Prostaglandin D\textsubscript{2} (PGD\textsubscript{2}) is released following exposure of asthmatics to allergen and acts via the adenylyl cyclase–coupled receptor for PGD\textsubscript{2} (DP receptor). In this study, it is reported that human eosinophils possess this receptor, which would be expected to inhibit their activation. In contrast, it was found that prostaglandin D\textsubscript{2} is a potent stimulator of eosinophil chemotaxis, actin polymerization, CD11b expression, and L-selectin shedding. These responses are specific for eosinophils, as neutrophils display little or no response to prostaglandin D\textsubscript{2}. They were not due to interaction with receptors for other prostanoids, as prostaglandins E\textsubscript{2} and F\textsubscript{2\alpha}, U46619 (a thromboxane A\textsubscript{2} analogue), and carbaprostacyclin (a prostacyclin analogue) displayed little or no activity. Furthermore, they were not shared by the selective DP receptor agonist BW245C and were not prevented by the selective DP receptor antagonist BWA868C, indicating that they were not mediated by DP receptors. In contrast, the prostaglandin D\textsubscript{2} metabolite 13,14-dihydro-15-oxoprostaglandin D\textsubscript{2} induced eosinophil activation but did not stimulate DP receptor–mediated adenosine 3’\,5’–cyclic monophosphate (cAMP) formation. These results indicate that in addition to the classic inhibitory DP\textsubscript{1} receptor, eosinophils possess a second, novel DP\textsubscript{2} receptor that is associated with PGD\textsubscript{2}–induced cell activation. These 2 receptors appear to interact to regulate eosinophil responses to PGD\textsubscript{2} as blockade of DP\textsubscript{1} receptor–mediated cAMP production by BWA868C resulted in enhanced DP\textsubscript{2} receptor–mediated stimulation of CD11b expression. The balance between DP\textsubscript{1} and DP\textsubscript{2} receptors could determine the degree to which prostaglandin D\textsubscript{2} can activate eosinophils and may play a role in eosinophil recruitment in asthma. (Blood. 2001;98:1942-1948) © 2001 by The American Society of Hematology

Introduction

Prostaglandins (PGs), formed by the actions of cyclo-oxygenases 1 and 2 on arachidonic acid, have biological effects on many types of cells through the actions of 8 known receptors. PGE\textsubscript{2} has 4 receptor subtypes (EP\textsubscript{1-4} receptors), whereas PGs D\textsubscript{2}, F\textsubscript{2\alpha}, I\textsubscript{2}, and thromboxane (TX) A\textsubscript{2} each have a single receptor (DP, FP, IP, and TP receptors, respectively).\textsuperscript{1} The DP receptor is coupled positively to adenylyl cyclase through G\textsubscript{s},\textsuperscript{1} and this results in a strong inhibitory effect on platelet aggregation\textsuperscript{2} as well as bronchodilator\textsuperscript{3} and vasodilator\textsuperscript{4} effects in humans. The DP receptor and lipocalin-type PGD\textsubscript{synthase are both abundant in the central nervous system, where PGD\textsubscript{2} plays a role in regulating sleep\textsuperscript{5} and pain perception.\textsuperscript{6}

