Hill S, Patel A, Reakes S, Sangane H, Dougall IG, Phillips TT, Salter S, Schmidt J, Arrowsmith EC, Carrillo JJ, Bell FM, Paine SW, Weaver R: Discovery of potent CRTH2 (DP₂) receptor antagonists. *Bioorg Med Chem Lett* 2006;16:4287–4290.
- 138 Stearns BA, Baccei C, Bain G, Broadhead A, Clark RC, Coate H, Evans JF, Fagan P, Hutchinson JH, King C, Lee C, Lorrain DS, Prasit P, Prodanovich P, Santini A, Scott JM, Stock NS, Truong YP: Novel tricyclic antagonists of the prostaglandin D₂ receptor DP₂ with efficacy in a murine model of allergic rhinitis. *Bioorg Med Chem Lett* 2009;19:4647–4651.
- 139 Sandham DA, Adcock C, Bala K, Barker L, Brown Z, Dubois G, Budd D, Cox B, Fairhurst RA, Furegati M, Leblanc C, Manini J, Profit R, Reilly J, Stringer R, Schmidt A, Turner KL, Watson SJ, Willis J, Williams G, Wilson C: 7-azaindole-3-acetic acid derivatives: potent and selective CRTH2 receptor antagonists. *Bioorg Med Chem Lett* 2009;19:4794–4798.
- 140 Bauer PH, Cheng JB, Gladue RP, Li B, Neote KS, Zhang J: Methods for the identification of compounds useful for the treatment of disease states mediated by prostaglandin D₂. *Eur Patent No EP1170594 A2*. 2002.
- 141 Bonnert RV, Patel A, Thom S: Phenoxyacetic acid derivatives. *Int Patent No WO2005/018529A3*. 2005.
- 142 Sandham DA, Aldcroft C, Baettig U, Barker L, Beer D, Bhalay G, Brown Z, Dubois G, Budd D, Bidlake L, Campbell E, Cox B, Everatt B, Harrison D, Leblanc CJ, Manini J, Profit R, Stringer R, Thompson KS, Turner KL, Tweed MF, Walker C, Watson SJ, Whitebread S, Willis J, Williams G, Wilson C: 2-cycloalkyl phenoxyacetic acid CRTH2 receptor antagonists. *Bioorg Med Chem Lett* 2007;17:4347–4350.
- 143 Awad MMA, Bazin M, Feru F, Goldstein SW, Kuhn CF: Tetrahydroquinoline derivatives as CRTH2 antagonists. *Int Patent No WO2004/035543*. 2004.
- 144 Mimura H, Ikemura T, Kotera O, Sawada M, Tashiro S, Fuse E, Ueno K, Manabe H, Ohshima E, Karasawa A, Miyaji H: 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 D₂. *J Pharmacol Exp Ther* 2005;314:244–251.

- 145 Barnes NB, Pavord IP, Cuchalin AC, et al: A randomised, double-blind, placebo-controlled study of the CRTH2 antagonist OC000459 on moderate persistent asthma (abstract 3267). ERS Annual Congress, Vienna, 2009.
- 146 Torisu K, Kobayashi K, Iwahashi M, Egashira H, Nakai Y, Okada Y, Nanbu F, Ohuchida S, Nakai H, Toda M: Development of a prostaglandin D₂ receptor antagonist: discovery of a new chemical lead. *Eur J Med Chem* 2005;40:505–519.
- 147 Sturino CF, O'Neill G, Lachance N, Boyd M, Berthelette C, Labelle M, Li L, Roy B, Scheigetz J, Tsou N, Aubin Y, Bateman KP, Chauret N, Day SH, Lévesque JF, Seto C, Silva JH, Trimble LA, Carrière MC, Denis D, Greig G, Kargman S, Lamontagne S, Mathieu MC, Sawyer N, Slipetz D, Abraham WM, Jones T, McAuliffe M, Piechuta H, Nicoll-Griffith DA, Wang Z, Zamboni R, Young RN, Metters KM: Discovery of a potent and selective prostaglandin D₂ receptor antagonist. *J Med Chem* 2007;50:794–806.
- 148 Beaulieu C, Guay D, Wang Z, Leblanc Y, Roy P, Dufresne C, Zamboni R, Berthelette C, Day S, Tsou N, Denis D, Greig G, Mathieu MC, O'Neill G: Identification of prostaglandin D₂ receptor antagonists based on a tetrahydropyridoindole scaffold. *Bioorg Med Chem Lett* 2008;18:2696–2700.
- 149 Philip G, van Adelsberg J, Loeys T, Liu N, Wong P, Lai E, Dass SB, Reiss TF: Clinical studies of the DP₁ antagonist laropiprant in asthma and allergic rhinitis. *J Allergy Clin Immunol* 2009;124:942–948.e9.
- 150 Kamanna VS, Ganji SH, Kashyap ML: The mechanism and mitigation of niacin-induced flushing. *Int J Clin Pract* 2009;63:1369–1377.
- 151 Jones RL, Giembycz MA, Woodward DF: Prostanoid receptor antagonists: development strategies and therapeutic applications. *Br J Pharmacol* 2009;158:104–145.
- 152 Liu J, Fu Z, Wang Y, Schmitt M, Huang A, Marshall D, Tonn G, Seitz L, Sullivan T, Lucy Tang H, Collins T, Medina J: Discovery and optimization of CRTH2 and DP dual antagonists. *Bioorg Med Chem Lett* 2009;19:6419–6423.