

Selective modulation of chemokinesis, degranulation, and apoptosis in eosinophils through the PGD₂ receptors CRTH2 and DP

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Background: PGD₂ is the major prostanoid released by mast cells during an allergic response. Its role in the allergic response, however, remains unclear.

Objective: Because the accumulation of eosinophils is a feature of allergic reactions, we investigated the role of PGD₂ in the modulation of eosinophil function.

Methods: Circulating human eosinophils were isolated and challenged with PGD₂. The effects of PGD₂ on various eosinophil functions were then analyzed.

Results: PGD₂ binds with high affinity preferentially to 2 receptors, DP and chemoattractant receptor-homologous molecule expressed on T_H2 cells (CRTH2). We show that both DP and CRTH2 are detectable on circulating eosinophils. We demonstrate that PGD₂ (1-10 nmol/L) induces a rapid change in human eosinophil morphology and an increase in chemokinesis and promotes eosinophil degranulation. These effects are induced by the CRTH2-selective agonist 13-14-dihydro-15-keto-PGD₂ (DK-PGD₂) but not by the DP-selective agonist BW245C. These results suggest a role for CRTH2 in the modulation of eosinophil movement and in triggering the release of cytotoxic proteins. Finally, we demonstrate that BW245C, but not DK-PGD₂, can delay the onset of apoptosis in cultured eosinophils, presumably through interaction with DP.

Conclusion: These data support the hypothesis that PGD₂ controls eosinophil functions through 2 pharmacologically distinct receptors with independent functions. Blockade of PGD₂-mediated effects on human eosinophils may reduce the damage caused by these cells during an allergic response, but inhibition of both receptors may be required. (*J Allergy Clin Immunol* 2001;108:982-8.)

Key words: Eosinophils, G-protein-coupled receptor, receptors, CRTH2, DP, PGD₂, chemokinesis, degranulation, apoptosis

PGD₂ is released in large amounts when sensitized mast cells are challenged with allergen.^{1,2} The precise role of PGD₂ in the allergic response, however, remains to be determined. A hallmark of certain allergic responses is an infiltration of eosinophils.³ After activation,

Abbreviations used

cAMP: Cyclic adenosine monophosphate

CRTH2: Chemoattractant receptor-homologous molecule expressed on T_H2 cells

DK-PGD₂: 13-14-Dihydro-15-keto-PGD₂

ECP: Eosinophil cationic protein

EDN: Eosinophil-derived neurotoxin

GPCR: G-protein-coupled receptor

PAF: Platelet-activating factor

eosinophils release cytotoxic granular proteins, such as major basic protein, eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN; also called eosinophil protein X), and eosinophil peroxidase, through degranulation.⁴ The release of these histotoxic proteins can lead to severe damage at the site of infiltration, and eosinophil infiltration may contribute to the establishment of a chronic state of hyperreactivity in response to subsequent allergen challenges.³ Eosinophil infiltration into the airways can be induced by a variety of different mediators, including PGD₂.⁵ This observation suggests that PGD₂ may act directly on eosinophils. In agreement with this hypothesis, Goetzl et al⁶ have shown that PGD₂ stimulates the capacity of eosinophils to move in the absence of a chemical gradient through chemokinesis.

PGD₂ interacts preferentially with 2 G-protein-coupled receptors (GPCRs) from distinct GPCR subfamilies: DP, a prostanoid receptor, and chemoattractant receptor-homologous molecule expressed on T_H2 cells (CRTH2), a chemokine-related receptor.^{7,8} Hirai et al⁸ have recently reported that CRTH2, but not DP, is present on human eosinophils. They also show that PGD₂ activation of CRTH2 stimulates eosinophil migration. Studies on DP-deficient mice predict a role for the DP receptor in eosinophil function. This is based on the observation that allergen-sensitized DP-deficient mice display a greatly reduced infiltration of eosinophils into the lung after allergen challenge compared with wild-type mice.⁹ This suggests that both receptors may be involved in eosinophil function.

In this report we delineate distinct roles for DP and CRTH2 in controlling eosinophil function. The effects mediated by these receptors can be separated by selective

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pharmacologic agents. We demonstrate that both CRTH2 and DP can be found on the surface of human eosinophils. We provide evidence that activation of the DP receptor reduces eosinophil apoptosis, whereas activation of CRTH2 induces morphologic changes, chemokinesis, and degranulation of eosinophils. Our findings suggest that mast cell–derived PGD₂ has the potential to modulate eosinophil activity through both DP and CRTH2 receptors. These data have important implications for the design of therapeutics targeted at blocking PGD₂-mediated allergic responses.