Despite the DP receptor–mediated bronchodilator effect of PGD\textsubscript{2}, there is evidence that PGD\textsubscript{2} may contribute to the pathogenesis of asthma. Hematopoietic-type PGD\textsubscript{2} synthase is abundant in mast cells,\textsuperscript{7} dendritic cells,\textsuperscript{8} and certain subpopulations of T\textsubscript{H}2 lymphocytes,\textsuperscript{9} all of which play critical roles in this disease.\textsuperscript{10} Hematopoietic-type PGD\textsubscript{2} synthase is abundant in mast cells,\textsuperscript{7} dendritic cells,\textsuperscript{8} and certain subpopulations of T\textsubscript{H}2 lymphocytes,\textsuperscript{9} all of which play critical roles in this disease.\textsuperscript{10} Hematopoietic-type PGD\textsubscript{2} synthase is abundant in mast cells,\textsuperscript{7} dendritic cells,\textsuperscript{8} and certain subpopulations of T\textsubscript{H}2 lymphocytes,\textsuperscript{9} all of which play critical roles in this disease.\textsuperscript{10} PGD\textsubscript{2} is released from immunologically stimulated mast cells\textsuperscript{11} and T\textsubscript{H}2 cells,\textsuperscript{9} and its levels in bronchoalveolar lavage fluid increase dramatically following allergen challenge of asthmatic subjects.\textsuperscript{12} Recent evidence that DP receptor knockout mice are resistant to the pulmonary effects of antigen challenge provides further support for a role of PGD\textsubscript{2} in asthma.\textsuperscript{13} Pulmonary infiltration of eosinophils and lymphocytes, levels of T\textsubscript{H}2 lymphocyte-derived cytokines, and hyperresponsiveness were all dramatically lower in the DP receptor–deficient mice compared with control wild-type mice.\textsuperscript{13} The reduction in eosinophil recruitment is interesting in view of the key role played by these cells in the pathophysiology of asthma owing to their release of proinflammatory cytokines, bronchoconstrictr cytoseulin leukotrienes (LTs), degradative enzymes, and other factors.\textsuperscript{14} Furthermore, in dogs, PGD\textsubscript{2} caused a rapid reduction in circulating eosinophil levels,\textsuperscript{15} whereas tracheal superfusion of PGD\textsubscript{2} in vivo induced the recruitment of eosinophils but not neutrophils into the superfusate.\textsuperscript{16} It is unclear from the above studies whether the in vivo effects of PGD\textsubscript{2} are due to direct effects on eosinophils or are caused by the release of mediators from other cells. However, PGD\textsubscript{2} has been reported to directly stimulate calcium mobilization and LTC\textsubscript{4} release in vitro in human eosinophils.\textsuperscript{17}

The stimulatory effects of PGD\textsubscript{2} on eosinophils are somewhat surprising, as this prostaglandin is thought to act via DP receptor–mediated stimulation of adenylyl cyclase.\textsuperscript{1} A number of studies have clearly shown that agents that raise adenosine 3’\,5’–cyclic monophosphate (cAMP) levels in eosinophils inhibit various responses, including LTC\textsubscript{4} release, chemotaxis, and degranulation.\textsuperscript{18,19} This raises the possibility that PGD\textsubscript{2} can activate eosinophils by a mechanism unrelated to the DP receptor. The objectives of the current study were to determine whether PGD\textsubscript{2} has a direct chemotactic effect on eosinophils and, if so, to investigate the hypothesis that this effect is mediated by a novel receptor for this compound.
Materials and methods

Materials

Prostaglandins and BW245C were purchased from Cayman Chemical (Ann Arbor, MI). BWA868C was kindly provided by Glaxo Wellcome (Stevenage, United Kingdom), and 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-ETE) was synthesized chemically.20 5-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)phallacidin (NB-D-phallacidin) was obtained from Molecular Probes (Eugene, OR). Fluorescein isothiocyanate (FITC)—labeled mouse anti-human CD11b (Becton Dickinson Facscalibur instrument). For personal use only.on September 14, 2017. by guest

Preparation of blood cells

Unfractionated leukocytes were prepared by removal of red blood cells from whole blood, obtained from healthy human subjects, by treatment with Dextran T-500 (Amersham-Pharma Biotech, Piscataway, NJ) for 45 minutes at 4°C.21 This preparation was used for measurements of CD11b and L-selectin. Granulocytes were prepared by centrifugation of red cells–depleted leukocytes over Ficoll-Paque (Amersham-Pharma Biotech), followed by removal of remaining red cells by hypotonic lysis as described previously.21 This fraction was used for experiments on neutrophil chemotaxis. Purified eosinophils were obtained by treating granulocytes with anti-CD16–labeled paramagnetic microbeads (Miltenyi Biotec, Bergisch-Gladbach, Germany) and removing the labeled neutrophils on a column containing a steel matrix placed in a permanent magnet (MACS, Miltenyi Biotec). Eosinophils (95% ± 4% pure) were obtained in the pass-through fraction22 and were used for experiments evaluating chemotaxis, actin polymerization, and cAMP formation.

Measurement of chemotactic responses

Cell migration was measured as previously described23 by means of 48-well microchemotaxis chambers (Neuro Probe, Cabin John, MD) and Sartorius cellulose nitrate filters (8-μm pore size; 140-μm thickness) (Neuro Probe). Agonists were added to the bottom well in a volume of 30 μL phosphate-buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH2PO4, and 8.1 mM Na2HPO4 at a pH of 7.4) containing 1 mM CaCl2, 1 mM MgCl2, and 0.3% bovine serum albumin, whereas eosinophils or neutrophils (150,000 cells in 55 μL RPMI containing 0.4% ovalbumin) were added to each of the top wells. Following incubation for 2 hours at 37°C, the filters were fixed with mercuric chloride and stained with hematoxylin and chromotrope 2R.24 The numbers of cells on the bottom surfaces of the filters were counted in 5 different fields at a magnification of 400 × for each incubation, each of which was performed in duplicate.

