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The Role of the Prostaglandin D₄ Receptor, DP, in Eosinophil Trafficking

Petra Schratl,* Julia F. Royer,* Evi Kostenis,† Trond Ulven,§ Eva M. Sturm,* Maria Waldhoer,* Gerald Hoeffer,† Rufina Schuligoi,* Irmgard Th. Lippe,* Bernhard A. Peskar,* and Akos Heinemann²*

Prostaglandin (PG) D₄ is a major mast cell product that acts via two receptors, the D-type prostanoid (DP) and the chemotactic receptor-homologous molecule expressed on Th2 cells (CRTH2) receptors. Whereas CRTH2 mediates the chemotaxis of eosinophils, basophils, and Th2 lymphocytes, the role of DP has remained unclear. We report in this study that, in addition to CRTH2, the DP receptor plays an important role in eosinophil trafficking. First, we investigated the release of eosinophils from bone marrow using the in situ perfused guinea pig hind limb preparation. PGD₂ induced the rapid release of eosinophils from bone marrow and this effect was inhibited by either the DP receptor antagonist BW868c or the CRTH2 receptor antagonist ramatroban. In contrast, BW868c did not inhibit the release of bone marrow eosinophils when this was induced by the CRTH2-selective agonist 13,14-dihydro-15-keto-PGD₂. In additional experiments, we isolated bone marrow eosinophils from the femoral cavity and found that these cells migrated toward PGD₂. We also observed that BW868c inhibited this response to a similar extent as ramatroban. Finally, using immunohistochemistry we could demonstrate that eosinophils in human bone marrow specimens expressed DP and CRTH2 receptors at similar levels. Eosinophils isolated from human peripheral blood likewise expressed DP receptor protein but at lower levels than CRTH2. In agreement with this, the chemotaxis of human peripheral blood eosinophils was inhibited both by BW868c and ramatroban. These findings suggest that DP receptors comigrate with CRTH2 the mobilization of eosinophils from bone marrow and their chemotaxis, which might provide the rationale for DP antagonists in the treatment of allergic disease. *The Journal of Immunology, 2007, 179: 4792–4799.*

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pig (30), and DP-deficient mice exhibit reduced pulmonary inflammation in response to allergen (31). These in vivo studies hence point to a proinflammatory role of DP but are difficult to explain based on the known functional responses of DP receptor activation.

We have previously shown that 1,2-PGJ2 and PGD2 markedly prime eosinophils for chemotaxis toward other chemoattractants, such as the CCR3 ligand eotaxin, and that 1,2-PGJ2 is capable of mediating the rapid mobilization of eosinophils from the isolated hind limb of guinea pigs (8). These observations suggested novel mechanisms by which PGD2 or its metabolites generated at sites of allergic inflammation could act as systemic signals for the supply of eosinophils from the bone marrow and enhance their extravasation to the tissue. Hence we set out to investigate the differential roles of CRTH2 and DP in the mobilization of eosinophils from the bone marrow and chemotaxis. Our data demonstrate that the DP receptor plays an important modulator role in the recruitment of eosinophils into the tissue. Thus, DP antagonists might be useful to treat conditions associated with eosinophilic inflammation such as asthma or other allergic diseases.

Materials and Methods

Animals

Adult Dunkin-Harley guinea pigs (either sex, 350–450 g body weight) were obtained from the Research Institute for Laboratory Animal Breeding (Medical University of Vienna, Himberg, Austria).

