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9α,11β-PGF_2 and its stereoisomer $PGF_{2\alpha}$ are novel agonists of the chemoattractant receptor, CRTH2

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Abstract CRTH2 is a recently described chemoattractant receptor for the prostaglandin, PGD_2, expressed by Th2 cells, eosinophils and basophils, and believed to play a role in allergic inflammation. Here we describe the potency of several PGD_2 metabolites at the receptor to induce cell migration and activation. We report for the first time that the PGD_2 metabolite, 9α,11β-PGF_2, and its stereoisomer, $PGF_{2\alpha}$, are CRTH2 agonists. 9α,11β-PGF_2 is a major metabolite produced in vivo following allergen challenge, whilst $PGF_{2\alpha}$ is generated independently of PGD synthetase, with implications for CRTH2 signalling in the presence or absence of PGD_2 production.

Keywords: CRTH2; Prostaglandin; Eosinophil; Chemoattractant; Inflammation

1. Introduction

PGD_2 is the major prostaglandin (PG) released by mast cells following activation [1], and mice over-expressing PGD synthase exhibit marked eosinophil and T-lymphocyte recruitment to the lung [2], implying a role for the PG in allergic inflammation. PGD_2 signals through the DP receptor [3], and in a model of allergic airway inflammation, mice lacking DP failed to develop airway hyperreactivity and showed reduced cell infiltration [4]. Recently, a novel PGD_2 receptor, chemoattractant receptor homologous molecule expressed by Th2 cells (CRTH2), was identified [5], which is expressed by eosinophils, Th2 cells and basophils [6] and facilitates the migration of these cells in response to PGD_2 [5].

PGD_2 is a labile molecule [7,8], and the PGD_2 metabolites, Δ^{12}-PGD_2, 15d-PGD_2, 15d-PGJ_2, Δ^{12}-PGJ_2 and PGJ_2 (depicted in Fig. 1), have been shown to bind to CRTH2 [5,9,10] and to activate eosinophils [10–12]. 9α,11β-PGF_2 is generated from PGD_2 by the action of PGF synthase [13] and is one of the major metabolites of PGD_2 formed in vivo [13,14], being found in the urine and plasma of asthmatics following allergen challenge [15,16]. $PGF_{2\alpha}$ is a stereoisomer of 9α,11β-PGF_2 (Fig. 1), produced from PGH_2 by the action of PGF synthase [17], and from PGE_2 by the action of PGE 9-ketoreductase [18]. $PGF_{2\alpha}$, 9α,11β-PGF_2 and PGD_2 have been reported to cause smooth muscle contraction with similar potency [19], and the three PGs exhibit potent bronchoconstrictor activity [20]. 9α,11β-PGF_2 and $PGF_{2\alpha}$ exhibit activity at DP, albeit with lower affinity than PGD_2 [19,21]. In addition, 9α,11β-PGF_2 has been demonstrated to induce the upregulation of CD11b on eosinophils [11], and to inhibit cAMP generation in CRTH2 transfected cells [9], whilst $PGF_{2\alpha}$ induced actin polymerisation and CD11b upregulation in eosinophils [22], and in CRTH2 transfectants, induced calcium mobilisation and inhibited cAMP generation, actions presumably mediated by CRTH2. Here we provide further evidence that 9α,11β-PGF_2 and $PGF_{2\alpha}$ are CRTH2 agonists, and characterise their effects at this receptor.

2. Materials and methods

2.1. Materials

Unless otherwise stated, all reagents were from Sigma–Aldrich (Poole, UK). PGs and ramatroban were from Cayman Chemicals (MI, USA), and tissue culture reagents were from Invitrogen (Paisley, UK).

2.2. Cell culture

Naïve BaF/3 cells and those stably expressing CRTH2 (CRTH2.BaF/3 cells) were cultured as described [23].

2.3. Chemotaxis assay

Assays were carried out as previously described [23]. Briefly, agonists were diluted in RPMI + 0.1% BSA and placed in the wells of a 96 well Neuroprobe chemotaxis plate (Receptor Technologies, UK). 2×10^5 cells were placed on top of the filter and the plate incubated at 37 °C, 5% CO_2, in a humidified box for 5 h. Cells traversing the filter were counted on a haemocytometer. Results are expressed as the percentage of migrating cells, following the subtraction of basal migration, to buffer alone.