Measurement of actin polymerization

Eosinophil F-actin was measured by means of NB-D-phallacidin, which binds strongly to F-actin, the polymerized form of actin, but not to unpolymerized G-actin.25 Eosinophils (3 × 107 cells in 260 μL) were incubated with agonists for 20 seconds, followed by fixation with formaldehyde (30 μL of a 37% solution) at room temperature for 15 minutes. F-actin was then stained by incubation with lysophosphatidylcholine (30 μg in 15 μL) and NB-D-phallacidin (49 pmol in 6.2 μL; final concentration, 0.15 μM) overnight in the dark at 4°C. The cells were then centrifuged at 700g for 5 minutes and resuspended in PBS (0.5 mL). The fluorescence intensity of the stained eosinophils was quantified by flow cytometry with a Becton Dickinson Facscalibur instrument.

Measurement of surface expression of CD11b and L-selectin

CD11b and L-selectin were measured as described previously.26 Unfractionated leukocytes (0.5 mL; 107/mL) in PBS containing Ca2+ and Mg2+ were incubated with agonists for either 10 minutes (L-selectin) or 15 minutes (CD11b). The incubations were terminated by the addition of ice-cold Facscalibur (Becton Dickinson) and centrifugation. Following incubation of the pellets for 10 minutes at 4°C with mouse plasma (5 μL), the cells were incubated for 30 minutes at 4°C with PE-labeled anti–VLA-4 (5 μL) along with an FITC-labeled antibody (10 μL) to either CD11b or L-selectin or the appropriate isotype-matched control FITC-labeled antibody. After incubation with OptiLyse C (0.25 mL) (Beckman-Coulter) for 15 minutes, the cells were centrifuged and fixed in 1% formaldehyde in PBS (0.4 mL). The distribution of fluorescence intensities among 60,000 cells was measured by flow cytometry. Eosinophils were gated out on the basis of their granularity (high side scatter) and labeling with anti–VLA-4 (PE fluorescence). CD11b or L-selectin was then measured in the eosinophil region on the basis of fluorescence due to FITC as previously described.26

Results

PGD2 is a potent and selective eosinophil chemoattractant

We first investigated the effects of PGD2 on the migration of human eosinophils, which were purified from a polymorphonuclear cell fraction by removal of neutrophils with iron-labeled anti-CD16.22 Chemotactic effects were evaluated by means of a modified Boyden chamber assay in which cells that had migrated through a nitrocellulose filter were stained and counted. As a positive control, we used 5-oxo-ETE, which we have shown to be the most efficacious eosinophil chemoattractant among lipid mediators, inducing a maximal response about 3 times greater than that of platelet-activating factor (PAF).23 and about 50% higher than the chemokine eotaxin.26 The present experiments indicate that PGD2 is a potent eosinophil chemoattractant with a median effective concentration (EC50) of 5 nM (Figure 1A). Although the maximal response of PGD2 is about one third that of 5-oxo-ETE, it is very similar to that of PAF.23 Furthermore, unlike 5-oxo-ETE24 and PAF,26 PGD2 does not have an appreciable effect on neutrophil migration (Figure 1B), which puts it in a unique position among lipid mediators as a selective eosinophil chemoattractant. This selective effect on eosinophil activation is reminiscent of the potent eosinophil chemoattractant eotaxin.26

The chemotactic effects of PGD2 on eosinophils are not mediated by receptors for other prostanooids

The stimulatory effect of PGD2 on eosinophils raised the question of whether it is acting via the classical DP receptor, which signals through Gs-mediated stimulation of adenylyl cyclase, as this would be expected to inhibit, rather than induce, eosinophil activation.18,19 As stimulatory effects of PGD2 have often been attributed to other prostanooid receptors, including FP11,12 and TP13,34 receptors, we tested the effects of PGF2α, PGE2, and U46619, a TP receptor...
the numbers of different individuals indicated for PGD2 (cells per 5 high-power fields. Experiments were performed in duplicate on cells from 6 cells migrating through the filter in control experiments with vehicle alone was 26 relatively little additional effect observed at higher concentrations. The number of concentration of 1 μM 5-oxo-ETE induces close to the maximal response, with relatively little additional effect observed at higher concentrations. The number of cells migrating through the filter in control experiments with vehicle alone was 26 ± 4 cells per 5 high-power fields. Experiments were performed in duplicate on cells from the numbers of different individuals indicated for PGD2 (●; n = 8), 5-oxo-ETE (○, 5o-ETE; n = 8), PGF2a (▲; n = 4), PGE2 (■; n = 3), and the TXA2 agonist U46619 (□; n = 3).

