

Differential modulation of human basophil functions through prostaglandin D₂ receptors DP and chemoattractant receptor-homologous molecule expressed on Th2 cells/DP2

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Summary

Background Both prostaglandin (PG) D receptor (DP) and CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells)/DP2 are high-affinity receptors for PGD₂. Previous studies have demonstrated that PGD₂ enhances releasability and induces CRTH2/DP2-mediated migration in human basophils, but the precise effects of PGD₂ on basophils as well as receptor usage have not been fully clarified.

Objective We comprehensively explored the roles of DP and CRTH2/DP2 in basophil functions by using selective agonists and antagonists for each receptor.

Methods DP and CRTH2/DP2 transcripts were quantified by real-time PCR. We studied the effects of selective agonists (DP: BW245C; CRTH2/DP2: 13,14-dihydro-15-keto (DK)-PGD₂) and/or antagonists (DP: BWA868C; CRTH2/DP2: ramatroban) on Ca²⁺ mobilization, migration, degranulation, CD11b expression and survival of human basophils.

Results Basophils expressed transcripts of both DP and CRTH2/DP2, but the levels of CRTH2/DP2 transcripts were ca. 100-fold higher compared with DP transcripts. Ca²⁺ influx was induced in basophils by either PGD₂ or DK-PGD₂/CRTH2 agonist but not by BW245C/DP agonist. Basophils treated with PGD₂ were completely desensitized to subsequent stimulation with DK-PGD₂, but not *vice versa*. DK-PGD₂ as well as PGD₂ up-regulated CD11b expression, induced migration and enhanced degranulation, and those effects were completely antagonized by ramatroban/CRTH2 antagonist. In contrast, BW245C/DP agonist exhibited an inhibitory effect on basophil migration and IgE-mediated degranulation, and the migration inhibitory effect was effectively antagonized by BWA868C/DP antagonist. On the other hand, while PGD₂ significantly shortened the basophil lifespan, neither DK-PGD₂/CRTH2 agonist nor BW245C/DP agonist did.

Conclusion CRTH2/DP2 is primarily responsible for the pro-inflammatory effects of PGD₂ on human basophils, while DP introduces negative signals capable of antagonizing the effects of CRTH2/DP2 in these cells. The effects of PGD₂ on longevity imply a mechanism(s) other than via DP or CRTH2/DP2. CRTH2/DP2 on basophils may afford opportunities for therapeutic targeting in allergic inflammation.

Keywords apoptosis, basophils, Ca²⁺ influx, CD11b, chemotaxis, CRTH2, degranulation, DP, eosinophils, prostaglandin

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Introduction

Although basophils comprise the least abundant circulating leucocytes, substantial evidence is accumulating that these cells are active participants in the inflammation associated

with allergic diseases. The pathophysiological significance of basophils in allergic disorders has become more apparent with the recognition of allergic dual-phase reactions. Studies in humans have revealed the influx of basophils as well as eosinophils to inflammatory sites several hours after experimental allergen challenge [1]. Furthermore, analyses of chemical mediators have demonstrated selective increases in basophil-derived mediators during late-phase reactions [2]. The concept of a contribution by basophils to the late-phase reaction has been further strengthened by recent experiments using basophil-specific mAbs [3–5].

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Prostaglandin (PG) D₂ is an inflammatory mediator synthesized from arachidonic acid via enzymatic conversion by cyclooxygenase and PGD synthase [6]. To date a number of cellular sources of PGD₂ have been identified, and this substance is acknowledged as a particularly important mediator derived from mast cells. In fact, PGD₂ is a major lipid mediator of mast cells that is *de novo* synthesized during their activation process [7]. Since activation of mast cells represents a hallmark aspect of allergic immediate-phase reactions, PGD₂ may play a connecting role between immediate- and late-phase reactions through the recruitment and activation of basophils. Indeed, an earlier study revealed a priming effect of PGD₂ on basophil histamine release [8]. A more recent study has demonstrated that PGD₂ induces potent migratory responses in basophils [9].

PG receptors belong to a family of seven-transmembrane G-protein-coupled receptors, and redundant as well as promiscuous ligand-receptor interaction is observed in this receptor system. To date, two types of high-affinity receptors for PGD₂ have been cloned, i.e., the PGD receptor (DP) [10, 11] and CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells [9], also termed DP2 [12]). DP is present in various types of haematological and non-haematological cells [10, 11]. On the other hand, human CRTH2/DP2 has been specifically identified on haematological cells, i.e., basophils, eosinophils and Th2 cells [9], although the tissue expression pattern of CRTH2 in humans differs from that in rodents [13]. The latter study also demonstrated the expression of DP transcripts in basophils by RT-PCR [9]. Despite the presence of DP mRNA in basophils, however, CRTH2/DP2 was shown to be fully responsible for PGD₂-induced migratory responses in these cells [9].

A better understanding of PGD₂-mediated activation of basophils is potentially important for establishing a therapeutic strategy for allergic inflammation, but the precise effects of PGD₂ on basophils as well as receptor usage have not been fully clarified. In the present study, we used selective agonists and antagonists for each receptor to explore the roles of DP and CRTH2/DP2 in basophil functions.