2.4. Preparation of granulocytes

Granulocytes were prepared as described [24]. Peripheral venous blood from healthy volunteers was anticoagulated with trisodium citrate. Platelets were removed by centrifugation, and erythrocytes by dextran sedimentation. Leukocytes were separated according to

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Abbreviations: CRTH2, chemoattractant receptor homologous molecule expressed by Th2 cells; GAFS, gated auto-fluorescence forward scatter assay; PG, prostaglandin; PGDS, PGD_2 synthetase

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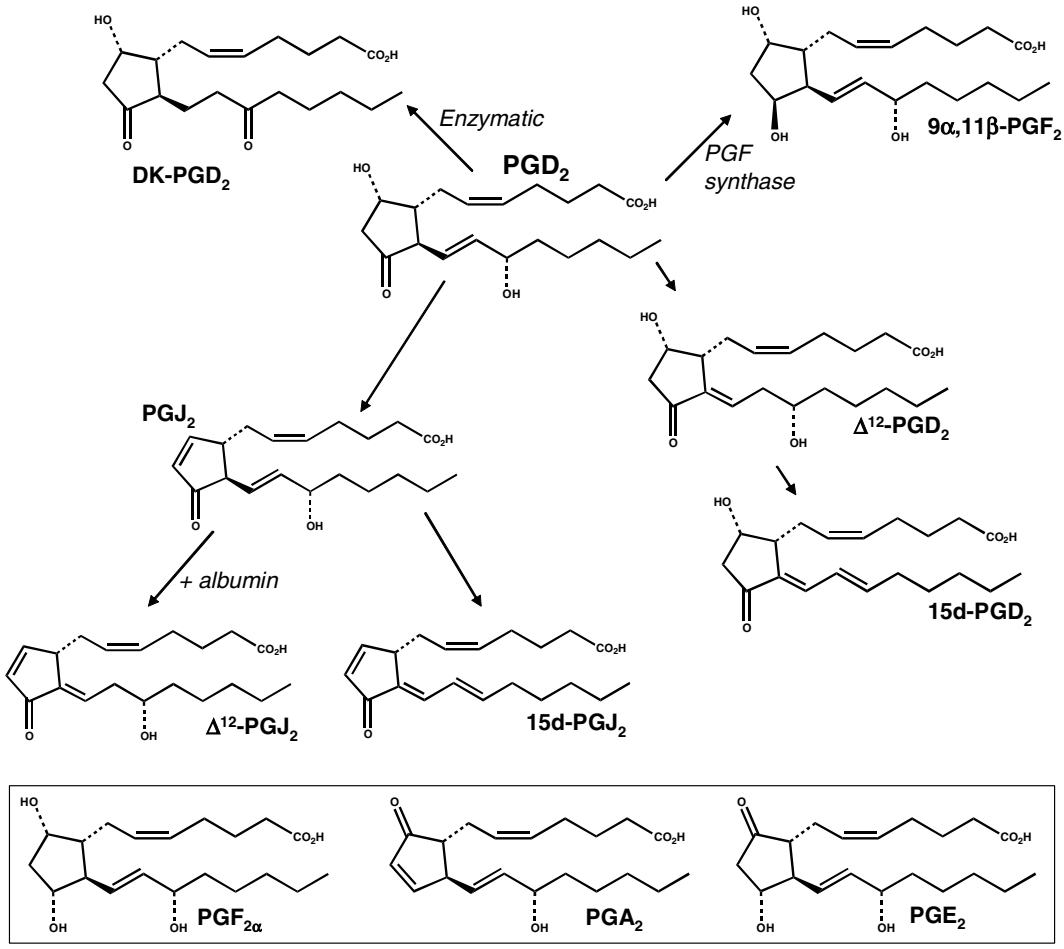

Fig. 1. The structures of the PGD_2 metabolites of interest, modified from [8], $PGF_{2\alpha}$, PGE_2 and PGA_2.

density by centrifugation over Histopaque 1077. Residual erythrocytes were removed by hypotonic lysis. Granulocytes were resuspended in handling buffer (0.1% BSA, 10 mM glucose, 10 mM HEPES in PBS without Ca^{2+} and Mg^{2+}, pH 7.4).