METHODS

Reagents

PGD₂, fluprostenol, and PGE₂ were obtained from Biomol Research Laboratories. BW245C, 13,14-dihydro-15-keto-PGD₂ (DK-PGD₂), and latanoprost (free acid) were from Cayman Chemical. Platelet-activating factor (PAF) was from Sigma. Recombinant human IL-5 was produced at the Merck Frosst Centre for Therapeutic Research by using a baculovirus system and purified by means of fast performance liquid chromatography.¹⁰

Eosinophil purification

Circulating eosinophils were isolated from heparinized venous blood from healthy volunteers. No attempt was made to separate atopic from nonatopic donors. Erythrocytes were removed with addition of Dextran T-500 (Pharmacia), and mononuclear cells were removed by means of centrifugation over Ficoll-Paque (Pharmacia), as described previously.¹¹ Residual erythrocytes were removed by means of hypotonic lysis. Eosinophils were isolated from the granulocyte fraction by using an immunomagnetic procedure, as described elsewhere.¹² The purity of the eosinophil fraction was evaluated by means of flow cytometry (CELL-DYN 3700 System) on the basis of size, complexity, granularity, and lobularity. In general, less than 5% contamination with neutrophils and lymphocytes was seen, and in most cases 0% basophils, monocytes, and platelets were reported.

RT-PCR and Southern blotting

Total RNA was obtained from isolated eosinophils (>95% pure) by using a total RNA isolation kit (RNeasy kit, Qiagen) and treating with Dnase (Gibco-BRL) before reverse transcription (Gene Amp kit, Perkin Elmer). Amplification of human DP and human CRTH2 by means of PCR (Advantage GC kit, Clontech) used the following primers: DP sense, 5'-ACAACTCGTTGTGCGCAAGCC; DP antisense, 5'-GCATCGCATAGAGGTTGCGC; CRTH2 sense, 5'-CTA-CATGTGCTGCTCCTGAAC; CRTH2 antisense, 5'-CAGGT-GAGCAGTAGAGCAC. The PCR reaction (50 µL) included a denaturation step (94°C for 1 minute) and 35 cycles (for DP) or 25 cycles (for CRTH2) of PCR (94°C for 30 seconds, 55°C for 30 seconds, and 68°C for 1 minute). PCR reactions were subjected to electrophoresis in agarose gels and then transferred to nylon N+Hybond membrane (Amersham). The membrane was hybridized with a phosphorous 32-labeled DNA fragment encoding the full-length human CRTH2 or human DP receptor in ExpressHyb solution (Clontech) overnight at 68°C. The blot was washed twice in 2× saline-sodium citrate (at 65°C) and twice in 0.2× standard saline citrate (at 65°C) for 30 minutes each. Results were revealed by means of autoradiography. RT-PCR with 18S ribosomal RNA-specific primers was conducted in parallel to ensure that an equivalent amount of RNA was used between each donor (data not shown).

In situ hybridization

Freshly isolated eosinophils (2×10^5) were layered onto a polylysine-coated glass slide by means of centrifugation

(Cytospin). Cells were then fixed in 4% (vol/vol) paraformaldehyde solution prepared in PBS (pH 7.4) for 20 minutes at room temperature. The slides were then processed as follows: 2 minutes in 3× PBS, 2 minutes in 1× PBS twice, and incubations of 5 minutes in 50%, 70%, 95%, and 100% (vol/vol) aqueous ethanol solutions. Slides were air-dried and stored at -80°C. The slides were equilibrated to room temperature and washed for 5 minutes in diethylpyrrocarbonate-treated water and twice in PBS.