Materials and methods

Reagents

All PGs, BW245C and U46619 were purchased from Cayman Chemical (Ann Arbor, MI, USA). Human recombinant eotaxin was purchased from PeproTech (London, UK). BWA868C and ramatroban ((+)-(3R)-3-(4-fluorobenzenesulphonamido)-1,2,3,4-tetra-hydrocarbazole-9-propionic acid) were synthesized by SOGO Pharmaceutical Co. Ltd (Tokyo, Japan) and Bayer Yakuhin Ltd (Kyoto, Japan), respectively. The other reagents used in the experiments were exactly the same as described previously [14].

Cell preparation

Basophils were isolated from venous blood obtained from consenting volunteers with no history of atopic diseases. For degranulation experiments, we used dextran-sedimented leucocytes as previously described [15]. For CD11b expression and migration experiments, basophils were semi-purified

by Percoll density centrifugation (1.079 and 1.070 g/mL) as previously described [16]. The purity of Percoll-separated basophil preparations was $15.4 \pm 2.1\%$ ($n = 19$). For experiments on real-time PCR, Ca²⁺ influx and apoptosis, Percoll-separated basophils were further purified by negative selection with magnetic cell sorting (MACS) beads (Basophil Isolation Kit, Miltenyi BioTech, Belgisch-Gladbach, Germany) as previously described [17]. The purity of MACS-separated basophil preparations was $99.1 \pm 0.2\%$ ($n = 12$). Eosinophils were purified by density gradient centrifugation followed by negative selection using anti-CD16-bound micro-magnetic beads (Miltenyi BioTech) as previously described [18]. The purity of eosinophils was $99.3 \pm 0.3\%$ ($n = 4$).

Real-time quantitative PCR analysis for CRTH2/DP2 and DP

Real-time quantitative PCR analysis was performed as previously described [18]. In brief, total RNA was extracted from MACS-separated basophils (purity: $99.2 \pm 0.3\%$) or eosinophils (purity: $99.3 \pm 0.3\%$) using a QIAGEN RNeasy Mini Kit (Qiagen, Hilden, Germany). Real-time PCR was performed using the ABI Prism 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA). Gene-specific primers and probes were purchased from Applied Biosystems. The primers and probes for DP (5'-CCGTGCTCTTCACTATGTGTTCTCT, 5'-CCTTGACATCCTTAAATGCTCCATA, and 5'-FAMCCCGTAATTTATCGCGCTT-3'/TAMRA) were designed according to the published sequences. The primers and probes for CRTH2/DP2 were designed by Applied Biosystems. A standard curve was constructed with serial dilutions of specific PCR products, which were obtained by amplifying basophil cDNA.

Ca²⁺ influx

Ca²⁺ influx was determined as previously described [16]. In brief, MACS-separated basophils (purity: $99.9 \pm 0.0\%$) were labelled with Fura-2 AM, and Ca²⁺ influx was measured using excitation at 340 and 380 nm on a Hitachi F-2500 fluorescence spectrometer (Hitachi Ltd, Tokyo, Japan).

Apoptosis

Apoptosis-blocking experiments were performed using MACS-separated basophils (purity: $99.1 \pm 0.3\%$) as previously described [17]. In brief, differential analysis was performed using a MEBCYTO-Apoptosis Kit (MBL, Nagoya, Japan) according to the manufacturer's instructions. Annexin V-positive/propidium iodide-negative cells were defined as apoptotic.

CD11b expression

CD11b expression experiments were conducted as previously described [18]. Briefly, Percoll-separated basophils were double stained with PE-conjugated anti-CD11b mAb (10 µg/mL) and biotinylated anti-FcεRIα-chain mAb (clone CRA-1, 5 µg/mL) for 60 min. The cells were further incubated with FITC-conjugated streptavidin (10 µg/mL) for 30 min at 4 °C. Finally, cells stained strongly with the anti-FcεRIα-chain mAb were analysed for the expression of CD11b.

Stained basophils were analysed using an EPICS XL SYSTEM II (Coulter, Miami, FL, USA). At least 3000 basophils were assessed to calculate the median value of fluorescence intensity. The median values of fluorescence intensity were converted to the numbers of molecules of equivalent soluble fluorochrome units (MESF). Surface receptor levels were semi-quantified using the following formula: $\Delta\text{MESF} = (\text{MESF of cells stained with anti-CD11b mAb}) - (\text{MESF of cells stained with control IgG})$.

Chemotaxis

Basophil chemotaxis was performed as previously described [16]. Briefly, Chemotaxicell (Kurabo, Osaka, Japan) with a 5- μm pore size was used for basophil chemotaxis assay. Percoll-separated basophils ($3 \times 10^4/100 \mu\text{L}$) were added to the upper chamber, and samples to be tested (300 μL) were placed in the lower chamber. After incubation for 2 h at 37°C, the migrated cells in the lower chambers were collected, stained with FITC-conjugated goat anti-human IgE (Biosource, Camarillo, CA, USA) and counted by flow cytometry. Migration was expressed as a percentage of inoculated cells after subtracting the spontaneous migration.

