Effects of prostaglandin D_2 and 5-lipoxygenase products on the expression of CD203c and CD11b by basophils

Guillaume Monneret, Radhia Boumiza, Sylvie Gravel , Chantal Cossette, Jacques Bienvenu, Joshua Rokach, and William S. Powell

Meakins-Christie Laboratories, Department of Medicine, McGill University, 3626 St. Urbain Street, Montreal, Quebec, Canada H2X 2P2 (S.G., C.C., W.S.P.), Immunology Laboratory, Lyon-Sud University Hospital, 69495 Pierre-Bénite Lyon, France (G.M., R.B., J.B.), Claude Pepper Institute and Department of Chemistry, Florida Institute of Technology, Melbourne, Florida 32901-6982, USA (J.R.)

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Corresponding author
William S. Powell
Meakins-Christie Laboratories, McGill University
3626 St. Urbain Street
Montreal, Quebec, Canada H2X 2P2

Telephone: 514-398-3864 ext. 094071
FAX: 514-398-7483
E-Mail: William.Powell@McGill.ca

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Abbreviations

5-oxo-ETE, 5-oxo-6,8,11,14-eicosatetraenoic acid; BW868C, 3-[(2-cyclohexyl-2-hydroxyethyl)amino]-2,5-dioxo-1-(phenylmethyl)-4-imidazolidine-heptanoic acid; BW245C, (4S)-(3-[(3R,S)-3-cyclohexyl-3-hydroxypropyl]-2,5-dioxo)-4-Imida zolidineheptanoic acid; CAY10399, 9-oxo-11a,16S-dihydroxy-17-cyclobutylprosta-5Z,13E-dien-1-oic acid; CRTH2, chemoattractant receptor-homologous molecule expressed on Th2 cells; LT, leukotriene; PC5, phycoerythrin-cyanin 5; PG, Prostaglandin; TX, thromboxane.

ABSTRACT

Basophils are important in allergic diseases such as asthma as they produce a variety of inflammatory mediators. Activation of these cells with IgE and fMLP results in a variety of responses, including increased surface expression of CD203c and CD11b and release of histamine. Although considerable information is available on the effects of eicosanoids on neutrophils, eosinophils, and monocytes, less is known about their effects on basophils. In the present study we examined the effects of various eicosanoids on the above basophil responses. Of the naturally occurring eicosanoids tested, PGD_2 (EC_{50}, 10 nM) was by far the most potent activator of CD203c expression, with other prostanoids having little effect. This response was mediated by the DP_2 receptor/CRTH2, as it was shared by the selective agonist 15R-methyl-PGD_2 (EC_{50}, 3 nM). The 5-lipoxygenase products leukotriene B_4 and 5-oxo-6,8,11,14-eicosatetraenoic acid also stimulated CD203c expression, but to a lesser extent than PGD_2, whereas leukotriene D_4 was inactive. Neither PGD_2 nor 5-oxo-6,8,11,14-eicosatetraenoic acid stimulated histamine release or CD63 expression on basophils. Both PGE_2 and the DP_1 receptor agonist BW245C strongly inhibited DP_2 receptor-mediated CD203c expression. The DP_1 receptor antagonist BWA868C enhanced PGD_2-induced CD203c expression, suggesting that interaction of PGD_2 with DP_1 receptors can limit activation of basophils by this prostaglandin. In conclusion, PGD_2 is the most potent inducer of basophil CD203c expression among eicosanoids, and may be a key mediator in asthma and other allergic diseases. The balance between DP_1 and DP_2 receptors may be important in determining the magnitude of basophil responses to this prostaglandin.