A 398-bp fragment representing the 5' terminal end of human DP cDNA was amplified by means of PCR and subcloned into the PCR II dual-promoter vector (Invitrogen). Digoxigenin-labeled riboprobes were synthesized with a digoxigenin-RNA-labeling kit from Boehringer Mannheim. In situ hybridization was carried out as described previously.¹³ Colorimetric detection of the hybridized riboprobe was performed with an alkaline phosphatase-linked anti-digoxigenin antibody (Boehringer Mannheim). All subsequent steps were carried out at room temperature. The slides were washed in detection buffer (100 mmol/L TRIS, pH 7.5; 150 mmol/L NaCl; and 0.1% [vol/vol] Tween), incubated with SuperBlock buffer (Biogenex) for 10 minutes, incubated for 2 hours with the antibody (1:75 dilution) in detection buffer, and then washed 3 times in detection buffer. The chromagen solution (Fast Red, Sigma Biochemicals) was then added, and the slides were left to incubate for 30 minutes. The reaction was stopped by washing in 10 mmol/L TRIS (pH 8.0) and 1 mmol/L EDTA. The cells were mounted with SlowFade (Molecular probes) and examined on a fluorescent microscope connected to a CCD camera.

Microscopy

Purified eosinophils were incubated in RPMI-1640 media supplemented with 0.5% (vol/vol) FBS. Test compounds were added to the cell suspension in dimethyl sulfoxide (Me₂SO) to a final vehicle concentration of 0.1% (vol/vol). Cells and test compound were then transferred to a 24-well dish and incubated for 15 minutes. Light microscopy was performed with an inverted Axiovert 25 (Zeiss), and images were obtained with a 35-mm SLR camera (ARIA CON-TAX, Kyocera corporation) using Kodak Elite Chrome 160T film.

Eosinophil chemokinesis

Purified eosinophils were resuspended at 3.0×10^6 cells/mL in RPMI-1640 medium supplemented with 0.5% (vol/vol) FBS. Test compounds were added to 100 µL of cells in Me₂SO to a final vehicle concentration of 0.1% (vol/vol) and incubated at room temperature for 5 minutes. Treated cells (100 µL) were then added to the top half of a chemotactic chamber (6.5-mm Transwell, 3.0-µm polycarbonate membrane from Costar), and 600 µL of RPMI supplemented with 0.5% (vol/vol) FBS was added to the bottom chamber. No chemoattractant was added to the lower chamber to measure chemokinesis alone. After a 2-hour incubation at 37°C in a CO₂ chamber, the upper chamber was discarded, and the number of cells that had migrated to the lower chamber was evaluated by counting the cells with a hemacytometer. The average number of migrating eosinophils from 2 chemotactic chambers was determined for each test condition. Chemokinesis efficiency is expressed as the number of transmigrating cells, with the agent divided by the number of transmigrating cells with vehicle only (fold-increase chemokinesis over background).

Eosinophil degranulation

Purified eosinophils were resuspended at 3.0×10^6 cells/mL in RPMI-1640 medium supplemented with 0.5% (vol/vol) FBS. Test compounds were added to 300 µL of cells in Me₂SO (final concentration of 0.1% [vol/vol]). The cells were immediately transferred to a 24-well plate. After a 1-hour incubation at 37°C in a CO₂ chamber, the cells were removed by means of centrifugation (4°C at 300g for 10 minutes). ECP in the supernatant was quantified with a dou-

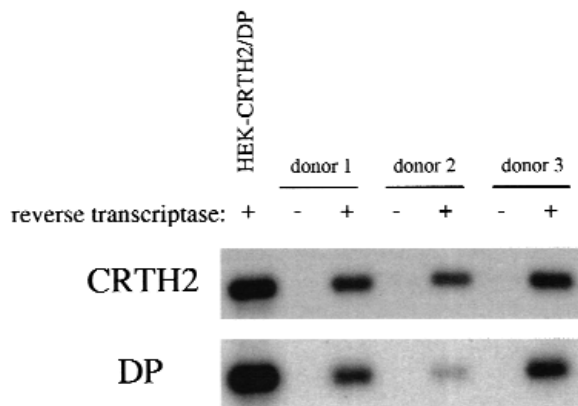


FIG 1. Both DP and CRTH2 mRNA are detected by means of RT-PCR/Southern hybridization in human eosinophils. RNA from HEK cells expressing recombinant CRTH2 or DP was used as a positive control in lane 1. Results from 3 of 7 donors tested are shown.