Degranulation

Basophil degranulation experiments were performed as previously described [15]. The released histamine was measured using an automated fluorometric technique. Histamine release was expressed as a percentage of the total cellular histamine after subtracting the spontaneous release (consistently less than 5%). Enhancement of histamine release was calculated by the following formula: % enhancement of histamine release = $100 \times (\text{histamine released by a stimulus plus reagent} - \text{histamine released by a stimulus}) / \text{total histamine content}$.

Statistics

All data are expressed as the mean $\pm$ SEM. Differences between values were analysed by the one-way ANOVA test. When this test indicated a significant difference, Fisher's protected least significant difference (PLSD) test was used to compare individual groups.

Results

mRNA expression of CRTH2/DP2 and DP in basophils

A previous report of RT-PCR by others showed that the DP transcript was expressed in basophils, while it was hardly detectable in eosinophils [9]. In our first series of experiments, we quantified the expression levels of transcripts of both DP and CRTH2/DP2 in basophils ($n = 5$) and eosinophils ($n = 4$) by using real-time PCR. Judging from the ratio of the copy number to the cell number, basophils expressed a higher level of DP transcripts compared with eosinophils (copy number/ 10^4 cells: 29.0 ± 6.5 and 0.5 ± 0.2 , for basophils and eosinophils, respectively). On the other hand, strong expression of CRTH2/DP2 mRNA was observed in both types of cells, and basophils expressed a slightly lower level of

CRTH2/DP2 transcripts compared with eosinophils (copy number/ 10^4 cells: 2765 ± 584 and 7053 ± 1731 , for basophils and eosinophils, respectively).

Ca²⁺ influx in basophils

In the next series of experiments, we studied the capacity of PGD₂, CRTH2/DP2-selective agonist 13,14-dihydro-15-keto PGD₂ (DK-PGD₂) and DP-selective agonist BW245C to mobilize Ca²⁺ in basophils. As shown in Figs 1a and b,

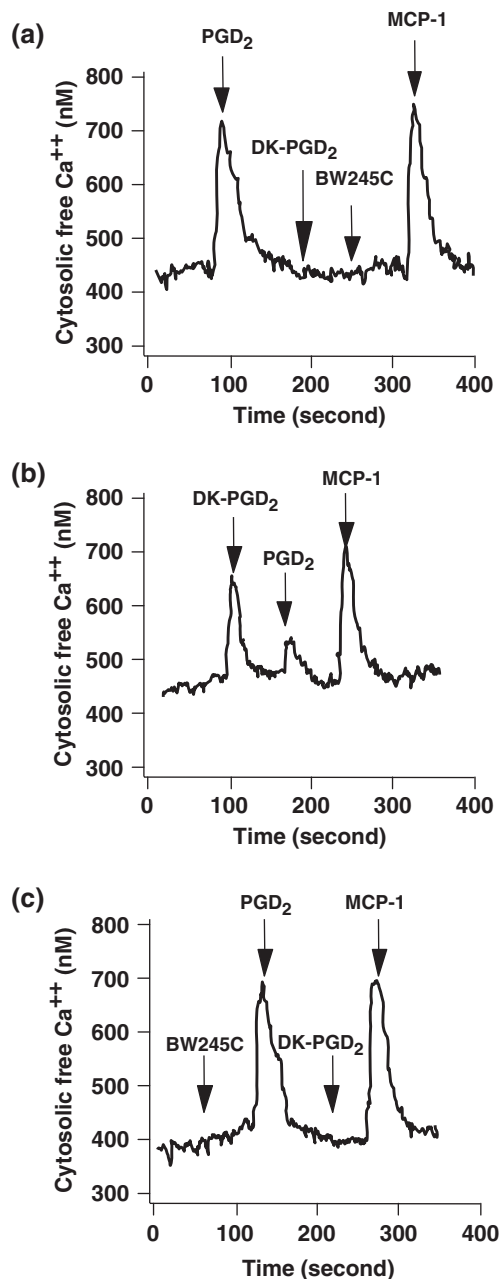


Fig. 1. Cross-desensitization between prostaglandin (PG) D₂, 13,14-dihydro-15-keto (DK)-PGD₂ and BW245C. Magnetic cell sorting (MACS)-separated basophils were incubated with Fura-2 AM (2 μM) at 37°C for 20 min. Cells were then stimulated with agonists (1 μM) as indicated by the arrows. Ca²⁺ influx was measured with a fluorescence spectrometer. The data shown are representative of three independent analyses from separate donors, each showing similar results.

apparent Ca^{2+} influx was observed in basophils stimulated with either PGD_2 or DK- PGD_2 . On the other hand, BW245C failed to induce significant Ca^{2+} influx in basophils (Fig. 1c). Basophils treated with PGD_2 were completely desensitized to subsequent stimulation with DK- PGD_2 , although these cells responded to the positive control, monocyte chemoattractant protein(MCP)-1 (Fig. 1a). In contrast, cells treated with DK- PGD_2 still responded to the subsequent stimulation with PGD_2 , indicating that PGD_2 is more potent at inducing CRTH2/DP2 desensitization than DK- PGD_2 (Fig. 1b).