INTRODUCTION

Basophils are similar to tissue mast cells in many respects, but unlike mast cells, do not synthesize PGD_2 (Schleimer, et al., 1985). They are found in the nasal mucosa and airways of allergic and asthmatic individuals, and rapidly infiltrate tissues following allergen provocation (Kleinjan, et al., 2000). Although mast cells are very prominent in the initial response to allergen, basophils appear to be more important in the late response (Schleimer, et al., 1985). Basophils express high levels of high affinity IgE receptors and are key cells in allergic reactions, especially late-phase reactions (Falcone, et al., 2000). Following allergen stimulation, they release various inflammatory mediators, including histamine and LTC_4 (Bochner, 2000) and are major sources of IL-4 and IL-13 (Devouassoux, et al., 1999). The adhesion molecule CD11b (Bochner and Sterbinsky, 1991) and the basophil activation marker CD203c (Bühring, et al., 1999) are also upregulated following immunological activation of these cells.

Among hematopoietic cells expression of CD203c is restricted to basophils, mast cells, and their $CD34^+$ precursors, and it is the only selective marker for cells of this lineage (Bühring, et al., 1999). This molecule is also known as ecto-nucleotide phosphodiesterase/pyrophosphatase 3 (E-NPP3) (Bühring, et al., 2001) and is also highly expressed in the prostate and uterus in humans (Goding, 2000). Little is known about its role in basophils, although there is evidence that it may be involved in basophil adhesion and in the differentiation of basophil progenitor cells (Bühring, et al., 2004). Although differing results have been obtained on the degree of surface expression of CD203c on unactivated basophils (Boumiza, et al., 2003; Bühring, et al., 1999), it is clearly highly expressed on basophils from sensitized subjects following treatment with allergen (Boumiza, et al., 2003; Bühring, et al., 1999; Hauswirth, et al., 2002).

Eicosanoids are lipid mediators derived principally from arachidonic acid. There are two major groups of these compounds: cyclooxygenase products, including prostaglandins (PGs) and thromboxane A_2 (TXA_2), and 5-lipoxygenase products, including leukotrienes (LTs) and 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-ETE) and lipoxins (Funk, 2001). The actions of eicosanoids are mediated by a large number of receptors that are each highly selective for a specific member of this family of lipid mediators. Prostaglandins D_2, E_2, $F_{2\alpha}$, and I_2 and TXA_2 each have selective receptors, and some (PGD_2 and PGE_2) have multiple receptors (Narumiya and FitzGerald, 2001). There are two specific receptors for each of LTB_4 and LTD_4 (Funk, 2001) and another receptor for 5-oxo-ETE (Powell, et al., 1993; Hosoi, et al., 2002). Although PGs D_2 and E_2, LTs B_4 and D_4, and 5-oxo-ETE are all known to have effects on a variety of inflammatory cells, relatively little is know about the responses of basophils to these mediators.

The present study focuses on the effects of eicosanoids on the expression of CD203c and CD11b and histamine release by basophils. We were particularly interested in the effects of PGD_2 on these responses, as basophils express both receptors for this substance: the DP_1 receptor and the DP_2 receptor (also known as chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2)). (Hirai, et al., 2001). The DP_1 receptor is coupled to G_s, resulting in elevation of cAMP levels in platelets and other cell types following stimulation (Narumiya and FitzGerald, 2001). Although the role of this receptor in regulating basophil responses has not previously been investigated, it could potentially have an inhibitory effect as do other agents that act through stimulation of adenylyl cyclase (Peachell, et al., 1988). Recently our group (Monneret, et al., 2001) and Hirai et al (Hirai, et al., 2001) independently discovered a second PGD_2 receptor.

Activation of the DP_2 receptor in eosinophils elicits a variety of responses, including chemotaxis, Ca^{++} mobilization, CD11b expression, actin polymerization, and shedding of L-selectin (Monneret, et al., 2001). It also selectively induces the migration of eosinophils and basophils from a preparation of neutrophil-depleted leukocytes, as well as the migration of differentiated Th2 cells (Hirai, et al., 2001). We report here that among eicosanoids, PGD_2 is the most potent activator of CD203c expression in basophils. However, at high concentrations of PGD_2 this response is tempered by the DP_1 receptor, which exerts a negative effect on basophil activation.