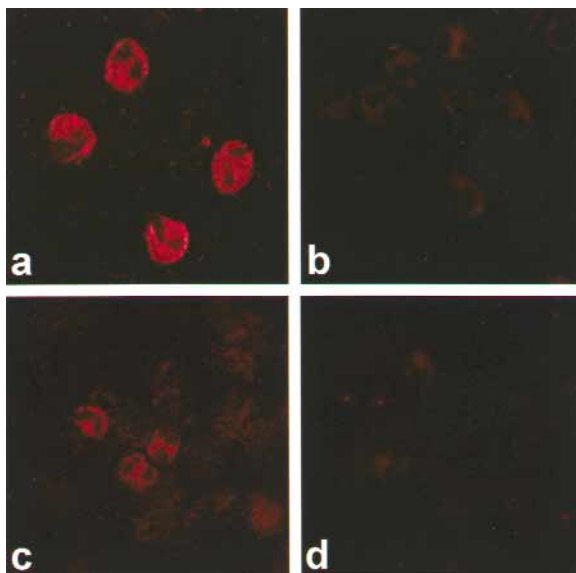


FIG 2. DP mRNA is detected by means of in situ hybridization in human eosinophils (A and B) or granulocytes (C and D) by using a DP-specific antisense probe (A and C). The sense probe was used as a negative control (B and D).

ble-antibody RIA (Pharmacia), according to the manufacturer's protocol. To measure the total content of ECP, 30 μ L of 5% (vol/vol) Nonidet P-40 (Calbiochem) was added to eosinophils at a final concentration of 0.5% (vol/vol). For each experiment, each condition was tested in duplicate. The results were expressed as the amount of ECP detected under the various conditions minus the value obtained with the vehicle-treated cells (amount of ECP over background).

In vitro eosinophil apoptosis assay

Purified eosinophils were resuspended at 2.0×10^5 cells/mL in RPMI-1640 medium supplemented with 10% (vol/vol) FBS, 2 mmol/L glutamine, and 100 units of penicillin and streptomycin. Test compounds were added in Me₂SO (final concentration of 0.1% [vol/vol]), and cells were incubated at 37°C in a CO₂ chamber for 36 hours. The extent of apoptosis in the eosinophil population was evaluated with the TACS Annexin-V-FITC apoptosis detection kit (R&D systems). Nonapoptotic cells are not stained with either

Annexin-V FITC or propidium iodide. Early apoptotic cells are stained with Annexin-V FITC but not propidium iodide (green fluorescence). Late apoptotic cells are stained with both Annexin-V FITC and propidium iodide (dual green and red fluorescence). Necrotic cells are only stained with propidium iodide (red fluorescence). Labeled eosinophils were analyzed in a FACS Calibur system from Becton Dickinson. Cells that have not reached the stage of late apoptosis (thus not positive for both Annexin V and propidium iodide staining) were considered to be viable. The results were expressed as the percentage of nonlate apoptotic eosinophils in the treated population minus the percentage of nonlate apoptotic eosinophils in the vehicle-treated population.

Statistical analyses

Repeated-measures ANOVA, followed by paired *t* tests, were performed. *P* values of less than .05 signify statistical significance.

RESULTS

Human eosinophils express both DP and CRTH2

RT-PCR was performed on total RNA from human eosinophils (>95% purity) to establish which PGD₂-binding receptors are expressed by human eosinophils. The identity of the PCR products was confirmed by means of Southern blot detection with DP- and CRTH2-specific probes. CRTH2 and DP mRNA were detected in eosinophils from all 7 donors tested. Results from 3 donors are shown in Fig 1. CRTH2 mRNA was easily detected on agarose gel after 25 cycles of PCR. DP mRNA was not seen on agarose gel after 35 cycles of PCR and required Southern blotting for visual detection. In situ hybridization studies were therefore performed with the eosinophil cell fraction to ensure that the relatively small amount of DP mRNA that was detected by means of RT-PCR was not produced by a noneosinophil contaminating cell type. The eosinophil fraction (>95% pure) was positively stained with a DP-specific antisense probe coupled with colorimetric detection by using a digoxigenin-labeled antisense riboprobe (Fig 2, A). Staining was not observed with the negative control sense riboprobe (Fig 2, B). In situ hybridization was also performed on the granulocyte fraction, in which neutrophils (>90%) predominate over eosinophils (<10%). The DP antisense riboprobe was clearly detected in only a few cells with the bilobal nucleus typical of eosinophils but not in the remaining neutrophils (Fig 2, C). Again, there was no staining with the negative control sense riboprobe on the granulocyte fraction (Fig 2, D). In conclusion, DP mRNA, although seen at a very low level with RT-PCR, is clearly detected with in situ hybridization in individual eosinophils.