**PGD2 selectively stimulates CD11b expression on eosinophils**

We also investigated the effects of PGD2 on other processes involved in the infiltration of eosinophils into tissues, including expression of the cellular adhesion molecule CD11b, which is important for the adherence of eosinophils to endothelial cells. Measurement of CD11b permitted more extensive experiments to be conducted because this does not require purification of eosinophils, which are present in blood in relatively small numbers. With the use of flow cytometry, it is possible to make simultaneous measurements on both eosinophils and neutrophils with the use of relatively small numbers of unfractionated leukocytes, while at the same time minimizing the artifactual activation of cells that occurs during purification.

Eosinophils were gated out from other cells on the basis of high side scatter owing to their granularity and labeling with PE-labeled anti–VLA-4 as shown by the dot plot in the inset to Figure 2A, and were readily distinguishable from neutrophils (Figure 2B, inset). PGD2 (1 μM) increased the expression of CD11b on eosinophils to about the same extent as an identical concentration of 5-oxo-ETE (Figure 2A). In contrast, PGD2 had only a very small effect on neutrophil expression of CD11b, whereas 5-oxo-ETE strongly stimulated its expression on these cells (Figure 2B).

Concentration-response curves for the effects of PGD2 and other eicosanoids on CD11b expression by eosinophils are shown in Figure 3A. PGD2 (EC50: 5 nM) has a potency similar to that of 5-oxo-ETE (EC50: 7.5 nM) and a maximal response about 25% lower. PGE2 and the TP receptor agonist U46619 did not affect CD11b expression appreciably, whereas PGF2α had detectable effects only at concentrations of 100 nM or above and appeared to be about 2 orders of magnitude less potent than PGD2 (Figure 3A).

Similarly, the IP receptor agonist carbaprostacyclin was also without effect (data not shown). PGD2 had very little effect on agonist, on eosinophil migration. None of these compounds exhibited significant chemotactant effects on these cells, with the possible exception of PGF2α, at the highest concentration tested (Figure 1A). These results clearly indicate that PGD2 does not induce eosinophil migration by interacting with receptors for other prostanoids.

**CD11b expression by neutrophils**

CD11b expression was measured by flow cytometry on eosinophils (A) and neutrophils (B), which were distinguished from one another and from other leukocytes on the basis of side scatter (SSC), a measure of granularity, and staining with PE-labeled anti–VLA-4. Eosinophils and neutrophils were gated out as shown by the dot plots in the insets. Eosinophils are shown by the black dots in the inset to panel A, whereas neutrophils are shown by the black dots in panel B. CD11b expression by the gated cells was measured by means of FITC-labeled anti-CD11b. Histograms are shown for cells stained with an FITC-labeled isotype control antibody (black) and with FITC-labeled anti-CD11b for unstimulated cells (control; light gray shading), PGD2 (heavy line), and 5-oxo-ETE (5o-ETE, dark gray shading).

**PGD2 induces shedding of L-selectin from eosinophils but not neutrophils**

Activation of granulocytes by chemotactants often results in the shedding of L-selectin owing to the action of a metalloproteinase. Intact L-selectin was measured on eosinophils and neutrophils by

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**Figure 1. The selectivity of PGD2 as an eosinophil chemoattractant.** The chemotactic effects of PGD2 and other eicosanoids on human eosinophils (A) and human neutrophils (B) were examined by means of a modified Boyden chamber assay followed by staining with hematoxylin and chromotrope 2R and counting cells that had migrated through to the bottom of the filter under a microscope. The results are expressed as percentages (± SE) of the maximal responses to 5-oxo-ETE (493 ± 68 cells per 5 high-power fields). Although it is not apparent from the Figure, a concentration of 1 μM 5-oxo-ETE induces close to the maximal response, with relatively little additional effect observed at higher concentrations. The number of cells migrating through the filter in control experiments with vehicle alone was 26 ± 4 cells per 5 high-power fields. Experiments were performed in duplicate on cells from the numbers of different individuals indicated for PGD2 (●; n = 8), 5-oxo-ETE (○, 5o-ETE; n = 8), PGF2a (▲; n = 4), PGE2 (■; n = 3), and the TXA2 agonist U46619 (□; n = 3).