MATERIALS AND METHODS

Materials

Unless indicated otherwise, all of the eicosanoids as well as BW245C ((4S)-(3-[(3R,S)-3-cyclohexyl-3-hydroxypropyl]-2,5-dioxo)-4-Imida zolidineheptanoic acid), CAY10399 (9-oxo-11a,16S-dihydroxy-17-cyclobutylprosta-5Z,13E-dien-1-oic acid), and carbaprostacyclin were purchased from Cayman Chemical, Ann Arbor, MI. 5-Oxo-ETE was synthesized chemically as described previously (Khanapure, et al., 1998). BWA868C (3-[(2-cyclohexyl-2-hydroxyethyl)amino]-2,5-dioxo-1-(phenylmethyl)-4-imidazolidine-heptanoic acid) was kindly provided by GlaxoSmithKline, Stevenage, UK. fMLP was obtained from the Sigma Chemical Company, St. Louis, MO. The following monoclonal antibodies and their respective isotype controls were used: PE-conjugated anti-CD203c (Beckman Coulter France), phycoerythrin-cyanin 5 (PC5)-conjugated anti-human CD45 (Immunotech, Marseille, France), PE-conjugated anti-human CD63 (Immunotech), polyclonal FITC-conjugated anti-human IgE (BioSource International, Camarillo, CA), PE-conjugated anti-CD11b (Beckman-Coulter, Fullerton, CA), and PC5-conjugated anti-CD49d (Pharmingen, San Diego, CA).

Preparation of blood cells for flow cytometry

Blood (4 ml), collected from healthy volunteers using citrate as the anticoagulant, was diluted with 20 ml phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, and 8.1 mM Na_2HPO_4 at a pH of 7.4). Following centrifugation at 200 x *g* for 15 min at 4 ^{o}C, the pellet was resuspended in PBS containing $CaCl_2$ (1 mM) and $MgCl_2$ (1 mM) to a final volume of 4 ml. Aliquots of this suspension were used for all studies on expression of CD203c and CD11b.

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Measurement of CD203c expression

Aliquots (100 µl) of washed whole blood cells were preincubated at 37 ^{o}C for 5 min in the presence or absence of various inhibitors, followed by the addition of either vehicle or agonist in 10 µl PBS containing Ca^{++}, Mg^{++}, and 0.1% bovine serum albumin. After 10 min, the incubations were terminated by addition of ice-cold Facsflow (2 ml; Becton-Dickinson) and the tubes placed in ice. After centrifugation at 700 x *g* for 6 min at 4 ^{o}C the pellets were incubated in the dark with a mixture of FITC-labeled anti-IgE (1.25 µl), PE-labeled anti-CD203c (5 µl), and PBS (3.75 µl) for 30 min at 4 ^{o}C. Optilyse C (0.5 ml; Beckman-Coulter) was then added, and the mixtures kept in the dark at room temperature for 15 min. The samples were then centrifuged at 700 x *g* for 6 min at 4 ^{o}C and the pellets resuspended in 0.4 ml PBS containing 1 % formaldehyde. The distribution of fluorescence intensities due to PE-anti-CD203c labeling was measured by flow cytometry (FACSCalibur, Becton-Dickinson, San Jose, CA) in basophils, which were gated on the basis of intense labeling with FITC-anti-IgE and forward scatter as shown in Fig. 1. Positive cells were then further gated based on side scatter (Fig. 1). The results are expressed as percentages of the maximal response to PGD_2.

Measurement of CD11b expression

CD11b expression was measured exactly as described above except that the cells were stained by addition of a mixture of FITC-labeled anti-IgE (1.25 µl), PE-labeled anti-CD11b (10 µl), PC5-labeled anti-CD49d (5 µl), and PBS (3.75 µl). The distribution of fluorescence intensities due to PE-anti-CD11b labeling was measured by flow cytometry in basophils, which were identified as described above, and eosinophils, which were identified on the basis of high expression of CD49d and high side scatter.