PGD₂ induces a rapid change in eosinophil morphology through CRTH2

We observed that PGD₂ (<10 nmol/L) induces dramatic changes in cell morphology within minutes. Vehicle-treated eosinophils are spherical and only weakly adhere to the culture dish (Fig 3, A). In contrast, eosinophils treated with PGD₂ become flat, assume an amoeba-like

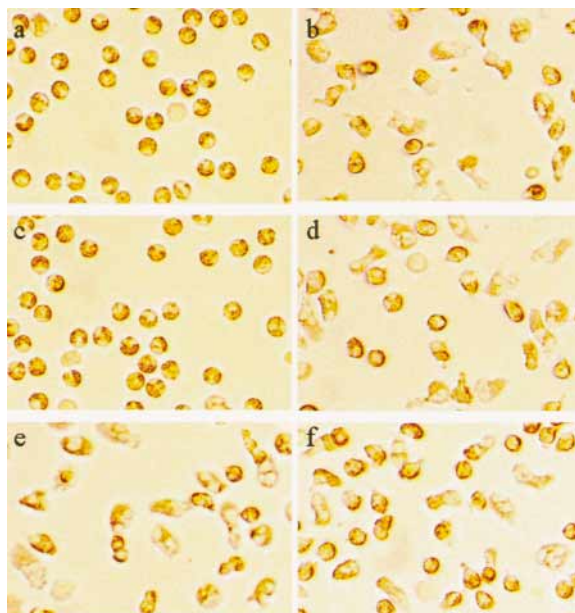


FIG 3. PGD₂ induces a rapid change in eosinophil morphology. Purified human eosinophils were incubated for 15 minutes with various agents in a 24-well dish. **A**, Vehicle-treated eosinophils; **B**, 10 nmol/L PGD₂; **C**, 1 μmol/L BW245C; **D**, 10 nmol/L DK-PGD₂; **E**, 100 nmol/L PAF; **F**, 1 ng/mL IL-5. A representative experiment from 20 donors tested is shown (original magnification, ×200).

shape, and show round structures that may represent secretory vesicles (Fig 3, *B*). This effect was seen on the majority of eosinophils from all donors analyzed ($n = 20$). PGD₂-treated eosinophils reverted to a spherical shape within 6 hours. These cells were resistant to morphology changes after a second PGD₂ challenge (data not shown). The DP-selective agonist BW245C did not affect eosinophil shape at concentrations as high as 1 μmol/L (Fig 2, *C*). In contrast, the CRTH2-selective agonist DK-PGD₂ induced a morphologic change identical to that observed with PGD₂ (Fig 3, *D*). Known activators of eosinophils, such as PAF (Fig 3, *E*) and IL-5 (Fig 3, *F*), also led to a rapid change in eosinophil morphology. Other prostanoid receptor agonists, such as PGE₂ (EP receptors), fluprostenol, and latanoprost (FP receptor), did not cause, even at concentrations of up to 10 μmol/L, any alterations of eosinophil morphology (data not shown). These data suggest that the morphologic changes induced by PGD₂ and DK-PGD₂ on eosinophils are mediated through the CRTH2, and not the DP, receptor.

PGD₂ increases eosinophil chemokinesis through CRTH2

Eosinophil shape changes induced by PAF and IL-5 are associated with increases in their chemotactic and chemokinetic capabilities.^{14,15} The ability of PGD₂ to modulate eosinophil cell movement as a chemoattractant, chemotactic, or chemokinetic agent was therefore evaluated. In our chemotaxis assays PGD₂ was added to the bottom chamber of a chemotactic unit, and eosinophils were placed in the top chamber. In contrast to chemoattractants,