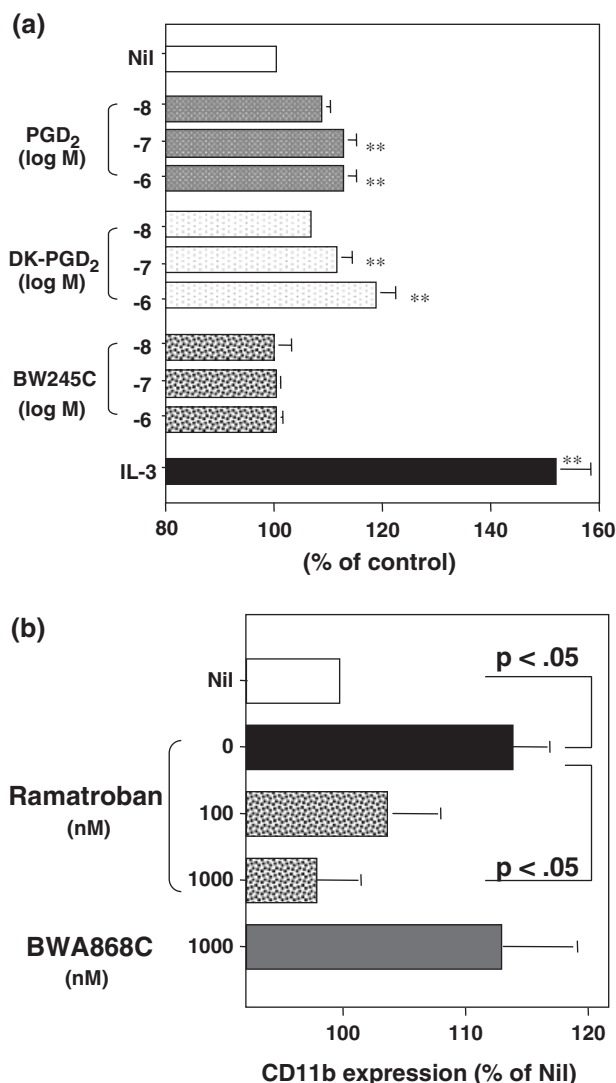


Fig. 2. Up-regulation of basophil CD11b expression by chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2)/prostaglandin (PG) D receptor (DP)2. (a) Effects of PGD_2 , 13,14-dihydro-15-keto (DK)- PGD_2 and BW245C on basophil CD11b expression. Percoll-separated basophils were incubated with PGD_2 , DK- PGD_2 , BW245C or the positive control, IL-3 (300 pM), at 37 °C for 30 min. Then the CD11b expression was measured by flow cytometry. The values are expressed as percentages of the expression on cells incubated in medium alone (Nil). ΔMESF values of control expression were $61\,840 \pm 1965$. Bars represent the SEM ($n = 6$). ** $P < 0.01$ vs. Nil. (b) Effects of antagonists of DP and CRTH2/DP2 on up-regulation of basophil CD11b expression. Percoll-separated basophils were incubated with ramatroban, BWA868C or vehicle at 37 °C for 10 min. After stimulation with PGD_2 (100 nM) for 30 min, the CD11b expression was measured by flow cytometry. The values are expressed as percentages of the expression on cells incubated and stimulated with buffer alone (Nil). ΔMESF values of Nil were $258\,900 \pm 19\,730$. Bars represent the SEM ($n = 4$).

Effects of PGD_2 on basophil functions

Previous reports by others have demonstrated that PGD_2 modulates histamine release [8] and induces a migratory response [9] in basophils, but any other effects of PGD_2 on basophils have been unclear. Therefore, we studied the effects of PGD_2 and selective agonists for each of DP and CRTH2/DP2 on other basophil functions, i.e., expression of CD11b and longevity. As shown in Fig. 2a, expression of basophil CD11b was up-regulated by PGD_2 . However, the effect of PGD_2 on CD11b expression was quite modest and significantly less potent compared with that of IL-3 ($P < 0.01$, IL-3 vs. 10^{-6} M of PGD_2). DK- PGD_2 also significantly up-regulated the expression of CD11b, while BW245C failed to stimulate the expression at all. Furthermore, PGD_2 -induced up-regulation of basophil CD11b was inhibited by pre-treatment with the CRTH2/DP2-selective antagonist ramatroban, but not with the DP-selective antagonist BWA868C (Fig. 2b). These results indicate an exclusive role for CRTH2/DP2 in basophil CD11b expression.

The results for basophil survival are depicted in Fig. 3. PGD_2 as well as the positive control, dexamethasone [17], significantly accelerated the increase in the number of apoptotic cells. However, neither DK- PGD_2 nor BW245C exerted any significant pro- or anti-apoptotic effects at all.