Measurement of CD63

Aliquots (100 µl) of whole blood, collected in heparin, were incubated for 10 min at 37 ^{o}C with agonists in PBS (10 µl). The tubes were then placed on ice and incubated with FITC-labeled anti-IgE, PE-labeled anti-CD63, and PC5-labeled anti-CD45 for 30 min at 4 ^{o}C in the dark. Red blood cells were then lysed using the Q-prep system (Beckman-Coulter, Hialeah, FL). The samples were analysed by flow cytometry using a Coulter EPICS XL instrument with System II software (Beckman-Coulter) as previously described (Boumiza, et al., 2003). A region enriched in basophils, which also contained lymphocytes and monocytes was first identified on the basis of side scatter and high expression of CD45. Basophils were then selected from this population based on high expression of IgE. The percentage of basophils that were positive for CD63 was then determined using a threshold that was defined by the PE fluorescence of cells treated with vehicle alone in the absence of any agonist.

Measurement of histamine release

Histamine was quantified using a radioimmunoassay kit from Immunotech following the procedure supplied by the manufacturer. Aliquots (100 µl) of whole blood were diluted with 300 µl of dilution buffer (provided by the manufacturer) and incubated for 30 min at 37°C with agonists dissolved in 200 µl of PBS. The tubes were then placed on ice and, following centrifugation, histamine was measured in the supernatants. Total histamine content was determined in a control tube after a water total cell lysis. Results obtained with agonists are expressed as percentages of total cellular histamine.

Data analysis

The results are presented as means ± SE. The statistical significance of the differences between treatments was assessed using two-way ANOVA with the Student-Newman-Keuls test being used for post-hoc analysis. A *P* value of less than 0.05 was considered to be statistically significant.

RESULTS

PGD_2 is a potent stimulus of CD203c expression in basophils

We investigated the effects of PGD_2 and other eicosanoids on the expression of CD203c in basophils in whole blood following removal of plasma. Basophils were identified on the basis of forward scatter and high expression of IgE using flow cytometry (Fig. 1A) and further selected by gating on side scatter as shown in the inset to Fig. 1A. The effect of PGD_2 (100 nM) on CD203c expression by basophils is shown in Fig. 1B. Unstimulated basophils displayed very low CD203c expression (shaded histogram), whereas basophils treated with PGD_2 strongly expressed this protein.

Concentration-response curves for PGD_2 and other prostanoids are shown in Fig. 2A. PGD_2 is a potent stimulator of CD203c expression, with an EC_{50} value of 10 ± 2 nM. Because there was some variability in expression levels among individual donors, the results are shown as percentages of the maximal response to PGD_2, which was 4.3 ± 0.6 times the basal level of CD203c. In contrast to PGD_2, PGE_2 and the thromboxane A_2 analog U46619 had no effect on CD203c expression. The prostacyclin analog and selective IP receptor agonist carbaprostacyclin was also without effect on CD203c expression (data not shown), whereas $PGF_{2\alpha}$ had a slight effect at concentrations of 1 μM or higher. The selective DP_2 receptor agonist 15R-methyl-PGD_2 was the most potent compound tested, with an EC_{50} value (3.2 ± 0.5 nM) about 3 times lower than that of PGD_2.

The effects of PGD_2 are compared to those of 5-lipoxygenase products and fMLP in Fig. 2B. In contrast to PGD_2, the response to fMLP was highly variable among subjects, as can be seen by

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the much larger standard errors. Low concentrations (≤ 10 nM) of these two substances induced similar increases in CD203c expression, whereas at higher concentrations (≥ 100 nM), fMLP was usually more effective. In contrast, all of the 5-lipoxygenase products tested were less active than PGD_2 in stimulating CD203c expression. LTB_4 (EC_{50}, 12 ± 2 nM) was nearly as potent at PGD_2, but only 43 ± 5% as efficacious. 5-Oxo-ETE (EC_{50}, 37 ± 12 nM) was less potent and had a maximal response that was 27 ± 7% that of PGD_2. LTD_4 had virtually no effect on CD203c expression by basophils.