Chemicals

All assay reagents were obtained from Sigma-Aldrich, unless specified. Assay buffer as used in the shape change and chemotaxis experiments was made from Dulbecco’s modified PBS (with 0.9 mM Ca2+ and 0.5 mM Mg2+; Invitrogen Life Technologies), 0.1% BSA, 10 mM HEPES, and 10 mM glucose (pH 7.4). For the perfusion of the guinea pig hind limb, modified Krebs-Ringer bicarbonate buffer was prepared with 10 mM Na2HPO4, 1.5 mM NaH2PO4, and 24 mM NaHCO3, and supplemented with 0.1% BSA, 10 mM glucose, 10 mM Na2HPO4, 1.5 mM NaH2PO4, and 24 mM NaHCO3, and supplemented with 0.1% BSA. Recombinant human eotaxin and synthetic guinea pig DR-2 and PGD2 were metabolically labeled with 2 μCi of [3H]Jadenine (Amersham; TRK311) in 24-well plates for 18–24 h at 37°C. They were then washed twice with PBS and stimulated with increasing concentrations of PGD2, in HEPES-buffered saline supplemented with 1 mM isobutylmethylxanthine at 30 min at 37°C. The reaction was stopped by adding 5% (w/v) ice-cold trichloroacetic acid supplemented with 0.1 mM cAMP and 0.1 mM ATP. [3H]cAMP was separated from the remaining nucleotides using anion exchange chromatography, and radioactivity was counted after addition of HyperSolv scintillation fluid (PerkinElmer Life and Analytical Sciences).

Leukocyte shape change assay

This study was approved by the Ethics Committee of the Medical University of Graz. Eosinophil shape change was recorded using human blood sampled from healthy volunteers or guinea pig blood obtained by cardiac puncture after an overdose of pentobarbital sodium (8). Ninety-microliter aliquots of citrated whole blood were stimulated with 10 μl of agonists for 4 min at 37°C. The samples were then transferred to ice and fixed with 250 μl of fixative solution followed by 100 μl of fixative solution channels (8). Shape change was determined as the increase of forward scatter compared with vehicle stimulation. The forward scatter values are shown as dimensionless arbitrary units.

Chemotaxis of human eosinophils

Preparations of polymorphonuclear leukocytes (PMNL) were prepared by dextran sedimentation and Histopaque gradients, and eosinophils were further purified by negative magnetic selection using an Ab mixture directed against CD14, CD16, CD19, CD56, glycothulin A from StemCell Technologies. The purity of the eosinophil preparations was usually above 97%, the contaminating cells being mononuclear cells. The viability of the cells was >98%. Thirty microliter of assay buffer or agonists were placed into the bottom wells of a 48-well micro-Bouydon chemotaxis chamber (NeuroProbe). Eosinophils were suspended in assay buffer at 2 × 106/ml, and 50 μl of the suspension were placed into the top wells of the plate, which was separated from the bottom wells by a 5-μm pore size polypyrrolidone-free polycarbonate filter. Baseline migration was determined in wells containing only assay buffer. The chamber was incubated at 37°C in a humidified CO2 incubator for 1 h and the membrane was carefully removed. Cells that had migrated to the bottom wells were enumerated by flow cytometry (FACSCalibur, BD Biosciences), and contaminating cells were gated out by side scatter and autofluorescence as previously described (8).

Chemotaxis of guinea pig bone marrow eosinophils

The femoral bone cavity was flushed, erythrocytes were lysed by hypotonic shock, and PMNLs were prepared by Histopaque gradients. The cells were resuspended in RPMI 1640 (1% FCS) at 10 × 106/ml, and 100-μl aliquots of the suspension were placed into Transwell inserts with 5-μm pore size polypyrrolidone-free polycarbonate filters (Corning). The cells were allowed to migrate toward 600 μl of assay buffer or agonists in the bottom wells of 24-well tissue culture plates for 1 h at 37°C in a humidified CO2 incubator. Baseline migration was determined in wells containing only assay buffer. Cells that had migrated to the bottom wells were enumerated by flow cytometry (FACSCalibur, BD Biosciences), and contaminating cells were gated out by side scatter and autofluorescence as previously described (8).

