Brief Definitive Report

Prostaglandin D2 Selectively Induces Chemotaxis in T Helper Type 2 Cells, Eosinophils, and Basophils via Seven-Transmembrane Receptor CRTH2

By Hiroyuki Hirai,*‡ Kazuya Tanaka,* Osamu Yoshie,‖ Kazuyuki Ogawa,* Kazumi Kenmotsu,* Yasushi Takamori,* Michiko Ichimasa,§ Kazuo Sugamura,¶ Masataka Nakamura,** Shoichi Takano,* and Kinya Nagata*

*From the *R&D Center, BML, Saitama 350-1101, Japan; the ‡Graduate School of Science and Engineering, and the §Department of Environmental Sciences, Faculty of Science, Ibaraki University, Ibaraki 310-8512, Japan; the ‖Department of Bacteriology, Kinki University School of Medicine, Osaka 589-8511, Japan; the ¶Department of Microbiology and Immunology, Tohoku University School of Medicine, Miyagi 980-8575, Japan; and the **Human Gene Sciences Center, Tokyo Medical and Dental University, Tokyo 113-8510, Japan*

Abstract

Prostaglandin (PG)D_2, which has long been implicated in allergic diseases, is currently considered to elicit its biological actions through the DP receptor (DP). Involvement of DP in the formation of allergic asthma was recently demonstrated with DP-deficient mice. However, proinflammatory functions of PGD_2 cannot be explained by DP alone. We show here that a seven-transmembrane receptor, CRTH2, which is preferentially expressed in T helper type 2 (Th2) cells, eosinophils, and basophils in humans, serves as the novel receptor for PGD_2. In response to PGD_2, CRTH2 induces intracellular Ca^{2+} mobilization and chemotaxis in Th2 cells in a Gαi-dependent manner. In addition, CRTH2, but not DP, mediates PGD_2-dependent cell migration of blood eosinophils and basophils. Thus, PGD_2 is likely involved in multiple aspects of allergic inflammation through its dual receptor systems, DP and CRTH2.

Key words: prostanoid receptor • Gαi-type G protein • T cells • cell migration • allergic inflammation

Introduction

Leukocytes migrate from the peripheral blood to sites of inflammation through interaction with various cellular and humoral components such as selectins, integrins, and locally produced chemoattractants (1, 2). Allergic inflammation is characterized by selective accumulation of Th2 cells, eosinophils, and basophils, and is often triggered by antigen-stimulated mast cells (3, 4), although the mechanisms supporting these phenomena are largely unclear.

Recently, we cloned a novel putative chemoattractant receptor named CRTH2, which is a seven-transmembrane G protein–coupled receptor structurally related to members of the *N*-formyl peptide receptor (FPR) subfamily (5). CRTH2 is intriguing in that it is selectively expressed in Th2 cells, T cytotoxic type 2 cells, eosinophils, and basophils (5–7). Furthermore, CRTH2 can mediate intracellular Ca^{2+} mobilization in response to a factor(s) released from activated mast cells, suggesting that CRTH2 may be closely involved in mast cell–mediated allergic inflammation (6).

In this study, we show that the mast cell–derived CRTH2 agonist is prostaglandin (PG)D_2. This result was somewhat surprising because PGD_2 is generally considered to exert its action via the DP receptor (DP; references 8–10) and because CRTH2 has no significant homology in amino acid sequence with DP or all other known prostanoid receptors (10). However, in view of the structure, it may be notable that, like CRTH2, some other lipid mediator receptors such as lipoxin (LX)A_4 receptor FPRL1 and the new leukotriene (LT)B_4 receptor BLT2 are also more closely akin to FPR than the prostanoid receptors (11, 12). PGD_2 is the major prostanoid that is produced by activated mast cells and thereby has long been implicated in allergic diseases (10, 13). Most recently, involvement of the DP-mediated activities of PGD_2 in the formation of allergic asthma has been demonstrated with DP-deficient mice

Address correspondence to Kinya Nagata, R&D Center, BML, 1361-1 Matoba, Kawagoe, Saitama 350-1101, Japan. Phone: 81-492-32-0440; Fax: 81-492-32-5480; E-mail: nagata@alk.co.jp

J. Exp. Med. © The Rockefeller University Press • 0022-1007/2001/01/255/07 $5.00
Volume 193, Number 2, January 15, 2001 255–261
http://www.jem.org/cgi/content/full/193/2/255

(14). However, contribution of DP to the direct effects of PGD_2 on leukocytes, such as induction of eosinophil migration (15), has not yet been established, raising new questions such as how these receptors are being used.

Our results show that although CRTH2 functions as a selective PGD_2 receptor as well as DP, it sharply differs from DP in signaling pathways. Furthermore, CRTH2 but not DP induces migration of Th2 cells, eosinophils, and basophils in response to PGD_2. Our findings elucidated a novel function of PGD_2 on T cell responses, and CRTH2 is the first receptor that directly and selectively connects a major lipid mediator of activated mast cells with Th2 cells, eosinophils, and basophils.

Materials and Methods

DP Cloning. Human DP cDNA was generated by reverse-transcription (RT)-PCR using a small intestine poly(A)$^+$ RNA (CLONTECH Laboratories, Inc.) as a template and the following primers: 5′-CTTCCGAAGCCTTCACTCCAGCCCTCT-GCTCCCG (sense) and 5′-GTTCTTTTCTAGAAAATGT-GACATATTCCTCAGCTTACC (antisense). The DP cDNA was cloned into the HindIII-XbaI sites of pRc/CMV (Invitrogen), yielding an expression plasmid pRc/DP.