PGD_2 stimulates expression of CD11b on basophils

PGD_2 is a potent stimulator of CD11b expression on basophils with an EC_{50} value of 11 ± 2 nM (Fig. 3A) and a maximal response of 140 ± 28% above control. In contrast, 5-oxo-ETE only weakly stimulates CD11b expression on these cells, with an EC_{50} of 95 ± 33 nM and a maximal response about 33 ± 13% that of PGD_2. To ensure that 5-oxo-ETE was still fully active in preparations of whole blood lacking only plasma, we tested its effects on eosinophils in the same samples as those used to measure basophil responses (Fig. 3B). 5-Oxo-ETE (EC_{50}, 23 ± 2 nM) was somewhat less potent than PGD_2 in stimulating CD11b expression by eosinophils, but induced a greater maximal response (47 ± 7% above that of PGD_2). The slightly lower potency of 5-oxo-ETE may have been due to non-specific binding to red blood cells in the samples, as we have consistently observed a small shift to the right in the concentration-response curve for 5-oxo-ETE-induced responses in whole blood compared to isolated leukocytes. As was the case for CD203c expression, LTD_4 failed to stimulate CD11b expression in basophils (data not shown).

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Activation of DP_1 receptors inhibits expression of CD203c by basophils

Although PGD_2 clearly stimulates expression of CD203c by basophils via DP_2 receptors, it is theoretically possible that this response could be tempered by activation of DP_1 receptors on these cells. We therefore investigated the effects of the selective DP_1 receptor agonist BW245C on the response of basophils to the selective DP_2 receptor agonist 15R-methyl-PGD_2. BW245C (1 μM) reduced the maximal response to 15R-methyl-PGD_2 by 50 ± 7% (Fig. 4A). The concentration-response curve for the inhibitory effect of BW245C on the response to a near maximal concentration of 15R-methyl-PGD_2 (10 nM) is shown in Fig. 4B. BW245C was a potent inhibitor of CD203c expression (IC_{50}, 8 ± 3 nM) with a maximal inhibitory effect of 77 ± 2%. BW245C also inhibited CD203c expression induced by fMLP (10 nM), but the maximal response (44 ± 3%) was less than that for inhibition of the response to 15R-methyl-PGD_2.

The DP_1 receptor antagonist BWA868C enhances PGD_2-elicited CD203c expression

Although the predominant response of basophils to PGD_2 is clearly stimulation, this prostaglandin could also activate the inhibitory G_s-coupled DP_1 receptor, which could potentially limit PGD_2-elicited CD203c expression. To examine this possibility, blood cells were preincubated with the selective DP_1 receptor antagonist BWA868C (1 μM) for 5 min prior to addition of PGD_2. BWA868C had no effect on CD203c expression on its own, and, if anything, tended to reduce expression induced by low concentrations of PGD_2 (not significant; Fig. 5A). However, at concentrations of PGD_2 at or above 1 μM, BWA868C significantly enhanced CD203c expression ($P < 0.01$), suggesting that interaction of PGD_2 with the DP_1 receptor can reduce the response of basophils to high concentrations of this prostaglandin. To further test this hypothesis, we examined the effect of BWA868C on the response of basophils to 15R- methyl-

PGD_2, which is devoid of DP_1 receptor agonist activity. In contrast to its stimulatory effect on PGD_2-induced CD203c expression, BWA868C slightly diminished CD203c expression in response to 15R-methyl-PGD_2 ($P < 0.02$) (Fig. 5B).

PGD_2 and 5-oxo-ETE do not stimulate histamine release or CD63 expression by basophils

The effects of PGD_2 and selective DP_1 and DP_2 receptor agonists on histamine release and expression of the degranulation marker CD63 were also examined. In contrast to its potent stimulatory effects on CD203c and CD11b expression, PGD_2 had little or no effect on histamine release from basophils in whole blood (Fig. 6A). BW245C (DP_1 agonist), 13,14-dihydro-15-keto-PGD_2 (DP_2 agonist), and 5-oxo-ETE were also without effect on histamine release. As a positive control we used fMLP, a potent stimulator of basophil degranulation, which stimulated the release of about 45% of the total histamine content in whole blood at a concentration of 100 nM. PGD_2, BW245C, 13,14-dihydro-15-keto-PGD_2, and 5-oxo-ETE were also without effect on the expression of CD63 by basophils, in contrast to fMLP, which induced 45% of basophils to express this marker (Fig. 6B).