The effects of cyclooxygenase products on basophil migration are shown in Fig. 4a. Consistent with a previous report by others [9], both PGD_2 and DK- PGD_2 induced potent migratory responses in basophils. On the other hand, none of the other PGs or thromboxane A2 mimetic U46619 showed any significant migratory effects. Concentration-response experiments demonstrated that DK- PGD_2 showed similar potency and efficacy to PGD_2 (data not shown). Furthermore, experiments using pharmacological antagonists showed that PGD_2 -induced basophil migration was sensitive to the CRTH2/DP2-selective antagonist ramatroban but resistant to the DP-selective antagonist BWA868C (Fig. 4b), lending further support to the concept that CRTH2/DP2

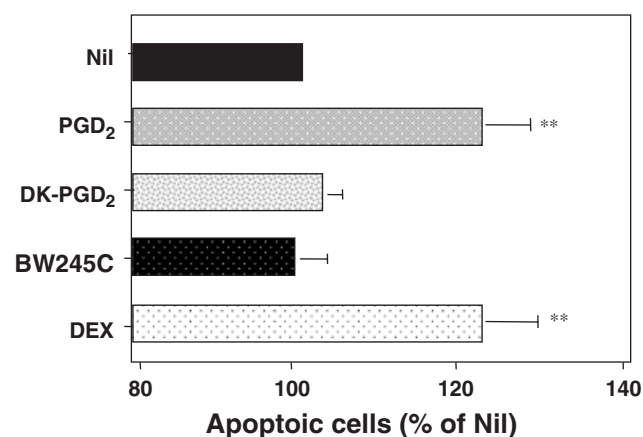


Fig. 3. Effects of prostaglandin (PG) D_2 , 13,14-dihydro-15-keto (DK)- PGD_2 and BW245C on basophil apoptosis. Magnetic cell sorting (MACS)-separated basophils were incubated with PGD_2 (1 μM , $n = 5$), BW245C (1 μM , $n = 4$), an apoptogenic reagent, i.e., dexamethasone (DEX, 100 nM, $n = 4$), or medium alone (Nil, $n = 6$) for 48 h. Then the apoptotic cells were determined as described in Materials and methods. The values are expressed as percentages of the control (Nil). The apoptotic cells in the control were $40.8 \pm 3.3\%$ of the total cells. Bars represent the SEM. ** $P < 0.01$ vs. Nil.

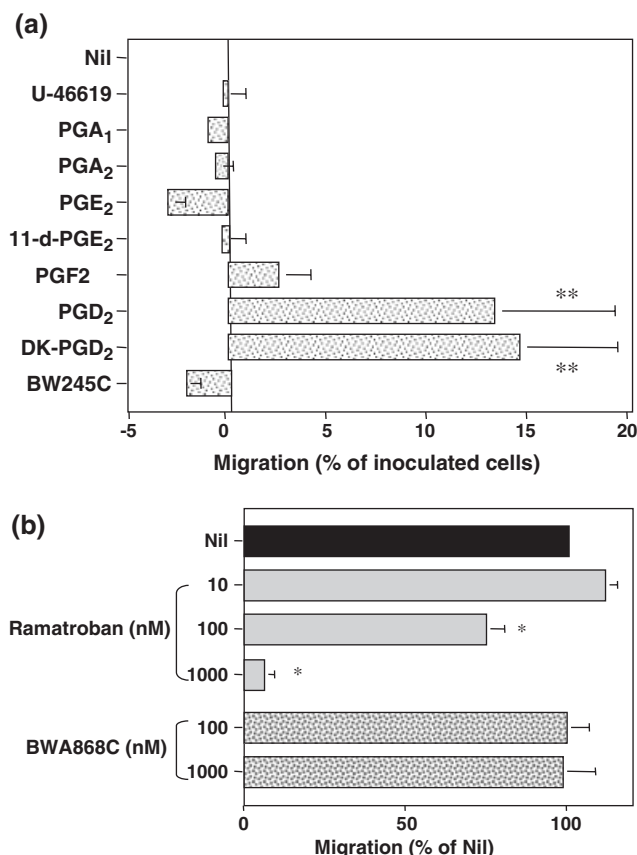


Fig. 4. Induction of basophil migration by chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2)/prostaglandin (PG) D receptor (DP)2. (a) Effects of prostanoids and U46619 on basophil migration. Migration assay was performed using Percoll-separated basophils and 100 nM of each reagent. Control net migration in the absence of any reagent (Nil) was $8.0 \pm 1.9\%$. Bars represent the SEM ($n = 4$). $^{**}P < 0.01$ vs. Nil. Data show the results of experiments conducted using PGs at a concentration of 100 nM. Other experiments at 10 and 1000 nM of PGs ($n = 4$) showed that significant migration was induced only by PGD₂ and 13,14-dihydro-15-keto (DK)-PGD₂. (b) Effects of antagonists of DP and CRTH2/DP2 on PGD₂-induced basophil migration. Percoll-separated basophils were incubated with ramatroban, BWA868C or vehicle at 37°C for 10 min. Migration assay was then carried out in the presence of PGD₂ (10 nM). The values are expressed as percentages of the net migration of cells pre-incubated with buffer alone (Nil). Control net migration was $15.6 \pm 4.1\%$. Bars represent the SEM ($n = 5$). $^{**}P < 0.01$, $^{*}P < 0.05$ vs. Nil.

is responsible for PGD₂-induced migratory responses. Although the DP-selective agonist BW245C alone failed to induce significant migratory responses (Fig. 4a), DP transduced negative signals for migration in basophils. When migration assays were carried out in the presence of graded concentrations of BW245C and a fixed concentration of eotaxin or DK-PGD₂, BW245C inhibited basophil migration induced by both reagents in a dose-dependent fashion (Figs 5a and b). Furthermore, the migration inhibitory effect of BW245C was effectively antagonized by pre-treatment of cells with DP-selective antagonist BWA868C (Fig. 5c).