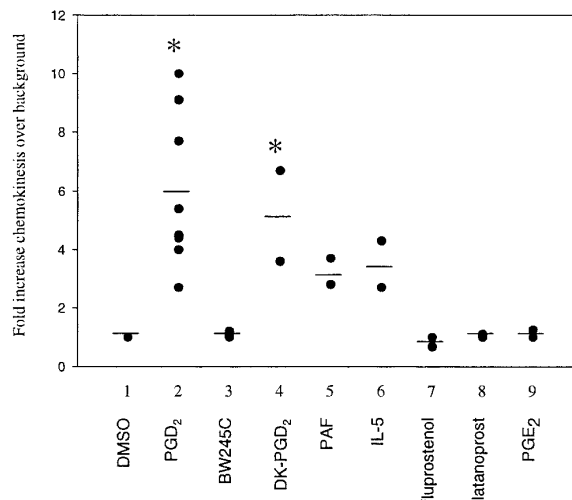


FIG 4. PGD₂ increases eosinophil chemokinesis. Lane 1, Vehicle-treated cells; lane 2, eosinophils treated with 100 nmol/L PGD₂; lane 3, 1 μmol/L BW245C; lane 4, 100 nmol/L DK-PGD₂; lane 5, 100 nmol/L PAF; lane 6, 1 ng/mL IL-5; lanes 7-9, 1 μmol/L of the indicated compounds. The mean response is indicated by a dash. The effect of PGD₂ is significant at a *P* value of less than .001 in repeated-measures ANOVA, followed by paired *t* tests. **P* < .05.

such as PAF and eotaxin, PGD₂ was not a chemoattractant because it did not attract eosinophils to the lower chamber of the chemotactic unit in a gradient-dependent manner (data not shown). We next evaluated whether pretreatment of eosinophils with PGD₂ would affect their chemotactic response. Eosinophils that had been preincubated with PGD₂ (up to 1 μmol/L from 5 minutes to 18 hours) did not have an altered chemotactic response to either PAF or eotaxin (data not shown). Finally, PGD₂ was evaluated as a chemokinetic agent. Chemokinesis is defined as an increase in cell motility in the absence of a chemical gradient. Eosinophils were incubated with PGD₂ for 5 minutes before their loading in the upper chamber of a chemotactic unit; no chemoattractant was added to the lower chamber. PGD₂ (100 nmol/L) increased eosinophil chemokinesis by 6-fold compared with cells treated with vehicle only (Fig 4). The chemokinetic effect was concentration dependent: 10 nmol/L and 1 μmol/L PGD₂ increased eosinophil chemokinesis by a factor of 5- and 9-fold, respectively (data not shown). PAF and IL-5 were also able to stimulate eosinophil chemokinesis (Fig 4), as previously reported.^{14,15} DK-PGD₂, but not BW245C, was effective in stimulating eosinophil chemokinesis (Fig 4). EP (PGE₂) and FP receptor (fluprostenol and latanoprost) agonists failed to modulate eosinophil migration. These data suggest that PGD₂ modulates eosinophil chemokinesis through the CRTH2 receptor in a DP-independent manner.

PGD₂ triggers eosinophil degranulation through CRTH2

Freshly isolated eosinophils were challenged with PGD₂, and the ECP released into the media was quantified with an ECP-specific RIA to test whether PGD₂ can

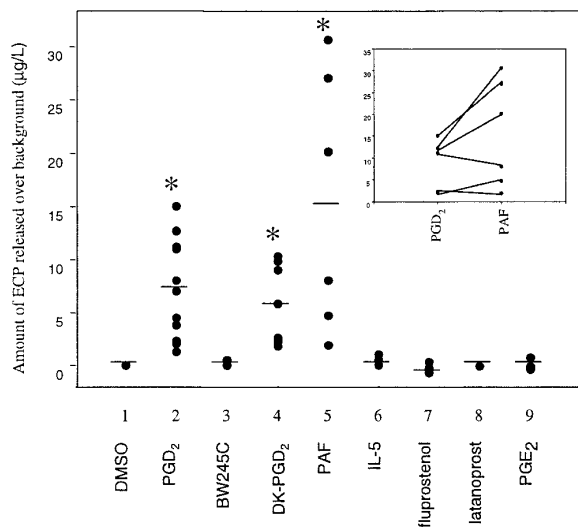


FIG 5. PGD₂ triggers eosinophil degranulation. Lane 1, Vehicle-treated cells; lane 2, eosinophils treated with 100 nmol/L PGD₂; lane 3, 1 µmol/L BW245C; lane 4, 100 nmol/L DK-PGD₂; lane 5, 100 nmol/L PAF; lane 6, 1 ng/mL IL-5; lanes 7-9, 1 µmol/L of the indicated compounds. The mean response is indicated by a dash. The effect of PGD₂ was tested on 11 donors ($P < .0003$, t test), and the effect of DK-PGD₂ was tested on 8 donors ($P < .01$). The value presented for PAF is the mean of 6 independent experiments ($P < .01$). *Inset*, The amount of ECP released by eosinophils challenged with PGD₂ for a given donor is linked to the value obtained with the same donor in response to PAF challenge. * $P < .05$. DMSO, Dimethyl sulfoxide.