2.5. Eosinophil gated-autofluorescence forward scatter assay (GAFS)

The GAFS assay was performed as described [25]. Granulocytes were incubated at room temperature for 1 h in handling buffer and resuspended in GAFS assay buffer (handling buffer with Ca^{2+} and Mg^{2+}). 5×10^5 cells were added to the indicated agonist, incubated for 4 min at 37 °C, and the cells fixed by the addition of 1× CellFix (Becton-Dickinson, Cowley, UK) which had been further diluted 1:4 with FACSFlow sheath fluid. Data were aquired on a FACSCalibur flow cytometer, with collection terminated once 500 high FL-2 events (eosinophils) had been acquired [25]. Agonist induced eosinophil shape change was calculated as a percentage of the baseline forward scatter (FSC).

2.6. Statistical analysis

Statistical analyses, curve fitting, and calculation of EC_{50} values was performed using Prism 3.0 (Graphpad Software Inc., San Diego, USA).

3. Results

3.1. Relative activities of PGD_2 metabolites at CRTH2

PGD_2, PGJ_2, Δ^{12}-PGJ_2, 15d-PGJ_2, DK-PGD_2, Δ^{12}-PGD_2 and 15d-PGD_2 induced the migration of BaF/3 cells stably expressing CRTH2 (Fig. 2) but had no effect on the migration of naïve cells (data not shown), implying that this response is mediated by CRTH2. Although two orders of magnitude less potent than PGD_2, the J-series PGs were the most efficacious agonists tested at inducing chemotaxis of these cells (Fig. 2,A–D).

As previously reported [23], PGD_2 induced a potent shape change response in eosinophils with activity in the sub-nanomolar range. The PGD_2 metabolites also induced eosinophil shape change (Fig. 3), with a rank order of potency of PGD_2 = 15d-PGD_2 > Δ^{12}-PGD_2 = DK-PGD_2 > PGJ_2 > Δ^{12}-PGJ_2 = 15d-PGJ_2. Calculated EC_{50} values for these responses are shown in Table 1. The responses appeared to be mediated via CRTH2 rather than DP as they were completely abrogated by pretreatment of the leukocytes with the CRTH2 antagonist, ramatroban (Table 1), which had no effect on the CCR3-mediated response to eotaxin (data not shown).

3.2. 9α,11β-PGF_2 and $PGF_{2\alpha}$ cause eosinophil activation

Both 9α,11β-PGF_2 and $PGF_{2\alpha}$ showed similar efficacy to PGD_2 in assays of eosinophil shape change, but were less potent than PGD_2 ($P < 0.0001$) with EC_{50} values of 1.56×10^{-7} and 1.47×10^{-7} M, respectively (Fig. 4A and B). Ramatroban inhibited these responses in a dose dependent manner (Fig. 4C and D), suggesting that they were