The results from our and other laboratories have demonstrated that IL-3 quantitatively and/or qualitatively modulates basophil histamine release [19]. However, even in the presence of IL-3 (300 pM), PGD₂ as high as 1 μ M failed to induce significant degranulation of basophils (data not shown). On the other hand, as reported by Peters et al. [8], PGD₂ primed basophils for increased releasability, albeit the

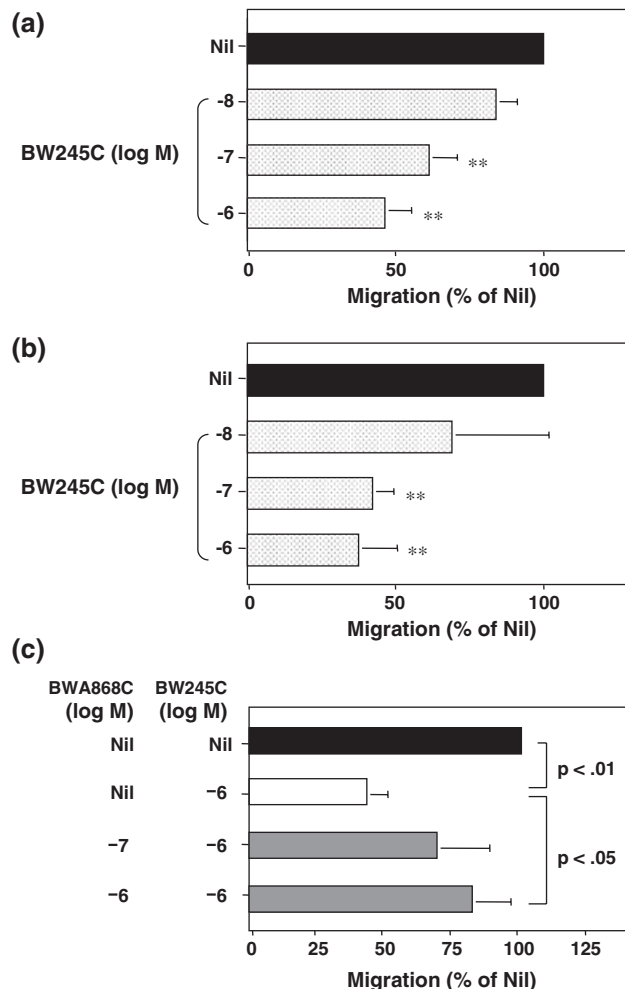


Fig. 5. Inhibition of basophil migration by prostaglandin (PG) D receptor (DP). (a, b) Effects of BW245C on eotaxin- (a) and 13,14-dihydro-15-keto (DK)-PGD₂-induced (b) basophil migration. Migration assays were performed in the presence of BW245C, eotaxin (a, 50 nM) or DK-PGD₂ (b, 30 nM). The values are expressed as percentages of the net migration of vehicle-treated cells in the absence of BW245C (Nil). Control net migration was $19.3 \pm 6.3\%$ (a) and $23.2 \pm 10.9\%$ (b) of the total basophils. Bars represent the SEM ($n = 5$). $^{**}P < 0.01$ vs. Nil. (c) Effects of BWA868C on BW245C-induced inhibition of basophil migration. Percoll-separated basophils were incubated with BWA868C or vehicle at 37°C for 10 min. Migration was induced by eotaxin (50 nM) in the presence or absence of BW245C (1 μ M). The values are expressed as percentages of the net migration of vehicle-treated cells in the absence of BW245C (Nil). Control net migration was $18.7 \pm 4.6\%$ of the total basophils. Bars represent the SEM ($n = 3$). $p < .01$, $p < .05$.

extent of the enhancement varied among secretagogues (Fig. 6a). While PGD₂ exerted little effect on degranulation initiated by anti-IgE, apparent enhancement was observed for both ionophore- and 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA)-mediated degranulation in PGD₂-stimulated cells. Increased basophil degranulation was also observed in cells treated with DK-PGD₂, and IgE-mediated degranulation was significantly enhanced by DK-PGD₂. On the other hand, BW245C significantly inhibited basophil degranulation initiated by anti-IgE. In addition, pre-treatment with ramatroban but not BWA868C abolished PGD₂-induced enhancement of basophil degranulation initiated by TPA (Fig. 6b), confirming the involvement of CRTH2/DP2 in the increased releasability induced by PGD₂.