PGE_2 and the IP receptor agonist carbaprostacyclin inhibit CD203c expression by basophils

We also investigated the potential inhibitory effects on CD203c expression of other prostanoids that act through G_s-coupled receptors, including PGE_2 and the stable PGI_2 analog, carbaprostacyclin. PGE_2 inhibited CD203c expression in response to 15R-methyl-PGD_2 with an EC_{50} of 53 ± 39 nM, about 5 times higher than that of BW245C (Fig. 7). However, PGE_2 was more efficacious, achieving virtually complete inhibition at a concentration of 10 μM. The response to PGE_2 appeared to be biphasic, with an effect being observed at concentrations as low

as 0.1 nM. The selective EP_2 receptor agonist CAY10399 (Tani, et al., 2001) also nearly completely inhibited the response to 15R-methyl-PGD_2 at the highest concentration tested (10 μM), but unlike PGE_2, had little effect at concentrations at or below 10 nM. The IP receptor agonist carbaprostacyclin had a similar effect but was somewhat less efficacious.

DISCUSSION

The effects of eicosanoids on the surface expression of CD203c and CD11b by basophils has been examined by flow cytometry in unfractionated human blood cells. Basophils can readily be selected from these cells on the basis of high expression of IgE and light scattering (Fig. 1). The identity of these cells as basophils was confirmed by their high expression of the selective basophil marker CD203c following stimulation. This approach has the advantage that there is little manipulation of the cells prior to treatment with agonists, thus minimizing nonspecific activation that often accompanies lengthy purification procedures.

The results show for the first time that PGD_2 is a highly potent activator of CD203c expression on basophils. PGD_2 was recently reported to have a modest stimulatory effect on CD11b expression by basophils (Yoshimura-Uchiyama, et al., 2004), but the maximal response (~15% above control) was considerably less that that observed in the present study (~140% above control), possibly because the basophils had been activated during centrifugation over Percoll. The stimulatory effect of PGD_2 on CD11b expression may contribute to basophil infiltration into tissues, following its allergen-induced release from mast cells. PGD_2 is considerably more active than any of the other eicosanoids tested, including agonists of all of the known prostanoid receptors, leukotriene receptors, and 5-oxo-ETE, which has its own distinct receptor. This is in contrast to most other leukocytes, which are activated to a greater extent by products of the 5-lipoxygenase pathway. For example, LTB_4 (Ford-Hutchinson, et al., 1980) and 5-oxo-ETE (Powell, et al., 1993) strongly activate neutrophils, whereas PGD_2 is inactive (Monneret, et al., 2001). LTB_4 is also a potent stimulator of monocyte activation, whereas 5-oxo-ETE has only a modest effect, and PGD_2 is ineffective. Although both PGD_2 and 5-oxo-ETE are potent

activators of eosinophils, 5-oxo-ETE induces a stronger chemoattractant response at higher concentrations (Monneret, et al., 2001). The DP_2 receptor is also highly expressed on Th2 cells, and PGD_2 is a potent chemoattractant for these cells (Hirai, et al., 2001).

Among the compounds tested, 15R-methyl-PGD_2 is the most potent inducer of CD203c expression by basophils. This compound, which is the most potent known agonist at the DP_2 receptor, also elicits a variety of responses in eosinophils, including cell migration, CD11b expression, calcium mobilization, and actin polymerization (Monneret, et al., 2003). The potency of 15R-methyl-PGD_2, in which the configuration of the 15-hydroxyl group is opposite to that of naturally-occurring prostaglandins, illustrates the different selectivity pattern of the DP_2 receptor compared to other prostaglandin receptors, for which the 15S configuration is required for optimal activity. Unlike other prostaglandin receptors, the DP_2 receptor is also selectively activated by indomethacin (Hirai, et al., 2002) and the TXB_2 metabolite 11-dehydro-TXB_2 (Bohm, et al., 2004).