Table I. Compounds used in this study and their receptor selectivity

<table>
<thead>
<tr>
<th>Name</th>
<th>Receptor of Selectivity and Rank Order of Affinity</th>
<th>Activity</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZK110841</td>
<td>DP</td>
<td>Agonist</td>
<td>(6)</td>
</tr>
<tr>
<td>BWA868c</td>
<td>DP</td>
<td>Agonist</td>
<td>(7)</td>
</tr>
<tr>
<td>SQ299548</td>
<td>TP</td>
<td>Antagonist</td>
<td>(51)</td>
</tr>
<tr>
<td>Ramatroban</td>
<td>TP ≈ CRTH2</td>
<td>Antagonist</td>
<td>(52)</td>
</tr>
<tr>
<td>DK-PGD2</td>
<td>CRTH2</td>
<td>Agonist</td>
<td>(6)</td>
</tr>
<tr>
<td>PGE2</td>
<td>CRTH2</td>
<td>Antagonist</td>
<td>(49)</td>
</tr>
<tr>
<td>BW245c</td>
<td>DP</td>
<td>Agonist</td>
<td>(6)</td>
</tr>
<tr>
<td>12PGJ2</td>
<td>DP</td>
<td>Agonist</td>
<td>(50)</td>
</tr>
<tr>
<td>Ramatroban</td>
<td>DP</td>
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<td>Antagonist</td>
<td>(52)</td>
</tr>
</tbody>
</table>
In situ perfusion of the guinea pig hind limb

The guinea pig hind limb was perfused as previously described (8, 34). The external iliac artery and vein were exposed and the caudal abdominal artery, superficial iliac circumflex artery, and pudendoepigastric trunk along with their satellite veins were ligated. Polyethylene cannulas (0.8 mm outside diameter) were inserted into the external iliac artery and vein. Modified Krebs-Ringer bicarbonate buffer (gassed with 95% O₂ and 5% CO₂, 37°C) was infused at 4 ml/min via the arterial cannula and removed from the venous cannula using a peristaltic pump. After an equilibration period of 20 min, the perfusate fractions were collected every 10 min and centrifuged at 300 × g for 10 min. The cell pellet was resuspended in Kimura’s stain and nucleated leukocytes and Kimura-positive eosinophils were counted in a Neubauer hemacytometer.

Immunofluorescence microscopy

The DP and CRTH2 receptors were visualized on human peripheral blood eosinophils by immunofluorescence following a double antibody staining protocol. For visualization of the CRTH2 receptor, a suspension of unfixed 1 × 10⁷ PMNL in PBS was blocked with human IgG (Sigma-Aldrich) for 30 min at 4°C. Cells were washed once with PBS by centrifugation at 400 × g for 5 min and then incubated with rat mAb against CRTH2 (clone BM16; BD Biosciences) diluted 1/25, or the isotype control rabbit IgG (BD Biosciences), and subsequently incubated with polyclonal rabbit anti-rabbit IgG; Invitrogen LifeTechnologies; dilution 1/1000, for 30 min in the dark at 4°C with the secondary Ab (Cy3-conjugated goat anti-rabbit secondary Ab; Chemicon; dilution 1/250). PMNL were washed twice with PBS, and cells were cyto-spun on cover slips. Subsequently, PMNL were fixed with 3.7% formaldehyde in PBS at 4°C overnight. After washing, the cells were blocked with powdered milk and human IgG (Sigma-Aldrich) in PBS for 60 min at room temperature. Cells were washed twice with PBS and then incubated with rabbit polyclonal Ab against the DP receptor (Sigma-Aldrich) diluted 1/125 or rabbit IgG (Sigma-Aldrich) as negative control in PBS with powdered milk. After incubation with primary Ab, PMNL were washed six times with PBS and then incubated for 30 min in the dark at room temperature with secondary Ab (AlexaFluor 488 goat anti-rabbit IgG; Invitrogen LifeTechnologies; dilution 1/500). Finally, after six washes with PBS, the nuclei were stained with 4’,6’-diamidino-2-phenylindole (DAPI; Invitrogen LifeTechnologies). The fluorescent signal was recorded with an Olympus IX70 fluorescence microscope and an Olympus UPlanApo - 60/1.20 lens, using Olympus DP50-CU digital camera and Olympus CellP software. Images were further processed also with CellP for contrast and brightness adjustments.