Abs. CRTH2-specific rat mAbs BM16 and BM7 were described previously (5, 6). Both mAbs were generated by immunizing Wister rats with CRTH2-transfected rat T cell line TART-1, and their epitopes are still unclear.

Cells. Mast cell supernatants were prepared as reported previously (6). K562 and Jurkat lines were transfected with pRc/B19 (a CRTH2 expression vector; references 5, 6), pRc/DP, or pRc/CMV and cloned as described (5). Human Th1 and Th2 lines were generated from the PBMCs of healthy adults by stimulation with a purified protein derivative of *Mycobacterium tuberculosis* in the presence of IL-12 and IFN-γ (Th1 condition) or with an extract of *Dermatophagoides* in the presence of IL-4 and neutralizing anti–IFN-γ and anti–IL-12 mAbs (Th2 condition), then expanded by IL-2 and used at days 7–14 for examinations as described previously (5). In some chemotaxis assays, Th2 cells highly expressing CRTH2 were enriched by sorting with anti-CRTH2 mAb BM16 (5) a few days before use. PBMCs and granulocytes were isolated as described (6).

Ca^{2+} Mobilization. Ca^{2+} mobilization assays were performed as described (6). LTC_4, LTB_4, LXA_4, prostanoids, and their synthetic analogues were purchased from Cayman Chemical except for fluprostenol (Biomol) and PGD_2 methyl ester (Sigma-Aldrich). Thrombin and monocyte chemotactic protein 1 (MCP-1) were obtained from Sigma-Aldrich and PeproTech, respectively. For inactivation of G proteins, cells (2.5×10^6 cells/ml) were incubated with pertussis toxin (PTX; Sigma-Aldrich) or cholera toxin (CTX; Sigma-Aldrich) at 1 μg/ml for 2 h at 37°C.

^3H-PGD_2 Binding Analysis. Cells washed and suspended in HBSS containing 10 mM Hepes, pH 7.3 (5×10^6 cells/100 μl) were incubated with 1 nM ^3H-PGD_2 (Amersham Pharmacia Biotech) for 60 min on ice. Cells were then separated from free ^3H-PGD_2 by centrifugation on RPMI 1640 containing 1 M sucrose, and radioactivity bound to the cells was measured on a liquid scintillation counter. Nonspecific binding was determined in the presence of 10 μM cold PGD_2.

RT-PCR Analysis. Basophils and eosinophils were purified as described (6). Neutrophils (99% pure as determined by May-

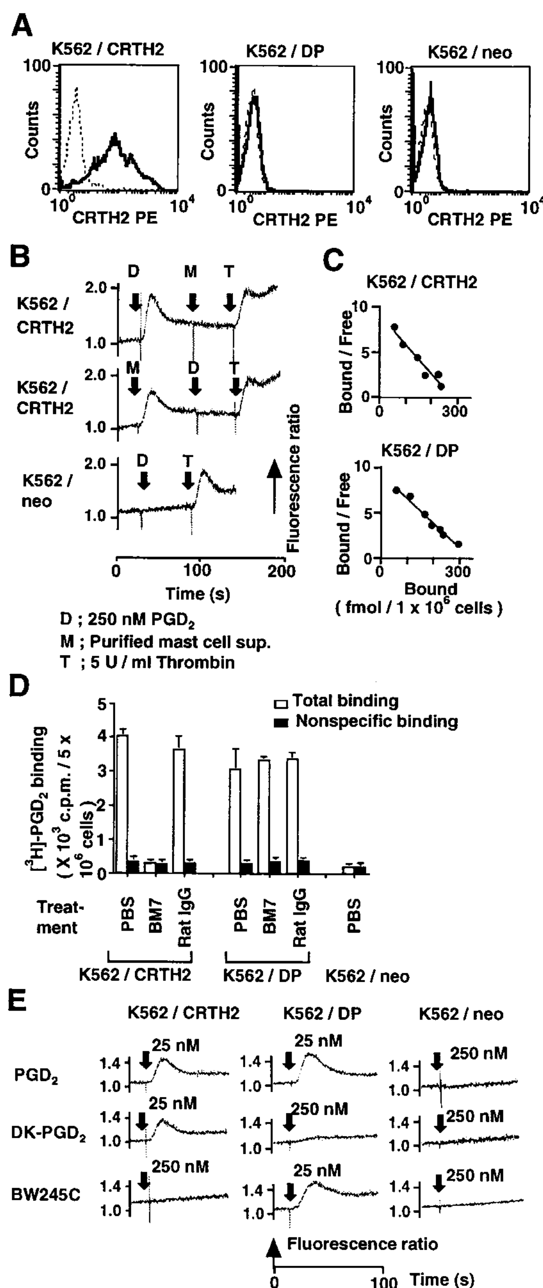

Figure 1. Binding of PGD_2 to CRTH2. (A) Expression levels of CRTH2 as determined by flow cytometry. Cells were stained with biotinylated BM16 (solid line) or control IgG2a (dotted line) as described (reference 6). (B) CRTH2 mediates Ca^{2+} mobilization by PGD_2 and purified mast cell supernatants. Thrombin was used as an irrelevant stimulant. Arrows indicate the time of stimulant addition. (C) Scatchard plot analysis for ^3H-PGD_2 binding to K562/CRTH2 or K562/DP. (D) CRTH2-mediated binding of ^3H-PGD_2 is selectively inhibited by BM7. Cells pretreated with PBS, BM7 (600 μg/ml), or normal rat IgG (600 μg/ml) at room temperature for 20 min were subjected to ^3H-PGD_2 binding analysis without washing (mean $\pm$ SD, $n = 3$). (E) DK-PGD_2 and BW245C serve as selective agonists for CRTH2 and DP, respectively, in Ca^{2+} mobilization assay.