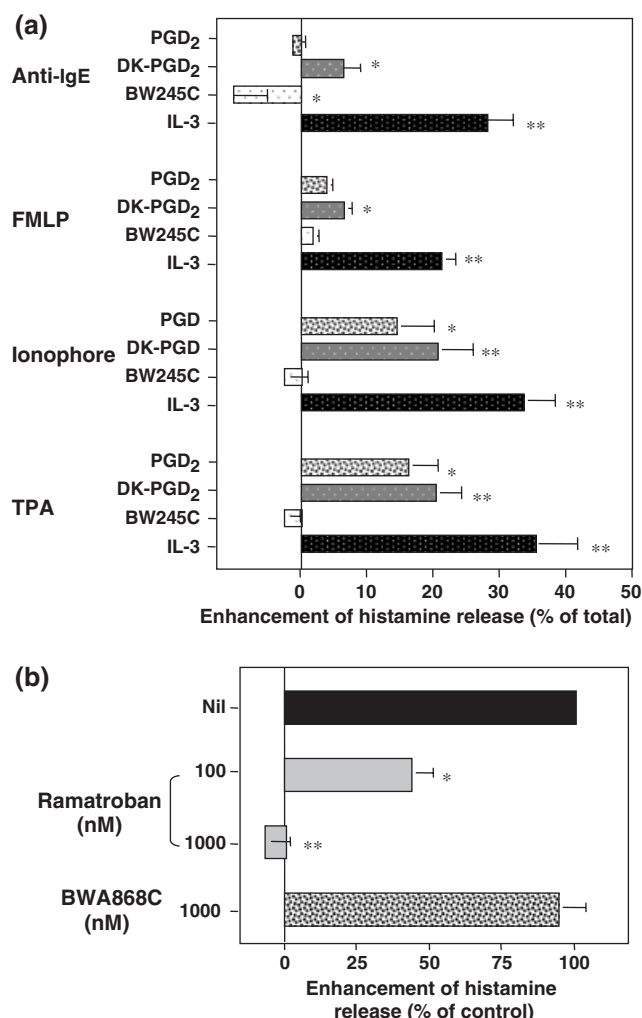


Fig. 6. Effects of prostaglandin (PG) D receptor (DP) and chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2)/DP2 on basophil degranulation. (a) Effects of PGD₂, 13,14-dihydro-15-keto (DK)-PGD₂ and BW245C on basophil degranulation. Dextran-sedimented leucocytes were pre-incubated with PGD₂ (1 μ M), DK-PGD₂ (1 μ M), BW245C (1 μ M), IL-3 (300 pm) or buffer alone at 37°C for 30 min. The cells were then stimulated with anti-IgE (1:30 000 or 1:10 000, n = 4), FMLP (100 nM, n = 5), ionophore (0.1 μ g/mL, n = 7) or 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) (10 ng/mL, n = 6) for an additional 45 min. Control release without any reagent was $29.6 \pm 7.0\%$, $30.2 \pm 12.5\%$, $47.3 \pm 5.7\%$ and $44.9 \pm 8.2\%$ for anti-IgE, formyl-methionyl-leucyl-phenylalanine, ionophore and TPA, respectively. Bars represent the SEM. ** P < 0.01, * P < 0.05 vs. controls. (b) Effects of antagonists of DP and CRTH2/DP2 on PGD₂-induced enhancement of basophil degranulation. Dextran-sedimented leucocytes were pre-incubated with ramatroban, BWA868C or buffer alone in the presence or absence of PGD₂ (30 nM) at 37°C for 30 min. The cells were then stimulated with TPA (10 ng/mL) for an additional 45 min. The values are expressed as percentages of the control enhancement of cells pre-incubated with buffer alone (Nil). Histamine release initiated by TPA was $24.4 \pm 7.3\%$ and $14.7 \pm 5.6\%$ in the presence and absence of PGD₂, respectively. Bars represent the SEM (n = 3). ** P < 0.01, * P < 0.05 vs. Nil.

Discussion

The effects of PGD₂ are mediated via at least two distinct receptor systems, i.e., DP and CRTH2/DP2. By using selective agonists and/or antagonists for each receptor, the current study was undertaken to explore the effects of PGD₂ on human basophils, focusing particularly on the receptor systems. PGD₂ binds to DP and CRTH2/DP2 with almost

equal affinity (K_a -values: 1.7 and 2.4 nM for DP and CRTH2/DP2, respectively) [13]. On the other hand, DK-PGD₂ binds to CRTH2/DP2 with more than 1000-fold higher affinity than to DP (K_a -values: 6374 and 2.9 nM for DP and CRTH2/DP2, respectively), and BW245C binds exclusively to DP (K_a -values: 0.4 and >80 000 nM for DP and CRTH2/DP2, respectively) [13]. Furthermore, ramatroban, originally identified as a thromboxane-type prostanoid receptor antagonist, has recently been demonstrated to be a potent and selective antagonist of CRTH2/DP2 (IC₅₀ values for inhibition of Ca²⁺ mobilization in transfected cells: >10 000 and 30 nM for DP and CRTH2/DP2, respectively) [20].