Immunohistochemistry

The expression of DP and CRTH2 receptors was investigated in human bone marrow. Sections (3 μm) were prepared from EDTA decalcified material, deparaffinized by xylene, deparaffinized by xylene, rehydrated in graded alcohols, and sublimated to microwave epitope retrieval (10 min at 750 W using pH 9.0 Ag retrieval buffer 2367 obtained from Dako). After treatment in 3% H₂O₂ for 10 min, sections were incubated for 30 min using goat polyclonal Ab against eosinophil peroxidase (EPX; Santa Cruz Biotechnology) diluted 1/25, rabbit polyclonal Ab against the DP receptor (Sigma-Aldrich) diluted 1/200 and rat mAb against CRTH2 (clone BM16; BD Biosciences) diluted 1/10 in Dako Ab diluent. After enhancement using MultiLink (Dako E0453) diluted 1/100 and biotinylated StreptABComplex (Dako K5001) or alkaline phosphatase red (Dako K5005) diluted 1/100 for 30 min each, detection was performed using peroxidase substrate chromogen ready-to-use (Dako K5001) or alkaline phosphatase (fast red) under microscopic control. After each development procedure, blocking was performed for 5 min using 3% H₂O₂ with extensive rinsing using Tris-HCl buffer (pH 7.6) in between. Images were taken on a Nikon Eclipse E600 microscope with a Nikon Plan 40×0.65 lens using Nikon DS-5M-U1 digital camera and Nikon Digital Sight DS-U1 software. Images were further processed with Adobe Photoshop CS, used for additional white balance, contrast, and brightness adjustments.

Calculations and data analysis

IC₅₀ and EC₅₀ values were determined by nonlinear regression analysis using the Prism 3.0 software (GraphPad Software). Antagonistic potencies in functional assays are given as pIC₅₀ values that were obtained by competing with increasing concentrations of inhibitor compound for an agonist concentration required to elicit ~75–80% of the maximal agonist efficacy. IC₅₀ values generated by this procedure are very closely matching the true affinity of antagonists determined by the analysis according to Arunlaksana and Schild (35). Data are shown as the mean ± SEM except where otherwise stated. Statistical comparisons of groups were performed using two-way ANOVA for repeated measurements or the Mann-Whitney U test. Probability values of p < 0.05 were considered statistically significant.

Results

Pharmacological characterization of BWA868c

While BWA868c has been investigated before with respect to its affinity and selectivity toward human DP and CRTH2 receptors (36), guinea pig DP has been characterized so far only in functional assays. Thus we expressed the guinea pig DP receptor in HEK293 and 1321N1 cells for binding experiments and assay of cAMP accumulation, respectively. As expected, BWA868c and the DP-selective agonist BW245c exhibited high affinity for the guinea pig DP receptor, with log Kᵢ values of −7.95 ± 0.08 and −8.95 ± 0.06, respectively, and displaced PGD₂ from the receptor (Fig. 1A). In assays of cAMP accumulation, BW245c was a full agonist of DP comparable with PGD₂, with pEC₅₀ values of −8.89 ± 0.11 and −8.48 ± 0.09, respectively. In contrast, BWA868c caused a minute degree of cAMP accumulation at higher concentrations (Fig. 1B), which was compatible with partial agonism already reported for human DP (20).

The selectivity of BWA868c against guinea pig CRTH2 was investigated in eosinophils, which naturally express CRTH2, using the shape change assay. Stimulation with chemoattractants results in immediate responses of granulocytes including reorganization of the cytoskeleton and shape change, which can be detected by flow cytometry (8). PGD₂ was a potent and highly effective inducer of eosinophil shape change of guinea pig eosinophils, although with a 3- to 10-fold lower potency than in human whole blood (Fig. 2). Ramatroban inhibited the PGD₂-induced shape change of human and guinea pig eosinophils (Fig. 2), but had no effect on eotaxin-induced shape change (data not shown, n = 4). In contrast, BWA868c had no effect on shape change responses of guinea pig and human eosinophils, irrespectively of the stimulant used (PGD₂, Fig. 2; eotaxin, data not shown, n = 4). Since the DP agonist BW245c did not cause shape change (data not shown, n = 4), it seems that eosinophil shape change responses to PGD₂ are exclusively mediated by CRTH2.