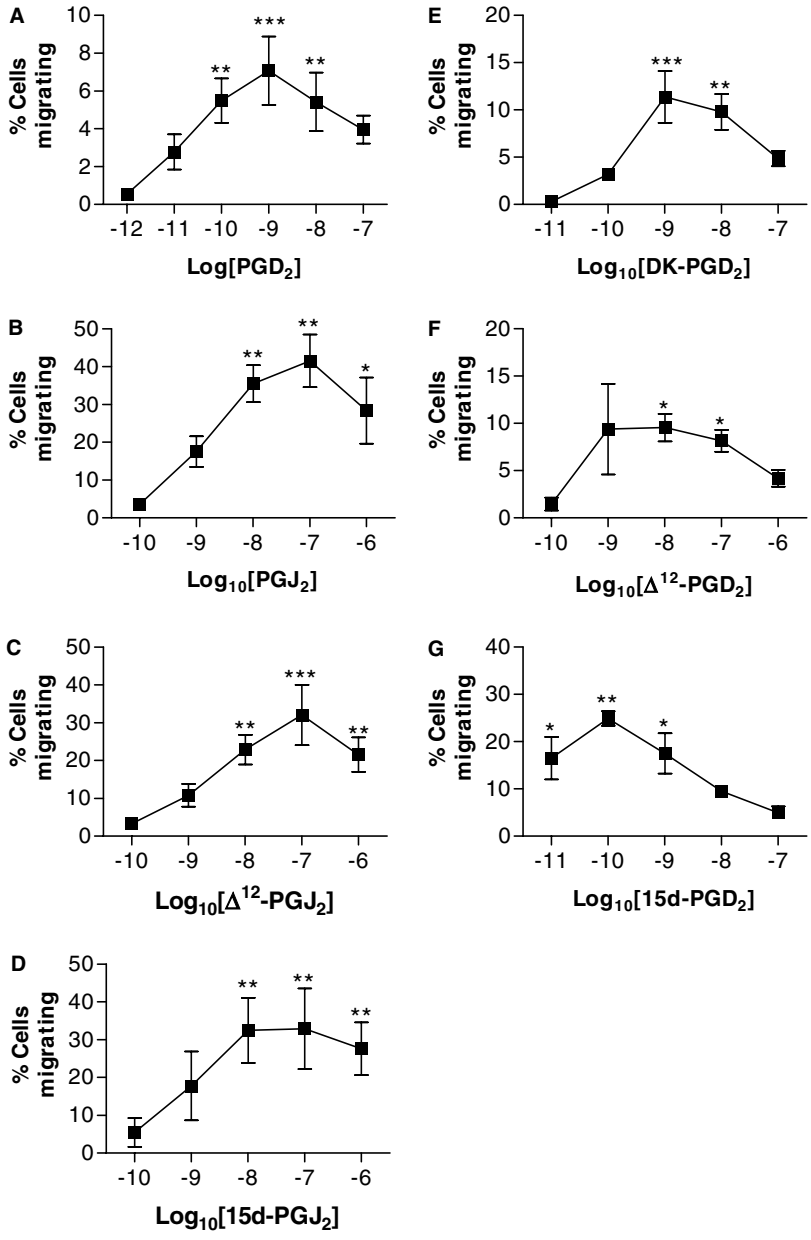

Fig. 2. The migratory response of CRTH2.BaF/3 cells in response to PGD_2 and some of its metabolites is shown. Results are the means ± S.E.M. of $n = 4$–8 experiments. * indicates $P < 0.05$, **, $P < 0.01$, and ***, $P < 0.001$, by ANOVA with Friedman post test, comparing the number of cells migrating to PG with the number migrating to buffer alone.

Table 1
The EC_{50} values of PG-induced eosinophil shape change were calculated and are given with the 95% confidence intervals ($n = 5$–8)

	EC_{50}(M)	95% CI for EC_{50}(M)	$IC_{50}$for ramatroban (M)
PGD_2	3.94×10^{-10}	1.95×10^{-10}–7.98×10^{-10}	2.73×10^{-9} (1 nM)
DK-PGD_2	$1.13 \times 10^{-9*}$	5.26×10^{-10}–2.45×10^{-9}	1.06×10^{-8} (10 nM)
PGJ_2	$2.22 \times 10^{-9**}$	8.99×10^{-10}–5.49×10^{-9}	1.04×10^{-8}(10 nM)
Δ^{12}-PGJ_2	$3.74 \times 10^{-9***}$	2.43×10^{-9}–5.75×10^{-9}	3.40×10^{-9} (10 nM)
15d-PGJ_2	$8.37 \times 10^{-9***}$	3.97×10^{-9}–1.76×10^{-8}	1.97×10^{-8}(100 nM)
Δ^{12}-PGD_2	$7.34 \times 10^{-10*}$	2.86×10^{-10}–1.88×10^{-9}	4.40×10^{-9}(3 nM)
15d-PGD_2	2.39×10^{-10}	8.98×10^{-11}–6.35×10^{-10}	6.15×10^{-8}(10 nM)

EC_{50} values of the tested PG were compared to that of PGD_2 by t test. * indicates $P < 0.05$, **, $P < 0.005$, and ***, $P < 0.0001$. IC_{50} values for the inhibition of the response by a 10 min pretreatment with ramatroban are also given, with the PG concentrations used to generate a response indicated in parenthesis ($n = 4$).