**Figure 2. Selective stimulation of CD11b expression on eosinophils by PGD2.** Concentration-response curves for the effects of PGD2 and other eicosanoids on CD11b expression by eosinophils are shown in Figure 3A. PGD2 (EC50: 5 nM) has a potency similar to that of 5-oxo-ETE (EC50: 7.5 nM) and a maximal response about 25% lower. PGE2 and the TP receptor agonist U46619 did not affect CD11b expression appreciably, whereas PGF2α had detectable effects only at concentrations of 100 nM or above and appeared to be about 2 orders of magnitude less potent than PGD2 (Figure 3A). Similarly, the IP receptor agonist carbaprostacyclin was also without effect (data not shown). PGD2 had very little effect on agonist, on eosinophil migration. None of these compounds exhibited significant chemotactant effects on these cells, with the possible exception of PGF2α, at the highest concentration tested (Figure 1A). These results clearly indicate that PGD2 does not induce eosinophil migration by interacting with receptors for other prostanoids.

**Figure 3. Effects of PGD2 and other eicosanoids on CD11b expression by eosinophils and neutrophils.** Concentration-response curves for the effects of PGD2 and other eicosanoids on CD11b expression by eosinophils are shown in Figure 3A. PGD2 (EC50: 5 nM) has a potency similar to that of 5-oxo-ETE (EC50: 7.5 nM) and a maximal response about 25% lower. PGE2 and the TP receptor agonist U46619 did not affect CD11b expression appreciably, whereas PGF2α had detectable effects only at concentrations of 100 nM or above and appeared to be about 2 orders of magnitude less potent than PGD2 (Figure 3A). Similarly, the IP receptor agonist carbaprostacyclin was also without effect (data not shown). PGD2 had very little effect on agonist, on eosinophil migration. None of these compounds exhibited significant chemotactant effects on these cells, with the possible exception of PGF2α, at the highest concentration tested (Figure 1A). These results clearly indicate that PGD2 does not induce eosinophil migration by interacting with receptors for other prostanoids.

**PGD2 selectively stimulates CD11b expression on eosinophils**

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gating out these cells by means of flow cytometry as discussed above for CD11b. PGD2 induced L-selectin shedding from eosinophils, although to a lesser extent than 5-oxo-ETE (Figure 4A). PGE2 did not appreciably affect L-selectin on eosinophils. In contrast, PGD2, like PGE2, had no effect on L-selectin shedding in neutrophils, whereas 5-oxo-ETE strongly stimulated this response (Figure 4B).

**PGD2 stimulates actin polymerization in eosinophils**

Migration of leukocytes into tissues involves dramatic changes in cell shape that are dependent on actin polymerization. PGD2 strongly induced the formation of polymerized F-actin in purified eosinophils with an EC50 (6.5 nM) and maximal response very similar to those of 5-oxo-ETE (EC50, 10 nM) (Figure 5). This is quite interesting in view of our recent findings that 5-oxo-ETE induces a significantly stronger actin polymerization response in eosinophils than a variety of other chemoattractants, including PAF26 and ejected. In contrast to PGD2, PGE2 had no effect on actin polymerization, whereas PGF2α was active only at the highest concentration tested (1 μM).

**Eosinophils possess DP receptors**

As it was not known whether eosinophils possess DP receptors, we investigated the effects of PGD2 and the highly selective DP receptor agonist BW245C on cAMP formation in these cells. BW245C has been reported to be slightly more potent than PGD2,38 to have over 300 times higher affinity for DP receptors compared with other prostanoid receptors.32 Both PGD2 and BW245C elevated cAMP levels in purified eosinophils, with the latter compound being slightly more potent (Figure 6A), clearly demonstrating the presence of classic Gs-coupled DP receptors on these cells. The effects of these compounds were comparable to that of PGE2, which stimulates adenyl cyclase by interacting with EP2 and EP4 receptors. The effects of PGD2 and BW245C on eosinophils were very similar to their effects on human platelets, which are known to possess DP receptors1 (Figure 6B). Consistent with previous reports,32 PGD2 was equipotent with PGD2 in stimulating cAMP formation in platelets, whereas BW245C was more potent. In contrast, the PGD2 metabolite 13,14-dihydro-15-oxo-PGD2 had no effect on cAMP formation in platelets.