Mobilization of eosinophils from the guinea pig femoral bone marrow

Using BWA868c and ramatroban as potent and selective antagonists for DP and CRTH2, respectively, we investigated which of the PGD₂ receptors was mediating the mobilization of mature eosinophils from bone marrow in the in situ perfused guinea pig hind limb preparation. PGD₂ caused an immediate increase of eosinophils in the perfusate when added during the period of 20–40 min

FIGURE 1. In vitro characterization of selective PGD₂ receptor ligands on recombinant guinea pig DP receptors. A, competition binding analysis in HEK293 cells expressing guinea pig DP. Values are the mean ± SD of three to six independent experiments performed in duplicate. Data are expressed as percentage of the maximal binding of [³H]PGD₂ obtained in the absence of the indicated competitor compounds. B, cAMP production of 1321N1 cells transfected with guinea pig DP. Values are the mean ± SD of two independent experiments performed in duplicate and are normalized to the maximal cAMP production achieved by PGD₂.
after the start of the experiments, whereas the vehicle of the prostanoid alone had no effect (Fig. 3A); 10 nM of PGD₂ caused a similar degree of eosinophil release as 1 nM eotaxin. The release of noneosinophilic cells from the bone marrow was not increased by PGD₂ or eotaxin, whereas LTB₄ nonselectively induced the release of both eosinophils and other nucleated cells (Fig. 3A).

To determine the role of CRTH₂, DP, and TP receptors in eosinophil mobilization from bone marrow, PGD₂ antagonists or their vehicle were infused throughout the experiment, and PGD₂ was added to the perfusate for the period of 20–40 min after the start of the perfusion. The ability of PGD₂ to increase the release of eosinophils from bone marrow was not altered by the TP-selective antagonist SQ29548 (100 nM), but it was inhibited by the CRTH₂ antagonist ramatroban (1 μM) by 70–80%, and also by the DP antagonist BWA868c (100 nM) to a similar extent (Fig. 3B). In contrast, the DP antagonist BWA868c did not inhibit the response to DK-PGD₂ (Fig. 3B). Therefore, these data show that PGD₂-induced release of eosinophils is mediated both by CRTH₂ and DP receptors, while in agreement with its known selectivity for CRTH₂, DK-PGD₂ mobilized eosinophils solely via CRTH₂.

Unexpectedly, the DP agonists BW245c or ZK110841 did not induce eosinophil release by themselves and the addition of BW245c to the infusion of DK-PGD₂ did not enhance the response of bone marrow eosinophils when compared with DK-PGD₂ alone (Fig. 4A). Hence, these findings raised the question whether BW245c was inactive on DP receptors in this preparation or might even behave as a DP antagonist. When infused throughout the experiment, BW245c (100 nM) in fact reduced the ability of PGD₂ to release eosinophils from the bone marrow, whereas the response to DK-PGD₂ was not affected (Fig. 4B). From these observations it may be inferred that BW245c behaves as an antagonist of DP receptors in guinea pig bone marrow.

Chemotaxis of eosinophils

To further investigate the mechanisms by which CRTH₂ and DP activation induce the release of eosinophils from bone marrow, we harvested the leukocytes from the cavity of the guinea pig femoral bone to determine their migration. Bone marrow eosinophils showed a chemotactic response to PGD₂ (3–300 nM) similar to
human peripheral blood eosinophils, whereas neutrophil chemotaxis was not stimulated by PGD$_2$ (Fig. 5A). Migration of bone marrow eosinophils was significantly inhibited by the CRTH2 antagonist ramatroban and, to a larger extent, also by the DP antagonist BWA868c (Fig. 5B). Human eosinophils purified from peripheral blood likewise migrated toward PGD$_2$, and this response was inhibited by ramatroban but not SQ29548 (Fig. 6A). BWA868c (100 nM) also attenuated the migration of human eosinophils by causing a 3- to 6-fold shift of the PGD$_2$ concentration-response curve to the right (Fig. 6B). The inhibitory effect of BWA868c was unrelated to nonspecific attenuation of eosinophil responsiveness, because BWA868c pretreatment did not alter the migration toward eotaxin (Fig. 6A) or LTB$_4$ (n = 5, data not shown). The DP-selective agonists BW245c (3–300 nM) or ZK110841 (10–30 nM) did not stimulate the chemotaxis of eosinophils, neither alone (Fig. 6B) nor in combination with DK-PGD$_2$ or eotaxin (data not shown). However, pretreatment of eosinophils with BW245c (100 nM) significantly attenuated the migratory response toward PGD$_2$ (Fig. 6C). This suggests again that, in contrast to PGD$_2$, conventional DP agonist do not activate chemotactic pathways in eosinophils, but might even act as antagonist at DP-mediated eosinophil responses.