Grünwald Giemsa staining) were purified from granulocytes with anti-CD16–coated magnetic beads (Miltenyi Biotec). Monocytes (98% pure), T cells (92% pure), and B cells (91% pure) were isolated from PBMCs with anti-CD14–, anti-CD3–, and anti-CD19–coupled magnetic beads (Miltenyi Biotec), respectively. RT-PCR was performed as described previously (5, 6). Primers for DP were 5′-GCAACCTCTATGCGATGCA (sense) and 5′-CAAGGCTCGGAGGTCTTCT (antisense). PCR conditions were 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min.

Chemotaxis Assay. This was performed on a 96-well microchemotaxis chamber with a 5-μm-pore filter (Neuroprobe) according to the manufacturer's instructions. In brief, cells (1–2 × 10^5 cells/50 μl) and test samples (29 μl) prepared in RPMI 1640 containing 10% FCS and 10 mM Hepes, pH 7.3, were applied to top and bottom wells, respectively, of the chamber. After incubation at 37°C for 1 h, cells in the bottom wells were counted by flow cytometry. In the case of blood leukocytes, cell types were determined by Wright's staining.

Results

CRTH2 Is a Novel Receptor for PGD_2. We previously found that mast cells produce a CRTH2-specific agonist(s) that can induce Ca^{2+} mobilization in CRTH2-transfected K562 (K562/CRTH2) but not mock-transfected K562 (K562/neo) (Fig. 1 A; reference 6). In a screening of known mast cell products (13, 16), we found that only PGD_2 can induce Ca^{2+} mobilization in K562/CRTH2 but not K562/neo at nanomolar concentrations (Fig. 1 B). Other mast cell–related and –unrelated inflammatory mediators such as histamine, serotonin, platelet-activating factor, LTB_4, LTC_4, regulated upon activation normal T cell expressed and secreted (RANTES), MCP-1, eotaxin, IL-8, LXA_4, fMLP, C5a, and angiotensin II did not induce any significant CRTH2-mediated Ca^{2+} mobilization (5; and data not shown). Treatment of mast cells with aspirin, a PG synthesis inhibitor, completely impaired production of the active component. The active component of the mast cell supernatants showed an elution profile identical to that of PGD_2 on a reverse phase chromatography using a μRPC C2/C18 SC 2.1/10 column (Amersham Pharmacia Biotech) (data not shown). Having once responded to PGD_2, K562/CRTH2 did not show any appreciable Ca^{2+} mobilization by the mast cell active fraction, and vice versa (cross-desensitization; Fig. 1 B). These observations demonstrate that PGD_2 is the CRTH2 agonist that is produced by activated mast cells.

Table I. *K_i Values of Various Compounds on the Specific Binding of ^3H-PGD_2 to CRTH2 and DP*

Ligand	K562/CRTH2	K562/DP
PGD_2	61 ± 23	45 ± 17
DK-PGD_2	160 ± 35	>30,000
PGJ_2	460 ± 160	64 ± 13
11β-$PGF_{2\alpha}$	20,000 ± 160	1,200 ± 470
Δ^{12}-PGJ_2	7,100 ± 1,300	5,200 ± 1,700
15-deoxy-$\Delta^{12,14}$-PGJ_2	2,300 ± 400	>30,000
PGE_2	3,100 ± 1,100	2,300 ± 1,100
$PGF_{2\alpha}$	2,000 ± 780	>30,000
PGA_2	>30,000	>30,000
U46619	>30,000	>30,000
BW245C	>100,000	50 ± 20
PGD_2 methyl ester	460 ± 120	270 ± 46
LTB_4	>30,000	>30,000
LXA_4	>30,000	>30,000

Inhibitor constant (K_i) values (nM ± SD, $n \geq 3$) are shown. IC_{50} values were determined by the binding of ^3H-PGD_2 to the indicated cells in the presence of various concentrations of competing compounds and converted to K_i values according to the equation $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is the concentration of ^3H-PGD_2 (1 nM) and K_d is its dissociation constant (taken as 31.3 nM for CRTH2 and 37.7 nM for DP) (reference 17).