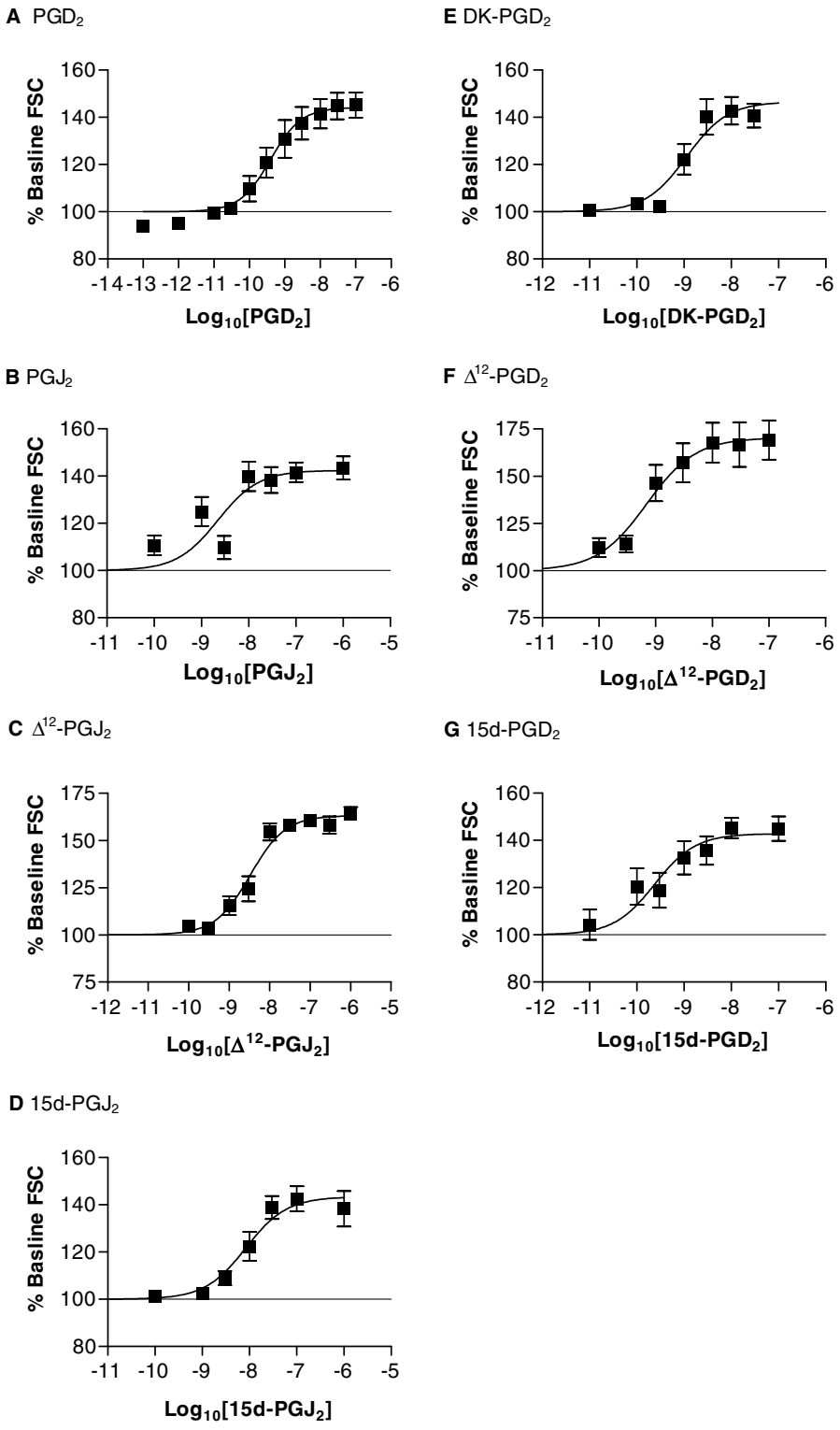

Fig. 3. Panels A–G show eosinophil shape change in response to a range of concentrations of PG. Results are the means ± S.E.M. of $n = 5–8$ experiments.

mediated via CRTH2. Ramatroban is also an antagonist of the TP receptor, but it seems unlikely that these responses are due to TP signalling as there is no evidence for TP on eosinophils [22].