Expression of CRTH2 and DP in human eosinophils

To further substantiate our findings of DP being involved in eosinophil release from bone marrow and eosinophil chemotaxis, we attempted to visualize the expression of DP and CRTH2 in human peripheral blood eosinophils and eosinophils in bone marrow biopsy specimens. Using immunohistochemistry, we identified DP and CRTH2 in normal bone marrow specimens of three different individuals (data not shown) and one patient suffering from hypereosinophilia of the bone marrow. In these samples, DP was located in mature eosinophils with typical bi-lobed nuclei that also stained positive for eosinophil peroxidase (Fig. 7A). CRTH2 could be observed in mature eosinophils and also in cells with larger, nonsegmented nuclei consistent with eosinophil precursors (Fig. 7B). Using double-staining immunofluorescence microscopy, we observed that eosinophils from peripheral blood expressed both DP and CRTH2, whereas neutrophils expressed DP only, although at higher levels than eosinophils (Fig. 8). In contrast, cells incubated with the respective isotype-matched primary control Abs followed by the secondary Abs did not exhibit positive staining.
A with the chemotactic activity of PGD2 as the prostanoid induced
migration of eosinophils from the bone marrow was apparently linked
demonstrates the DP selectivity of this antagonist. The mobiliza-
tion of eosinophils from the bone marrow, but BWA868c did not inhibit this response, which
might be the blockade of DP receptors on endothelial cells, the
effect of BWA868c on eosinophil mobilization from bone marrow
femoral cavity. One possible mechanism underlying the inhibitory
the in vitro migration of eosinophils isolated from the guinea pig
involved in the migration of bone marrow eosinophils at the cel-
lular level, because either BWA868c or ramatroban inhibited the in
vitro chemotaxis of isolated bone marrow eosinophils. This was
unexpected because the DP receptor has not been implicated in the
chemotactic response of eosinophils to PGD2 before. Therefore we
further investigated the role of DP in the migration of human eo-
sinophils purified from peripheral blood. As expected, the CRTH2
antagonist markedly attenuated the PGD2-induced chemotaxis of
human eosinophils purified from peripheral blood, whereas the TP-
selective antagonist SQ29548 had no effect. However, the DP an-
tagontant BWA868c also significantly reduced the migration toward
PGD2, by inducing a 6-fold rightward shift of the concentration response curve to PGD2 without altering the maximal response.

Our data suggested for the first time that, in addition to CRTH2,
the alternate PGD2 receptor DP might also be involved in eosin-
ophil chemotaxis and mobilization of eosinophils from the bone
marrow. This conclusion was based on the observation that
BWA868c inhibited the chemotaxis and mobilization from bone
marrow of eosinophils that had been stimulated with PGD2.
BWA868c has previously been characterized as a highly selective
antagonist at human DP receptors with virtually no affinity for
human CRTH2 (36). The same seems to hold true for the guinea
pig, since BWA868c potently inhibited the binding of PGD2 to
recombinant guinea pig DP, but it did not inhibit the PGD2-in-
duced shape change of guinea pig eosinophils, a response solely
mediated by CRTH2. Moreover, BWA868c did not inhibit the che-
motaxis of human eosinophils toward eotaxin and LTB4. Finally,
the selectivity of the DP antagonist was also demonstrated by its
lack of effect when eosinophil mobilization from bone marrow was
induced by the selective CRTH2 agonist, DK-PGD2.