**The effects of PGD2 on eosinophil migration and CD11b expression are not mediated by DP receptors**

The relative potencies of the above compounds on CD11b expression in eosinophils were quite different from their potencies in stimulating platelet adenyl cyclase. In contrast to its potent effects on platelet cAMP levels, BW245C has virtually no effect on CD11b expression (Figure 7A). PGJ2, which is equipotent to PGD2 in stimulating platelet adenyl cyclase, is only about one tenth as potent in stimulating eosinophil CD11b expression. Furthermore, 13,14-dihydro-15-oxo-PGD2 was nearly as potent as PGD2 in stimulating CD11b expression, despite its lack of activity in platelets. The relative potencies of PGD2, PGJ2, and BW245C in stimulating eosinophil migration were nearly identical to those for simulation of CD11b expression (Figure 7B). The striking differences in the structure-activity relationships for PGD2-induced stimulation of DP receptor–dependent cAMP formation and stimulation of CD11b and chemotactic responses provide compelling evidence that the latter effects are mediated by a novel stimulatory PGD2 receptor on eosinophils.
than PGD2 in stimulating CD11b expression on eosinophils but has a second PGD2 receptor. CD11b expression provides additional evidence for the existence of platelets. The specificity of the novel receptor for PGD2-related no effect on DP receptor–mediated stimulation of cAMP formation, and PGJ2 (described in the legend to Figure 1. The results are means ± SE of determinations on cells from 6 individuals and are expressed as percentages of the maximal responses to PGD2.

Interaction of PGD2 with classic DP receptors attenuates PGD2-stimulated eosinophil activation

The existence of both stimulatory and inhibitory PGD2 receptors on eosinophils raised the possibility that these receptors could interact to regulate eosinophil responses to PGD2, with classic DP receptors serving to limit PGD2-induced eosinophil activation. To test this hypothesis, we used the highly selective DP receptor antagonist BWA868C.32,39 Although BWA868C alone had no effect on CD11b expression at concentrations up to 1 μM, it strongly stimulated PGD2-induced CD11b expression, increasing the maximal response by about 60% (P < .001) (Figure 8A). The EC50 for PGD2 appeared to be reduced slightly, from 8 nM (PGD2 alone) to 4 nM, but this difference was not significant. In contrast, BWA868C (100 nM) strongly inhibited PGD2-induced cAMP formation in eosinophils (Figure 8B). The enhanced CD11b response to PGD2, in the presence of the DP receptor antagonist suggests that PGD2-induced stimulation of adenylyl cyclase can attenuate its effect on eosinophil activation, mediated by the novel PGD2 receptor. Furthermore, the failure of the DP receptor antagonist to inhibit PGD2-stimulated CD11b expression provides additional evidence for the existence of a second PGD2 receptor.

Discussion

The present study clearly demonstrates the presence of a novel PGD2 receptor on human eosinophils that results in activation of these cells. This is supported by several lines of evidence: (1) PGD2 stimulates actin polymerization, CD11b expression, L-selectin shedding, and chemotaxis in eosinophils; (2) these effects are not mediated by receptors for other prostanoids, since they cannot be reproduced by agonists for these receptors, and (3) these effects are not mediated by the classic DP receptor, as they are neither induced by the potent DP receptor agonist BW245C nor inhibited by the potent DP receptor antagonist BWA868C. Furthermore, the PGD2 metabolite 13,14-dihydro-15-oxo-PGD2 is only slightly less potent than PGD2 in stimulating CD11b expression on eosinophils but has no effect on DP receptor–mediated stimulation of cAMP formation in platelets. The specificity of the novel receptor for PGD2–related ligands (PGD2 equals or exceeds 13,14-dihydro-15-oxo-PGD2, which equals or exceeds PGJ2, which is much greater than BW245C) is quite different from that of the classic DP receptor (BW245C equals or exceeds PGD2 = PGJ2, which is much greater than 13,14-dihydro-15-oxo-PGD2). It does not interact appreciably with ligands for EP (PGE2), IP (carbaprostacyclin), or TP (U46619) receptors. It would seem likely that PGF2α has weak activity at the novel PGD2 receptor, as it can activate eosinophils with about 1% of the potency of PGD2. It would seem unlikely that this is due to activation of FP receptors because of the low potency of PGF2α in activating eosinophils. Because of the specificity of this novel receptor for PGD2, we propose the designation DP2 receptor. The classic Gs-coupled DP receptor would then be designated as the DP receptor. In addition to DP2 receptors, we have shown for the first time that eosinophils also possess classic DP1 receptors coupled to adenylyl cyclase. These receptors display the same specificity for PGD2-like compounds as the platelet DP1 receptor, as shown in the present study and various other studies.32,38 However, the magnitude of the cAMP response appeared to be less than that observed for platelets, possibly owing to limitations of cell numbers and rapid metabolism of cAMP by phosphodiesterase-4, which is known to be present in eosinophils.40 It was necessary to inhibit this enzyme with rolipram to observe the effect of PGD2 on cAMP levels in eosinophils, but this was not necessary with platelets. The presence of both DP1 and DP2 receptors on eosinophils could explain previous reports that PGD2 has both stimulatory and inhibitory effects on these cells.41