induce degranulation of eosinophils. PGD₂ (10-100 nmol/L) significantly increased the release of ECP from eosinophils into the media (Fig 5). A broad range in PAF- and PGD₂-induced release of ECP was seen across the donors. The average extent of PGD₂-induced ECP release was about half that obtained with PAF. In a given donor the extent of degranulation induced by PGD₂ and PAF was proportional (Fig 5, *inset*). Under our experimental conditions, PGD₂ and PAF induced, on average, the release of about 4.8% and 10.0%, respectively, of the total amount of intracellular ECP. ECP was not released as a result of necrosis because lactate dehydrogenase, a marker for necrotic cell lysis, was not detected in the media (data not shown). In one experiment the release of another granule protein, EDN, was also monitored. Stimulation of eosinophils with 100 nmol/L PGD₂ or PAF lead to the release of 170 and 200 µg/L EDN, respectively. DK-PGD₂, but not BW245C, significantly increased the release of ECP (Fig 5). EP (PGE₂) and FP receptor (fluprostenol and latanoprost) agonists did not induce ECP release. These data suggest that PGD₂ induces eosinophil degranulation by means of a CRTH2-dependent mechanism.

A selective DP agonist delays the onset of apoptosis in cultured eosinophils

IL-5, PGE₂, and agents that increase intracellular cyclic adenosine monophosphate (cAMP) have been shown to reduce the extent of apoptosis in eosinophils.¹⁶⁻¹⁸

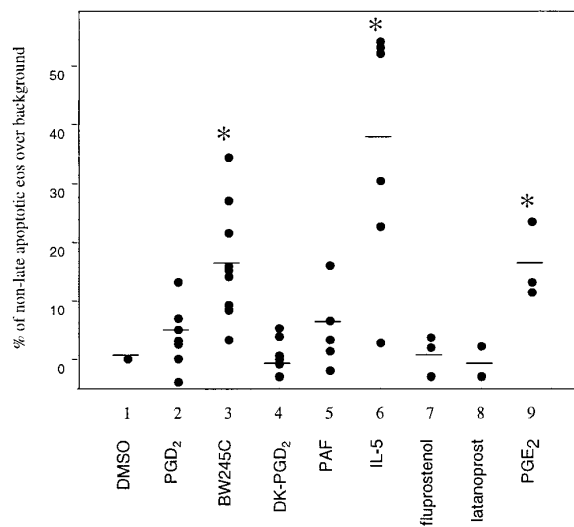


FIG 6. PGD₂ increases the survival of eosinophils in culture. Apoptosis was evaluated by means of flow cytometry (Annexin V/propidium iodide staining). Lane 1, Vehicle-treated cells; lanes 2-9, eosinophils treated with 1 µmol/L of the indicated compounds, except lane 6, where IL-5 was used at 1 ng/mL. The mean response is indicated by a dash. The effect of PGD₂ was tested on 7 donors ($P < .1$), and the effect of BW245C was tested on 9 donors ($P < .0005$). * $P < .05$. DMSO, Dimethyl sulfoxide.

We examined the capacity of PGD₂ to modulate apoptosis in eosinophils by quantifying the capacity of Annexin V to bind to phosphatidylserine on the outer membrane of apoptotic cells.¹⁹ Necrotic cell death was determined by means of propidium iodide uptake.²⁰ Annexin V-FITC and propidium iodide staining of eosinophils was evaluated by means of FACS analysis.

In our hands isolated eosinophils become apoptotic after approximately 12 hours when cultured in RPMI-1640 supplemented with 10% (vol/vol) FBS. After 48 hours, almost all the eosinophils are dead (data not shown). Addition of IL-5 or PGE₂ to the media increased the percentage of nonapoptotic eosinophils at 36 hours in culture (Fig 6). We observed that PGD₂ was a weak inhibitor of apoptotic cell death, whereas DK-PGD₂ had no significant effect (Fig 6). In contrast, the DP-specific agonist BW245C significantly increased the percentage of nonapoptotic eosinophils by 17%. FP agonists (fluprostenol and latanoprost) had no significant effect. The DP receptor therefore appears to be exclusively involved in regulating eosinophil apoptosis.