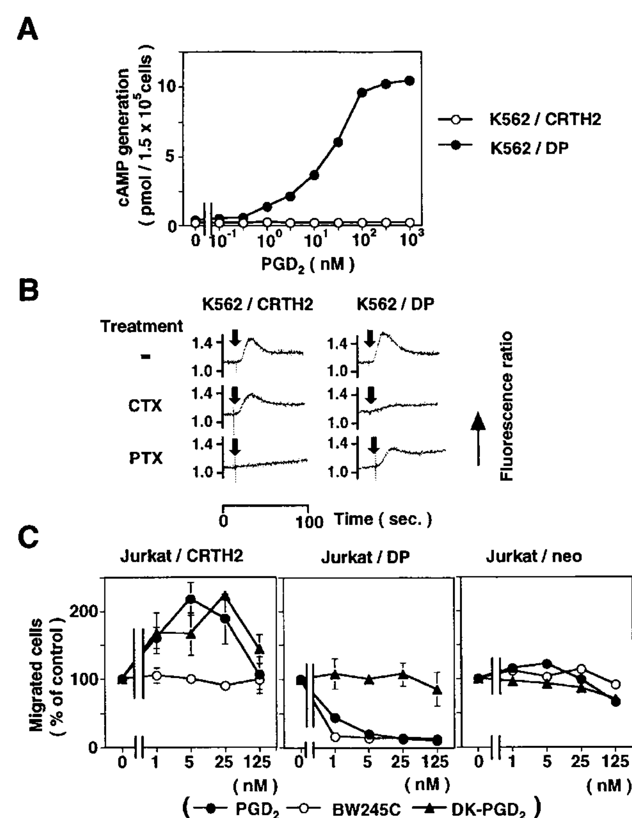

Figure 2. Functional discrimination between CRTH2 and DP. (A) DP but not CRTH2 mediates PGD_2-induced cAMP generation. Intracellular cAMP levels were determined by enzyme-linked immunoassay (mean, n = 2; Amersham Pharmacia Biotech). (B) CTX and PTX differentially affect PGD_2-induced (25 nM) Ca^{2+} mobilization in K562/CRTH2 and K562/DP. (C) Chemotactic responses to agonists (PGD_2, DK-PGD_2, and BW245C) are opposite in Jurkat/CRTH2 and Jurkat/DP (mean ± SD, n = 3). Numbers of migrated cells in controls were 727 ± 134 (Jurkat/CRTH2), 439 ± 50 (Jurkat/DP), and 661 ± 152 (Jurkat/neo).

The above finding was supported by the experiments using K562/CRTH2 and DP-transfected K562 (K562/DP; Fig. 1 A). Both lines exhibited specific binding of ^{3}H-PGD_2 with similar numbers of binding sites per cell and K_d values of 31.3 and 37.7 nM, respectively, whereas ^{3}H-PGD_2 did not bind to K562/neo (Fig. 1, C and D). A neutralizing CRTH2-specific mAb BM7 selectively inhibited the binding of ^{3}H-PGD_2 to K562/CRTH2 but not K562/DP, whereas control rat IgG had no effect on this assay (Fig. 1 D). To establish ligand specificity, binding affinities to the CRTH2 and DP of PGD_2 analogues, major prostanoids, a thromboxane (TX)A_2 receptor agonist U46619 (8), a synthetic DP agonist BW245C (8), LTB_4, and LXA_4 were determined by competitive binding assay (Table I). The results demonstrate that PGD_2 binds to CRTH2 as well as DP with the highest affinity among various prostanoids. Interestingly, one of the PGD_2 metabolites, 13,14-dihydro-15-keto-PGD_2 (DK-PGD_2), showed an affinity to CRTH2 as high as PGD_2 to CRTH2, whereas DP showed at least 600-fold lower affinity to DK-PGD_2 than PGD_2 as estimated by the competition assay. In contrast, BW245C was highly selective for DP.

Consistently, Ca^{2+} mobilization was induced in both K562/CRTH2 and K562/DP by PGD_2, whereas DK-PGD_2 and BW245C were selectively effective in K562/CRTH2 and K562/DP, respectively, in Ca^{2+} mobilization assay (Fig. 1 E). No significant Ca^{2+} mobilization was induced in K562/CRTH2 by up to 250 nM PGA_2, PGI_2, and TXB_2 (data not shown). From these findings, we have concluded that CRTH2 serves as the novel selective receptor for PGD_2. Furthermore, DK-PGD_2 and BW245C are useful reagents that specifically induce CRTH2- and DP-mediated responses, respectively.

Differences in Signaling Pathways Associated with CRTH2 and DP. To gain insight into functional differences between CRTH2 and DP, signaling pathways associated with