In the present study, we confirmed the earlier findings of others that human basophils express transcripts of both DP and CRTH2/DP2 [9]. However, in contrast to CRTH2/DP2-selective agonist DK-PGD₂, DP-selective agonist BW245C failed to induce significant Ca²⁺ mobilization in basophils (Fig. 1c). The limited expression of DP on basophils may explain the failure of BW245C to mobilize Ca²⁺ in basophils. In fact, our real-time PCR experiments showed that the expression level of DP transcripts was quite low (ca. 100-fold) compared with CRTH2/DP2 in basophils. CRTH2/DP2 was not only predominantly expressed in basophils, but also was responsible for most of PGD₂'s effects on these cells. A study by others demonstrated that basophil migration by PGD₂ is mediated via CRTH2/DP2 [9]. In the present study, we demonstrated for the first time that CRTH2/DP2 is also responsible for PGD₂-mediated up-regulation of CD11b expression (Fig. 2a) and enhancement of histamine release (Fig. 6) in addition to migratory responses (Fig. 4).

Negative regulatory effects of DP on chemotaxis have been reported in DP-transfected human cells [9]. Furthermore, *ex vivo* experiments have also implicated the migration inhibitory effects of DP on mouse dendritic cells [21]. Our present results for basophil migration are in line with those findings: migratory responses of basophils were significantly inhibited by BW245C, which was effectively antagonized by DP-selective antagonist BWA868C. The opposite effects seen between DP and CRTH2/DP2 may be a result of differences in the signalling pathways. Both DP and CRTH2/DP2 belong to a G-protein-coupled rhodopsin-type receptor family, but DP can couple to G α s and thus increase the intracellular cAMP concentration [10], while CRTH2/DP2 can couple to G α i [9] with the potential to decrease cAMP levels. DP-mediated inhibition of basophil chemotaxis may be involved in the increase in intracellular cAMP levels, since cAMP-elevating reagents such as phosphodiesterase inhibitors and β_2 -stimulants inhibit the motility of various leucocytes, including basophils [22, 23]. Elevation of cAMP also down-regulates the releasability of basophils [24]. The DP-mediated inhibition of IgE-mediated degranulation observed in the present study may also have been caused by elevation of cAMP levels.

Our laboratory has demonstrated that the regulatory profile of apoptosis of basophils is quite similar to that of eosinophils [17, 18]. Previous reports have shown conflicting results regarding the effects of PGD₂ on eosinophil survival. An earlier study indicated that both PGD₂ and BW245C are able to prolong the life-span of eosinophils, suggesting that DP activation mediates an anti-apoptotic effect in these cells [25]. In contrast, a more recent study by Ward et al. [26] demonstrated that PGD₂ potentially induces apoptosis in

eosinophils. Although BW245C was not tested in that study, the apoptogenic effect of PGD₂ is thought not to be mediated by either DP or CRTH2/DP2 [26]. Consistent with the study by Ward et al., we found that PGD₂ exerts pro-apoptotic effects on basophils (Fig. 3). Furthermore, in contrast to other basophil functions, both DK-PGD₂ and BW245C failed to exert positive effects on the life-span of basophils, suggesting that the effects of PGD₂ on basophil longevity are mediated by other known or unknown mechanism(s) besides activation of DP or CRTH2/DP2. Since induction of basophil apoptosis potentially contributes to the silent resolution of allergic inflammation, identification of mechanisms responsible for PGD₂-induced basophil apoptosis is important and merits further study.

A pro-/anti-inflammatory dichotomy was observed in the profile of the effects of DP and CRTH2/DP2. All of the effects of CRTH2/DP2, i.e., up-regulation of CD11b expression, enhancement of degranulation and induction of migration, are highly implicated in the development of allergic inflammation, thus indicating that CRTH2/DP2 activation primarily mediates the pro-inflammatory effects of PGD₂ on human basophils. On the other hand, DP activation of basophils conversely mediates anti-inflammatory effects: BW245C failed to exert positive effects on any of the basophil functions tested herein, but instead negatively regulated basophil chemotaxis (Figs 5a and b). This observation was somewhat unexpected, since DP knockout mice have been shown to develop reduced airway allergic inflammation [27]. It can be reasonably assumed that an *in vivo* role of DP is complicated, and the pro-inflammatory role of this receptor may reflect an overall action of DP, composed of various types of DP-expressing cells of varying significance. Thus, further study analysing the action of DP in these cells could be essential to understand the detailed *in vivo* role(s) of this receptor.

In summary, we have demonstrated dual properties of PGD₂ on human basophils: PGD₂ exerts pro-inflammatory (induction of migration, enhancement of degranulation and up-regulation of CD11b expression) as well as anti-inflammatory (promotion of apoptosis) effects on these cells. CRTH2/DP2 activation is necessary and sufficient for the pro-inflammatory effects of PGD₂, while DP activation may mediate the anti-inflammatory effects by negatively regulating basophil functions. Our results suggest that CRTH2/DP2 on basophils may afford opportunities for therapeutic targeting in allergic inflammation.

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