The ability of PGD2 to activate eosinophils while at the same time stimulating adenylyl cyclase is intriguing, as increased cAMP levels would be expected to attenuate eosinophil responses. The phosphodiesterase-4 inhibitor rolipram has been shown to inhibit eosinophil migration and CD11b expression in response to eotaxin, PAF, and C5a. Stimulation of adenylyl cyclase with forskolin or isoproterenol or addition of dibutyryl cAMP had similar effects. This raised the possibility that PGD2 could have opposing actions on eosinophils and that its stimulatory effect could be blunted by concomitant stimulation of adenylyl cyclase, especially at higher concentrations of PGD2. This hypothesis was confirmed
by the finding that blocking DP1 receptor–mediated cAMP formation in eosinophils with BWA868C resulted in a markedly enhanced DP2 receptor–mediated CD11b response to PGD2. Thus, it would appear that DP1 receptors could serve to negatively regulate DP2 receptor–mediated responses in eosinophils (Figure 9). The balance between inhibitory DP1 and stimulatory DP2 receptors is likely to determine the final response of these cells to PGD2. A reduction in expression of DP1 receptors, for example, could enhance PGD2-mediated stimulation of eosinophils, which could be important in diseases associated with eosinophil infiltration, such as asthma.

In contrast to its stimulatory effects on eosinophils, PGD2 appears to have an inhibitory effect on neutrophils. In the present study, we found that PGD2 induces little or no migration, CD11b expression, or L-selectin shedding in these cells. On the other hand, PGD2 has been reported to stimulate cAMP formation and to inhibit PAF responses in neutrophils.45 This suggests that neutrophils possess DP1 receptors, but lack appreciable numbers of DP2 receptors. It is possible that the opposite actions of PGD2 on neutrophils and eosinophils could further contribute to the selective accumulation of eosinophils in some conditions, such as asthma.

Although the effects of stimulation of DP1 receptors on neutrophils and eosinophils would be considered to be anti-inflammatory, it is clear that the overall role of DP1 receptors in inflammation is much more complex, as it also appears to be involved in promoting inflammation. Deletion of this receptor in mice markedly reduced the pulmonary eosinophilia and hyperresponsiveness that occurred following antigen challenge of control animals. This was accompanied by reduced levels of the Th2 cytokines interleukin (IL)–4, IL-5, and IL-13 but no change in the following antigen challenge. Because the DP1 receptor appears to contribute to eosinophil infiltration, it is likely to determine the final response of these cells to PGD2. Moreover, the present study demonstrates an interaction between inhibitory DP1 receptors and stimulatory DP2 receptors, resulting in attenuated DP1-mediated responses to PGD2.

In conclusion, we have shown that eosinophils possess both a novel DP2 receptor, which is responsible for the chemoattractant effect of PGD2, and the classic DP1 receptor, which appears to play a regulatory role in these cells. The balance between these receptors is likely to regulate the response of these cells to PGD2 and may be important in asthma. The role of DP1 receptors is obviously complex, as they appear to contribute to eosinophil infiltration indirectly through effects on Th2 cell cytokine production.11 The direct and indirect effects of PGD2 on eosinophil migration as well as its strategic location in mast cells and antigen presenting cells suggest an important role for this prostaglandin in asthma.

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Prostaglandin D$_2$ is a potent chemoattractant for human eosinophils that acts via a novel DP receptor

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