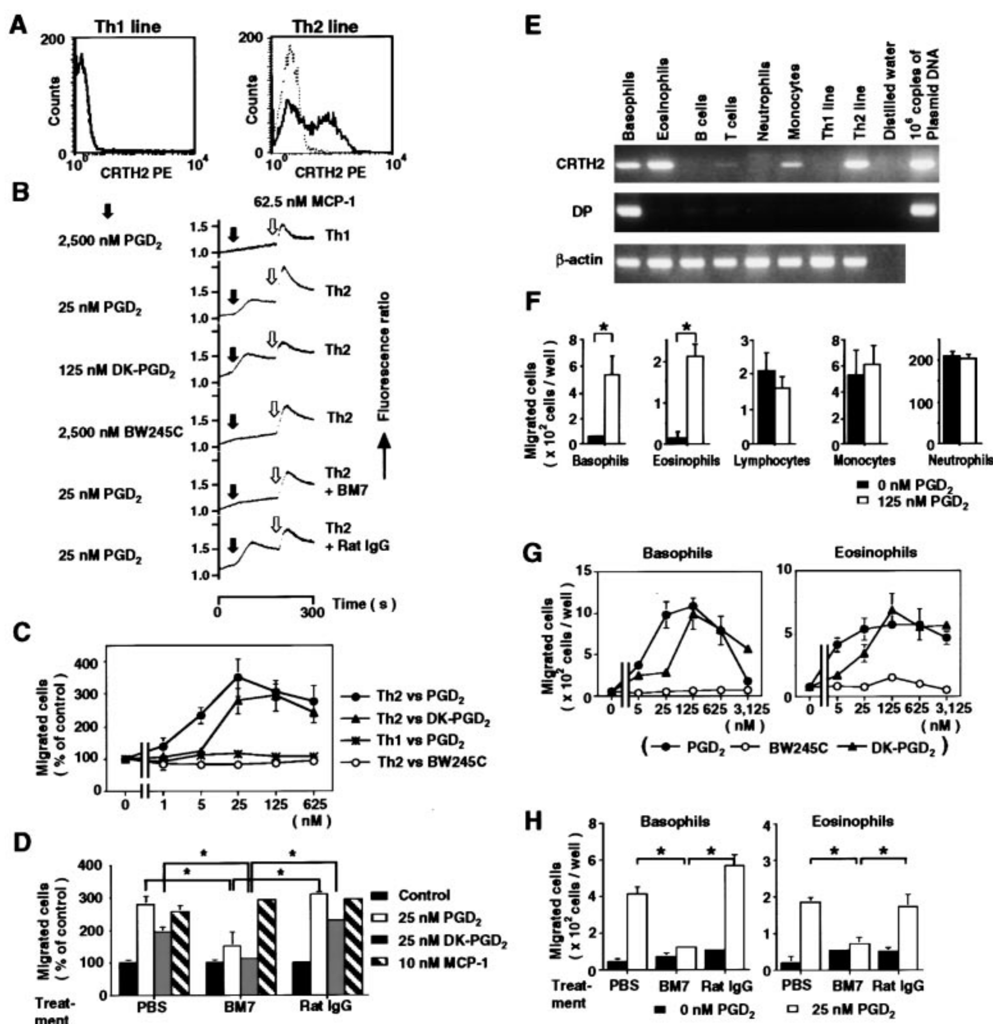

Figure 3. Selective responses of Th2, basophils, and eosinophils to PGD_2. (A) CRTH2 expression in representative Th1 and Th2 lines. Levels of CRTH2 expression are presented as described in the legend to Fig. 1 A. (B) PGD_2 and DK-PGD_2 selectively induce Ca^{2+} mobilization in Th2 cells via CRTH2. Arrows indicate the time of addition of the test samples (black arrows) and 62.5 nM MCP-1 (white arrows). MCP-1 was used as an irrelevant stimulant. BM7 and rat IgG (each 600 μg/ml) were added 20 min before the stimulant addition. (C) Chemotaxis to PGD_2 and DK-PGD_2 is selectively induced in Th2 but not Th1 lines (mean ± SD, $n = 3$). The numbers of migrated cells in controls were 4,015 ± 627 (Th1) and 1,374 ± 225 (Th2). (D) Chemotaxis to PGD_2 and DK-PGD_2 of Th2 cells is inhibited by BM7 (mean ± SD, $n = 3$). Th2 cells were treated with PBS, BM7, or normal rat IgG as described in B before being subjected to chemotaxis assay. Numbers of migrated cells in controls were 4,146 ± 433 (PBS), 3,283 ± 349 (BM7), and 3,246 ± 65 (normal rat IgG). (E) mRNA levels for CRTH2, DP, and β-actin (internal control) in peripheral blood leukocytes and Th lines as determined by RT-PCR. (F) Migration of basophils and eosinophils is induced by PGD_2 (mean ± SD, $n = 3$). (G) Migration of eosinophils and basophils to PGD_2 and DK-PGD_2 is dose dependent (mean ± SD, $n = 3$). In this experiment, neutrophil ($CD16^+$ cell)-depleted leukocyte samples were used. (H) PGD_2-induced migration of basophils and eosinophils is inhibited by BM7 (mean ± SD, $n = 3$). Neutrophil-depleted leukocytes were treated as described in D. Asterisks indicate statistical significance with a probability of <0.01 in Student's t test.

the two receptors were examined. DP induced cAMP generation in response to PGD_2 as reported previously (Fig. 2 A; reference 9), and DP-mediated Ca^{2+} mobilization was blocked by CTX but not by PTX as expected (Fig. 2 B). In contrast, PTX but not CTX inhibited CRTH2-mediated Ca^{2+} mobilization (Fig. 2 B), and no production of cAMP was seen by interaction of PGD_2 with CRTH2, as expected (Fig. 2 A). These results indicate that CRTH2 is coupled with Gαi-type G protein and DP with Gαs-type protein.

Since many seven-transmembrane Gαi-coupled receptors are involved in chemotaxis of leukocytes (1), we then addressed the issue of whether CRTH2 was responsible for chemotaxis. CRTH2-transfected Jurkat (Jurkat/CRTH2) was indeed attracted by PGD_2 and DK-PGD_2 but not by BW245C (Fig. 2 C). In sharp contrast, DP-transfected Jurkat (Jurkat/DP) was suppressed even in its spontaneous migration by PGD_2 or BW245C. Thus, differential effects of PGD_2 between CRTH2- and DP-expressing cells were clearly observed, presumably resulting from the difference in signaling molecules associated with the receptors.