3.3. 9α,11β-PGF_2 and $PGF_{2\alpha}$, but not PGA_2 or PGE_2, are agonists of CRTH2

9α,11β-PGF_2 and $PGF_{2\alpha}$ induced migration of the CRTH2 transfectants, with 100-fold less potency than PGD_2 (Fig. 5A

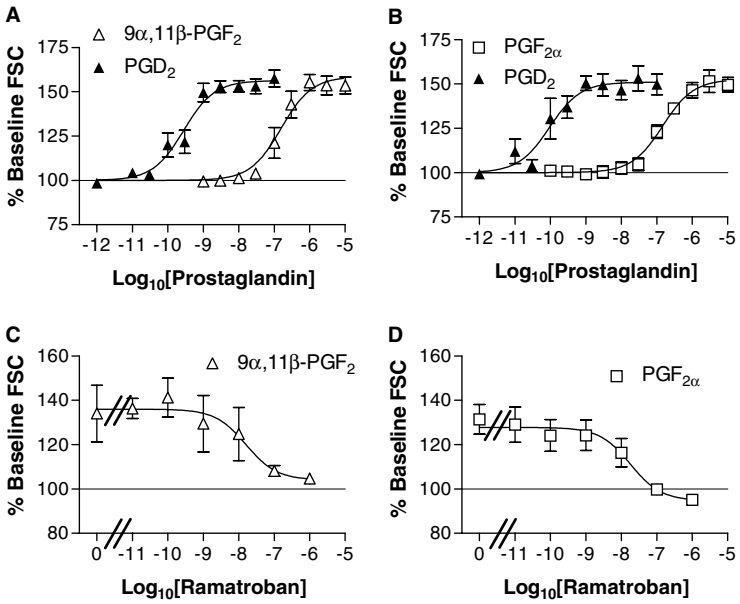

Fig. 4. Panels A and B show the eosinophil shape change induced by 9α,11β-PGF_2, $PGF_{2\alpha}$ and PGD_2. Panels C and D show eosinophil shape change to 1 μM 11β-$PGF_{2\alpha}$ or $PGF_{2\alpha}$, respectively, in the presence of the indicated ramatroban concentrations. Results are the means ± S.E.M. of $n = 4$ experiments.