DISCUSSION

PGD₂ interacts preferentially with 2 GPCRs: DP and CRTH2. We have shown that in eosinophils, shape change, chemokinesis, and degranulation are induced by both PGD₂ and DK-PGD₂. In contrast, PGD₂ and BW245C have anti-apoptotic effects on cultured eosinophils. BW245C²¹ and DK-PGD₂⁸ are reported to be highly selective agonists for DP and CRTH2, respectively, and PGD₂ has similar affinity for CRTH2 and DP.²² Taken together, these data strongly

suggest that CRTH2 mediates shape change, chemokinesis, and degranulation, whereas DP may control apoptotic events. PGD₂ also has affinity for another prostanoid receptor, FP. PGD₂ is not activating eosinophils through FP in this series of experiments because the highly selective FP agonists fluprostenol and latanoprost could not reproduce the PGD₂ effects. It is clear, however, that PGD₂ can potentially produce complex interactions in cells and tissues that coexpress CRTH2, DP, and FP.

Hirai et al⁸ have shown, by means of RT-PCR, that CRTH2, but not DP, is expressed in circulating eosinophils. Here we show, by means of both RT-PCR/Southern blotting and in situ hybridization, that DP can be found on eosinophils. The level of DP mRNA detected by RT-PCR is probably very low because a Southern blot was necessary to visualize the products of the PCR reaction. This may explain why Hirai et al failed to detect DP mRNA in human eosinophils by means of RT-PCR alone. In contrast, products of the CRTH2 RT-PCR reaction were clearly visible on agarose gel and did not require Southern blotting for detection.

Agents that elevate cAMP reduce eosinophil apoptosis.^{23,24} Both EP₂ and DP receptors are G α s-coupled, and both signal through increases in intracellular cAMP.^{7,25} Challenge of eosinophils with an EP₂-specific agonist,¹⁸ as well as with PGE₂ (Fig 6), increase their survival. Therefore it is not surprising that activation of the DP receptor is associated with increased cell survival in eosinophils (Fig 6). This is the only DP-mediated event that we could detect in eosinophils.

Hirai et al⁸ proposed that PGD₂ increases cell migration through the CRTH2 receptor. However, their experimental design does not distinguish whether PGD₂ is a chemotactic or chemokinetic agent. Hirai et al applied PGD₂ to the bottom chamber of the chemotactic unit. This would allow PGD₂ to be either a chemoattractant or to rapidly diffuse to the top chamber and induce chemokinesis. We observed that PGD₂ is not a chemoattractant itself nor does it stimulate the response of eosinophils to a chemoattractant, which is in agreement with a previous report.⁶ Instead, PGD₂ enhances the random migration of eosinophils in the absence of a chemoattractant gradient (chemokinesis; Fig 4).

We also show, for the first time, that PGD₂ can induce a rapid morphologic change in eosinophils (Fig 3). This morphologic change could be associated with the activation of the intracellular motile apparatus, as well as exocytosis, as proposed in the case of PAF-induced eosinophil shape change.²⁶

Factors that have been shown to induce eosinophil degranulation include the following: immune complexes (secretory IgA, IgG, and IgE),²⁷ complement fragment (C3b, C3bi, and C5a),²⁸ and PAF.²⁹ Factors that have been shown to prime eosinophils for degranulation include the following: GM-CSF, IL-3, and IL-5.³⁰ PGD₂ has been shown previously to prime eosinophils for enhanced release of leukotriene C₄ in response to the calcium ionophore A23187.³¹ We now show that PGD₂ is also a direct inducer of eosinophil degranulation (Fig 5).

Two important immune cell types involved in allergy, mast cells and T_H2 cells, both release PGD₂ in response to immunologic challenge.^{1,2,32} In allergic situations the released PGD₂ may facilitate eosinophil entry into the site of inflammation through DP-mediated vasodilation-extravasation, along with other circulating leukocytes.³³ The entry of eosinophils into the allergic site would be stimulated by the prochemokinetic activity of PGD₂ through CRTH2. The survival of resident eosinophils would be prolonged by the antiapoptotic effect of PGD₂ acting through DP. Finally, degranulation of eosinophils may be triggered by PGD₂ activation through CRTH2. If the PGD₂ effects observed on eosinophils in culture are an accurate reproduction of the role of PGD₂ in vivo, then pharmacologic blockade of PGD₂-mediated events at both the DP and CRTH2 receptors may reduce damage caused by eosinophils at a site of inflammation.

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