Functional Properties of CRTH2 in Normal Leukocytes. We next confirmed the above findings with normal leukocytes that naturally express CRTH2. Cultured Th2 but not Th1 cells showed specific binding of ^3H-PGD_2, which was blocked by anti-CRTH2 mAb BM7 (Fig. 3 A, and data not shown). In response to PGD_2 and DK-PGD_2, intracellular Ca^{2+} levels in Th2 cells increased (Fig. 3 B) and chemotactic migration was induced (Fig. 3 C). PGD_2 had no effect on Ca^{2+} mobilization and chemotactic migration of Th1 cells (Fig. 3, B and C). DP agonist BW245C had no effect on Ca^{2+} mobilization and chemotactic migration of Th2 cells (Fig. 3, B and C). The neutralizing anti-CRTH2 mAb BM7 nearly completely inhibited PGD_2- and DK-PGD_2–dependent Ca^{2+} mobilization and migration in Th2 cells without affecting their responses to an irrelevant chemoattractant, MCP-1 (Fig. 3, B and D). Similar results were obtained in all of five pairs of Th1 and Th2 lines from five different adults.

In RT-PCR analysis, CRTH2 was expressed highly in basophils and eosinophils as well as in Th2 lines and weakly in monocytes, whereas DP was expressed mainly in basophils (Fig. 3 E). When total peripheral blood leukocytes were tested for chemotactic migration, only basophils and eosinophils were significantly attracted by PGD_2 (Fig. 3 F). When partially purified (neutrophil-depleted) leukocytes were tested for the assay, both basophils and eosinophils more clearly exhibited their migratory responses towards PGD_2 and DK-PGD_2 (Fig. 3 G). BW245C, a selective agonist for DP, showed no chemotactic effect on either cell type (Fig. 3 G). Anti-CRTH2 mAb BM7 but not control rat IgG blocked the migratory responses to PGD_2 of basophils and eosinophils (Fig. 3 H). Circulating $CRTH2^+CD4^+$ lymphocytes also showed a tendency to migrate to PGD_2 or DK-PGD_2 at ~1.5–3-fold higher frequencies than to vehicle or BW245C (data not shown).

The migratory responses of the above three normal cell types to PGD_2 were completely abolished by PTX. A checkerboard analysis demonstrated that the effect of PGD_2 on Th2 cells was mostly chemotactic, whereas on basophils and eosinophils there was a partly chemokinetic effect as reported on eosinophils (15). PGD_2 is also known to weakly cross-react with receptors for PGE_2, $PGF_{2\alpha}$, and TXA_2 (18). However, involvement of these receptors in chemotaxis was ruled out because PGE_2, fluprostenol (a $PGF_{2\alpha}$ receptor–selective agonist; reference 8), and U46619 (a TXA_2 receptor–selective agonist; reference 8) had no significant chemotactic effect on these three cell types at nanomolar ranges. Taken together, these results indicate that CRTH2 mediates chemotactic/chemokinetic signal of PGD_2 in a Gαi-dependent manner in Th2 cells, eosinophils, and basophils.

Discussion

Prostanoid receptors have been classified into TX receptor (TP), PGE receptor (EP1, EP2, EP3, and EP4), PGF receptor (FP), PGI receptor (IP), and PGD receptor (DP; references 8, 10). In view of the structure, CRTH2 appears to be unique among members of prostanoid receptors in that it lacks any consensus amino acid sequence motifs that are shared by all other known prostanoid receptors (10). Indeed, a phylogenetic analysis shows that CRTH2 is more akin in the overall structure to the FPR subfamily members and receptors for LTs than known prostanoid receptors (Fig. 4). Furthermore, the chromosomal location of

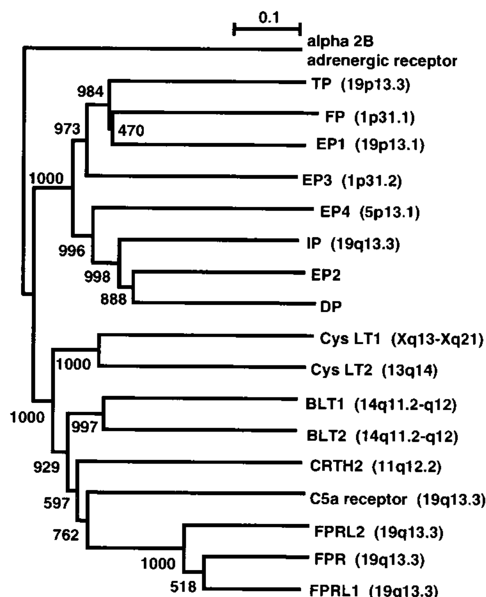

Figure 4. A phylogenetic tree for human receptors to classical chemoattractants and major prostanoids. The tree was constructed by the N-J method using CLUSTAL X software. The sequence of the human α2B adrenergic receptor was used as an outer group to obtain a root. Chromosomal locations are shown in parentheses. Accession nos. for the receptors are (from top to bottom) GenBank/EMBL/DDBJ AF005900, D38081, L24470, L22647, L27490, L28175, D25418, U19487, Q13258 (GenPept accession no.), AF119711, AF254664, AB008193, AB008193, AB008535, M62505, L14061, L10820, and M84562. Cys LT, cysteinyl LT receptor; FPRL, formyl peptide receptor–like receptor.