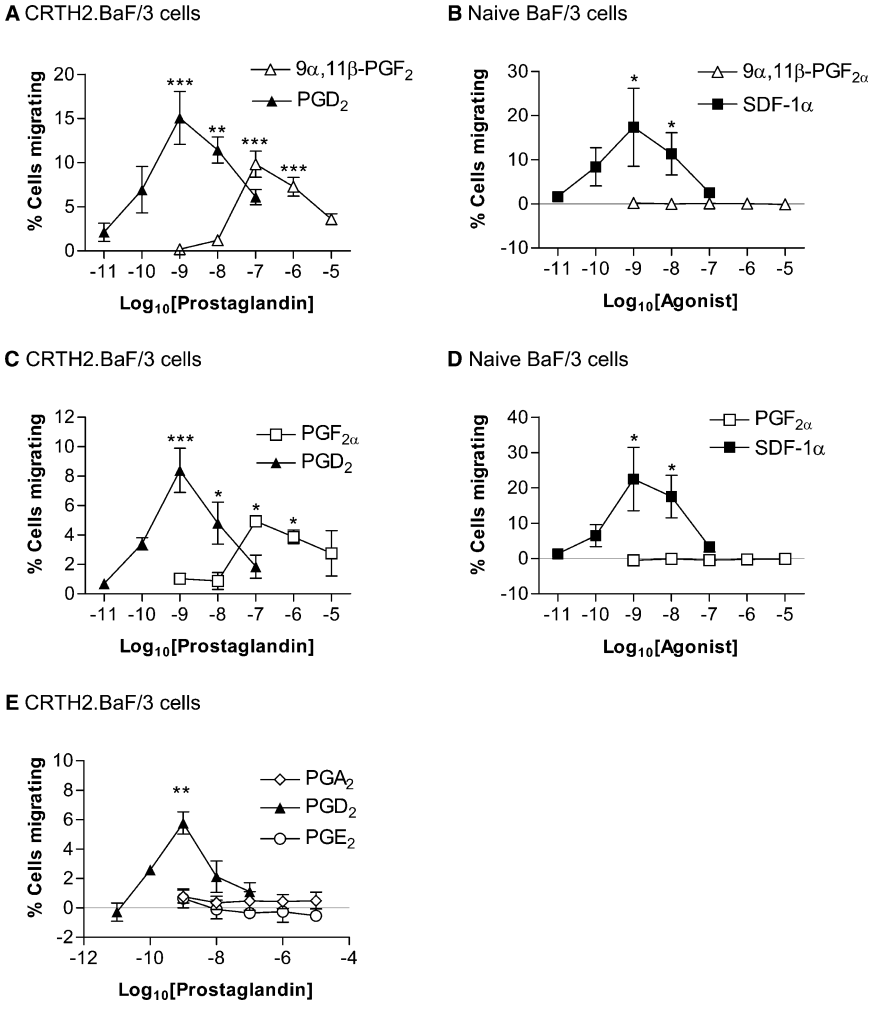

Fig. 5. Panels B and D, naïve Baf/3 cells, and A, C and E, CRTH2 expressing BaF/3 cells, were exposed to the indicated concentrations of PG for 5 h, and the resulting migration determined. Results are means ± S.E.M. of $n = 3$ (B, D and E) or $n = 5$ (A and C) experiments. * indicates $P < 0.05$ and **, $P < 0.01$ by ANOVA with Friedman post test comparing the number of cells migrating to PG to the number migrating to buffer.

and C). Naïve BaF/3 cells were unresponsive to both PGs, despite migrating to the chemokine SDF-1α via endogenous CXCR4 (Fig. 5B and D), implying that the migratory response is mediated via the presence of CRTH2.

As CRTH2 is activated by PGs containing D, J and F rings, it was of interest to establish whether PGs with the A or E ring could also activate the receptor. Neither PGA_2 nor PGE_2 (structures shown in Fig. 1) caused the migration of BaF/3 cells expressing CRTH2, at a range of concentrations active for the other CRTH2 agonists (Fig. 5,E and F).

4. Discussion

CRTH2 is a remarkably promiscuous receptor, with ligands including PGD_2 and its metabolites, a TXA_2 metabolite and the non-steroidal anti-inflammatory drug indomethacin [5,10–12,23,24,26]. $PGF_{2\alpha}$ and 9α,11β-$PGF_{2\alpha}$ have been previously demonstrated to stimulate CRTH2 transfectants [9] and eosinophils [11,22]. Here, we investigate the ability of the prostaglandins to induce the migration of CRTH2 transfected cells, and with the use of an antagonist, demonstrate for the first time that 9α,11β-PGF_2 $PGF_{2\alpha}$, and several PGD_2 metabolites stimulate eosinophils via CRTH2.

The J-series PGs caused eosinophil shape change and the migration of CRTH2 transfected BaF/3 cells with less potency than PGD_2 itself, reflecting the findings of previous studies [5,9,11]. Studies have shown that high concentrations of 9α,11β-PGF_2 and $PGF_{2\alpha}$ are able to displace radiolabelled PGD_2 from CRTH2 [5,9], in agreement with our findings that these PGs signal via CRTH2, although with lower potency than PGD_2. PGs containing the A or E ring were inactive at CRTH2, suggesting CRTH2 is not activated by PGs containing a ketone at carbon 9. In contrast, the identity of the group at carbon 11 appears to be less important, as the F-series PGs with a hydroxyl at this position rather than the ketone of PGD_2 are able to activate the receptor.

Whilst 9α,11β-$PGF_{2\alpha}$ is several orders of magnitude less potent than PGD_2, it is reported to be more stable in vivo, as following administration of PGD_2 to humans, 9α,11β-PGF_2 but not PGD_2 was found in the urine [14]. It is therefore likely that the metabolite may accumulate in vivo. Indeed, plasma levels of 1.4 μM have been reported in a patent with severe mastocytosis [27], whilst a mean concentration of 42.3 pM was found in the plasma of allergen challenged asthmatics [15]. It therefore seems plausible that levels of 9α,11β-$PGF_{2\alpha}$ at concentrations sufficient to activate CRTH2 are generated in vivo. Since PGD_2 is produced in the lung in response to allergen challenge [28], and PGF synthase is also expressed in the lung [29,30], local generation of 9α,11β-$PGF_{2\alpha}$ may play a role in allergic lung inflammation by activating eosinophils, Th2 cells and basophils, via CRTH2.

Interestingly, $PGF_{2\alpha}$ is the first PG produced in the absence of PGD synthase to be described as a CRTH2 ligand. Therefore, $PGF_{2\alpha}$, along with the thromboxane metabolite, 11d-TXB_2 [23] allow for the possibility of CRTH2 signalling in vivo in the absence of PGD_2 production, as $PGF_{2\alpha}$ is produced from either PGH_2 [17] or PGE_2 [18], and 11d-TXB_2 is formed as a TXB_2 metabolite. These data reinforce the potential importance of CRTH2 signalling in the regulation of allergic inflammation.

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