Whether eosinophils express the corresponding DP receptor
protein has not been investigated before. Using immunohistochem-
istry we found that eosinophils in human bone marrow and in
peripheral blood express both CRTH2 and DP receptors, which
further emphasizes the importance of DP in eosinophil trafficking.
BW245c and ZK110841 have previously been described as selec-
tive DP agonists, which can potently mimic those PGD2 effects
that are mediated by the DP receptor, such as inhibition of platelet
aggregation, vasodilation and ocular hypotension (24, 37, 38).
BW245c has been reported to delay eosinophil apoptosis at supra-
maximal (1 µM) concentration (10). In this study, we found that
BW245c and ZK110841 were not chemotactic for eosinophils, and
none of the DP agonists induced the mobilization of eosinophils
from in the in situ perfused guinea pig hind limb preparation. On
the contrary, BW245c actually inhibited the responses to PGD2,
i.e., the chemotaxis of peripheral blood eosinophils and eosinophil
release from bone marrow. However, eosinophil mobilization in-
duced by DK-PGD2 was not attenuated by BW245c.

These observations suggest that the DP receptor, that is involved
in eosinophil locomotion, behaves differently than the DP recep-
tors in the vasculature or platelets: although BWA868c is a DP
agonist in all these cell types (38, 39), BW245c is a DP agonist
in platelets and vascular smooth muscle (39, 40), but it does not
activate chemotactic pathways in eosinophils and even might act
as a DP antagonist in eosinophil responses. Similar disparities had
been observed in the conjunctiva, where PGD2 increased conjunc-
tival microvascular permeability in a BWA868c-sensitive manner,
despite the fact that BW245c failed to evoke a plasma exudation
response (25). These findings might be explained by unique cou-
pling of the DP receptor in eosinophils: previous studies by others
and our own data suggest that activation of CRTH2 in eosinophils
leads to shape change through Gαi G-proteins, phospholipase C,
Ca2+ mobilization from intracellular stores, MAP kinases, and

Discussion
In the current study, we show for the first time that the DP receptor
plays an important role in eosinophil trafficking, i.e., chemotaxis
and mobilization of eosinophils from the bone marrow. Hence, our
data provide a rationale for the use of DP receptor antagonists or
mixed CRTH2/DP antagonists in the treatment of allergic disease.
In this study, we observed that PGD2 induced the release of mature
eosinophils from the bone marrow and that this effect was inhibited
by either the CRTH2 antagonist ramatroban or the DP antagonist
BWA868c, but not the TP antagonist SQ29548. The CRTH2-se-
lective agonist DK-PGD2 also mobilized eosinophils from the
bone marrow, but BWA868c did not inhibit this response, which
demonstrates the DP selectivity of this antagonist. The mobiliza-
tion of eosinophils from the bone marrow was apparently linked
with the chemotactic activity of PGD2, as the prostanoid induced
the in vitro migration of eosinophils isolated from the guinea pig
femoral cavity. One possible mechanism underlying the inhibitory
effect of BWA868c on eosinophil mobilization from bone marrow
might be the blockade of DP receptors on endothelial cells, the
activation of which by PGD2 may support the release of bone
marrow cells to the blood stream when the eosinophils are stim-
ulated through CRTH2. However, both DP and CRTH2 seem to be
involved in the migration of bone marrow eosinophils at the cel-

FIGURE 8. Human peripheral blood eosinophils express DP and
CRTH2 receptors. In D, double-staining immunofluorescence shows an
eosinophil (Eo) and a neutrophil (N) stained positive for DP (green),
whereas B demonstrates that the same eosinophil also expresses CRTH2
(red). A and C are images taken with the respective control Abs. E and F
show overlay-images of A and C, and B and D, respectively.
actin polymerization (17, 41), while the CRTH2-mediated chemotaxis of eosinophils depends on $G_{i/o}$ (36). By contrast, DP is coupled to $G_s$ and activation of adenyl cyclase (3, 41). From our data it appears that the events leading to PGD$_2$-stimulated shape change are solely mediated by CRTH2 without an involvement of DP. Monneret et al. (41) have shown that both PGD$_2$ and BW245c stimulate cAMP formation in eosinophils in a BWA868c-sensitive manner. However, an increase of cAMP is likely to attenuate eosinophil chemotaxis (42) and blockade of DP with BWA868c am-

**Disclosures**

The authors have no financial conflict of interest.

**References**