CRTH2 (11q12.2) (sequence data available from GenBank/EMBL/DDBJ under accession no. AC004126) differs from those of other receptors listed in Fig. 4, suggesting that CRTH2 might form a novel receptor gene subfamily. The mechanisms by which CRTH2 binds PGD_2 with an affinity as high as DP remain to be clarified.

PGD_2 is currently considered to elicit its biological actions through DP (8, 10). In fact, well-established PGD_2-induced activities such as vasodilation, relaxation of other various smooth muscles, and inhibition of platelet aggregation are apparently mediated by DP because they are also induced by a DP-specific agonist BW245C (18, 19). However, several in vivo and ex vivo effects of PGD_2 such as an increase in microvascular permeability, eosinophil infiltration, and goblet cell depletion are not mimicked by BW245C (20). Thus, questions on the existence of a BW245C-insensitive PGD_2 receptor subtype(s) has been repeatedly proposed (20–22). In addition, although DK-PGD_2 is generally thought to be biologically inactive in many systems, several investigators actually observed its effects on some PGD_2-sensitive tissues (23). Our finding that CRTH2 is the novel PGD_2 receptor functioning differently from DP may lead to the resolution of such longstanding questions.

A notable difference between CRTH2 and DP is the signaling molecules: $G\alpha i$ is used for CRTH2 whereas $G\alpha s$ is for DP. As shown by many cell types with $G\alpha i$-coupled receptors, CRTH2 leads Th2 cell, eosinophil, and basophil induction of chemotaxis/chemokinesis in response to PGD_2. Consequently, these types of cells may accumulate at the sites of allergic inflammation (3, 4). Indeed, it was demonstrated that topical application of PGD_2 causes significant accumulation of eosinophils in the guinea pig conjunctiva and the dog trachea (20, 24). In contrast, DP-mediated PGD_2 signals caused reduction in spontaneous cell migration in DP-transfected Jurkat cells and had no effect on the migration of DP-expressing basophils in our study. These results suggest that DP-mediated signals may not, in nature, lead to chemotactic or chemokinetic behavior in leukocytes. Although PGD_2 has been reported to modulate various activities of neutrophils, we could not detect any substantial expression of CRTH2 or DP in blood neutrophils (25). However, further studies are required to finally establish the actual involvement of these receptors in the PGD_2-induced modulation of neutrophils.

Although the mechanisms by which DP contributes to the formation of allergic inflammation remain to be clarified, our results suggest that DP and CRTH2 may be cooperatively involved in allergic inflammation through different processes. A plausible scenario could be that PGD_2 is largely produced by mast cells upon antigen stimulation, inducing local vasodilation via DP, which enhances extravasation of blood leukocytes (26), followed by chemotactic migration of Th2 cells, eosinophils, and basophils via CRTH2 in cooperation with other chemotactic mediators such as CC chemokines TARC and eotaxin (27, 28). Thus, CRTH2 may be a new favorable target for allergic disease therapies. Generation of selective inhibitors for CRTH2 or CRTH2-deficient mice should help to elucidate the physiological and pathophysiological roles of the CRTH2/PGD_2 system and its relative importance in the host defense mechanisms.

We thank Drs. K. Katayama and F.B. Hoshino (BML) for drawing the phylogenetic tree.

Submitted: 31 August 2000
Revised: 15 November 2000
Accepted: 20 November 2000

References

1. Murphy, P.M. 1994. The molecular biology of leukocyte chemoattractant receptors. *Annu. Rev. Immunol.* 12:593–633.
2. Springer, T.A. 1994. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell.* 76:301–314.
3. Charlesworth, E.N., A.F. Hood, N.A. Soter, A. Kagey-Sobotka, P.S. Norman, and L.M. Lichtenstein. 1989. Cutaneous late-phase response to allergen. Mediator release and inflammatory cell infiltration. *J. Clin. Invest.* 83:1519–1526.
4. Robinson, D.S., Q. Hamid, S. Ying, A. Tsicopoulos, J. Barkans, A.M. Bentley, C. Corrigan, S.R. Durham, and A.B. Kay. 1992. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N. Engl. J. Med.* 326: 298–304.
5. Nagata, K., K. Tanaka, K. Ogawa, K. Kemmotsu, T. Imai, O. Yoshie, H. Abe, K. Tada, M. Nakamura, K. Sugamura, et al. 1999. Selective expression of a novel surface molecule by human Th2 cells in vivo. *J. Immunol.* 162:1278–1286.
6. Nagata, K., H. Hirai, K. Tanaka, K. Ogawa, T. Aso, K. Sugamura, M. Nakamura, and S. Takano. 1999. CRTH2, an orphan receptor of T-helper-2-cells, is expressed on basophils and eosinophils and responds to mast cell-derived factor(s). *FEBS. Lett.* 459:195–199.
7. Cosmi, L., F. Annunziato, M. Iwasaki, G. Galli, R. Manetti, E. Maggi, K. Nagata, and S. Romagnani. 2000. CRTH2 is the most reliable marker for the detection of circulating human type 2 Th and type 2 T cytotoxic cells in health and disease. *Eur. J. Immunol.* 30:2972–2979.
8. Coleman, R.A., I. Kennedy, P.P.A. Humphrey, K. Bunce, and P. Lumley. 1990. Prostanoids and their receptors. *In* Comprehensive Medical Chemistry: Membranes and Receptor. J.C. Emmett, editor. Pergamon Press, Oxford. 3:643–714.
9. Boie, Y., N. Sawyer, D.M. Slipetz, K.M. Metters, and M. Abramovitz. 1995. Molecular cloning and characterization of the human prostanoid DP receptor. *J. Biol. Chem.* 270: 18910–18916.
10. Narumiya, S., Y. Sugimoto, and F. Ushikubi. 1999. Prostanoid receptors: structures, properties, and functions. *Physiol. Rev.* 79:1193–1226.
11. Fiore, S., J.F. Maddox, H.D. Perez, and C.N. Serhan. 1994. Identification of a human cDNA encoding a functional high affinity lipoxin A_4 receptor. *J. Exp. Med.* 180:253–260.
12. Yokomizo, T., K. Kato, K. Terawaki, T. Izumi, and T. Shimizu. 2000. A second leukotriene B_4 receptor, BLT2. A new therapeutic target in inflammation and immunological disorders. *J. Exp. Med.* 192:421–432.
13. Lewis, R.A., N.A. Soter, P.T. Diamond, K.F. Austen, J.A. Oates, and L.J. Roberts II. 1982. Prostaglandin D_2 generation

after activation of rat and human mast cells with anti-IgE. *J. Immunol.* 129:1627–1631.
14. Matsuoka, T., M. Hirata, H. Tanaka, Y. Takahashi, T. Murata, K. Kabashima, Y. Sugimoto, T. Kobayashi, F. Ushikubi, Y. Aze, et al. 2000. Prostaglandin D_2 as a mediator of allergic asthma. *Science.* 287:2013–2017.
15. Goetzl, E.J., P.F. Weller, and F.H. Valone. 1979. Biochemical and functional bases of the regulatory and protective roles of the human eosinophils. *In* Advances in Inflamation Research. Proceedings of the International Congress of Inflammation. G. Weissmann, B. Samuelsson, and R. Paolletti, editors. Raven Press, New York. 14:157–167.
16. Larsen, G.L., and P.M. Henson. 1983. Mediators of inflammation. *Annu. Rev. Immunol.* 1:335–359.
17. Shimizu, T., A. Yamashita, and O. Hayaishi. 1982. Specific binding of prostaglandin D_2 to rat brain synaptic membrane. *J. Biol. Chem.* 257:13570–13575.
18. Wright, D.H., K.M. Metters, M. Abramovitz, and A.W. Ford-Hutchinson. 1998. Characterization of the recombinant human prostanoid DP receptor and identification of L-644,698, a novel selective DP agonist. *Br. J. Pharmacol.* 123:1317–1324.
19. Giles, H., and P. Leff. 1988. The biology and pharmacology of PGD_2. *Prostaglandins.* 35:277–300.
20. Woodward, D.F., S.B. Hawley, L.S. Williams, T.R. Ralston, C.E. Protzman, C.S. Spada, and A.L. Nieves. 1990. Studies on the ocular pharmacology of prostaglandin D_2. *Invest. Ophthalmol. Vis. Sci.* 31:138–146.
21. Narumiya, S., and N. Toda. 1985. Different responsiveness of prostaglandin D_2-sensitive systems to prostaglandin D_2 and its analogues. *Br. J. Pharmacol.* 85:367–375.
22. Rangachari, P.K., P.A. Betti, E.T. Prior, and L.J. Roberts II. 1995. Effects of a selective DP receptor agonist (BW 245C) and antagonist (BW A868C) on the canine colonic epithelium: an argument for a different DP receptor? *J. Pharmacol. Exp. Ther.* 275:611–617.
23. Rangachari, P.K., and P.A. Betti. 1993. Biological activity of metabolites of PGD_2 on canine proximal colon. *Am. J. Physiol.* 264: G886–894.
24. Emery, D.L., T.D. Djokic, P.D. Graf, and J.A. Nadel. 1989. Prostaglandin D_2 causes accumulation of eosinophils in the lumen of the dog trachea. *J. Appl. Physiol.* 67:959–962.
25. Simpkins, C.O., D.L. Mazorow, S.T. Alailima, E.A. Tate, W. Sweatt, M. Johnson, K. Shariff, and D.B. Millar. 1990. Prostaglandin D_2 modulates human neutrophil intracellular calcium flux and inhibits superoxide release via its ring carbonyl. *Life Sci.* 46:793–801.
26. Simpkins, C.O., D.L. Mantovani, A.F. Colotta, S. Sozzani, P. Allavena, and E. Dejana. 1994. Vasodilation in multistep paradigm of leucocyte extravasation. *Lancet.* 343:1499–1500.
27. Sallusto, F., A. Lanzavecchia, and C.R. Mackay. 1998. Chemokines and chemokine receptors in T-cell priming and Th1/Th2- mediated responses. *Immunol. Today.* 19:568–574.
28. Imai, T., M. Nagira, S. Takagi, M. Kakizaki, M. Nishimura, J. Wang, P.W. Gray, K. Matsushima, and O. Yoshie. 1999. Selective recruitment of CCR4-bearing Th2 cells toward antigen-presenting cells by the CC chemokines thymus and activation-regulated chemokine and macrophage-derived chemokine. *Int. Immunol.* 11:81–88.