The response of basophils to 5-oxo-ETE is clearly different from that of eosinophils, for which this substance is a potent agonist (Powell, et al., 1995). As previous studies with 5-oxo-ETE were conducted with purified or partially purified eosinophils, it is theoretically possible that, because of its hydrophobicity, it could be non-specifically adsorbed by red cells present in the preparations used in the current study, thus reducing its potency. However, when we examined eosinophil CD11b expression in the same preparation, we found that 5-oxo-ETE induced a stronger response than PGD_2, in spite of its much weaker effect on basophils. Thus unlike

eotaxin, which stimulates both types of cells (Uguccioni, et al., 1997; Yamada, et al., 1997), 5-oxo-ETE is more selective for eosinophils.

The present study is the first to demonstrate a rapid upregulation of CD203c expression in response to an endogenous inflammatory mediator. CD203c was first shown to be upregulated on basophils following cross-linking of the IgE receptor (Bühring, et al., 1999), and we have recently demonstrated that its expression is increased by the bacterial peptide fMLP (Boumiza, et al., 2003). In contrast, CD203c levels were unaffected by a large panel of cytokines (Bühring, et al., 1999), although more recent studies suggest that its expression is increased after treatment with IL-3 for 90 min (Bühring, et al., 2004) All previous studies have shown increased CD203c expression to occur in association with basophil degranulation. However, the current results demonstrate that these two responses can be dissociated, as PGD_2 is a potent activator of CD203c expression, but has no effect on either histamine release or CD63 expression. This is consistent with other studies in which it was found that PGD_2 or 13,14-dihydro-15-keto-PGD_2 on their own do not affect histamine release (Virgolini, et al., 1992; Yoshimura-Uchiyama, et al., 2004), although they can enhance the response of basophils to other stimuli, including antigen, PMA, and A23187 (Peters, et al., 1984)Yoshimura-Uchiyama, 2004 2154 /id}. The lack of a direct degranulatory response to PGD_2 cannot be explained by its interaction with the inhibitory DP_1 receptor, as the selective DP_2 receptor agonist 13,14-dihydro-15-keto-PGD_2 also failed to induce both histamine release and CD63 expression (Fig. 6).

A dissociation between degranulation and other basophil responses has also been noted for certain chemokines. Eotaxin, like PGD_2 (Hirai, et al., 2001) is a potent basophil chemoattractant,

but has only a modest (Uguccioni, et al., 1997) or no (Yamada, et al., 1997) effect on histamine release. In contrast, MCP-1 has only a modest effect on basophil migration, but is a potent inducer of histamine release (Uguccioni, et al., 1997; Yamada, et al., 1997). It is also possible that PGD_2 could elicit other responses in basophils such as LTC_4 release, as it does in eosinophils (Raible, et al., 1992). The cyclooxygenase inhibitor indomethacin, which activates the DP_2 receptor (Hirai, et al., 2002), has been reported to induce a shape change in basophils (Stubbs, et al., 2002), which might be consistent with stimulation of actin polymerization via this receptor.

As PGD_2 could interact with both stimulatory DP_2 receptors and inhibitory DP_1 receptors on basophils, the final response could be determined by the relative contributions of these two pathways. The present study shows for the first time that selective activation of the DP_1 receptor with BW245C (IC_{50}, 8 nM) results in inhibition of 15R-methyl-PGD_2-induced basophil activation. This effect is presumably mediated by stimulation of adenylyl cyclase, as elevation of cAMP levels by phosphodiesterase inhibitors inhibits basophil activation (Weston, et al., 1997). The stimulatory effect of the selective DP_1 receptor antagonist BWA868C on CD203c expression in response to higher concentrations of PGD_2 suggests a regulatory effect of the DP_1 receptor on PGD_2-induced basophil activation (Fig. 8). In contrast, BWA868C failed to enhance the responsiveness of basophils to 15R-methyl-PGD_2, which is devoid of DP_1 receptor activity, and instead had a slight inhibitory effect on this response, possibly due to weak antagonist activity at the DP_2 receptor. The failure of BWA868C to enhance the response of basophils to lower concentrations of PGD_2 may be because these concentrations of PGD_2 were insufficient to achieve inhibitory levels of cAMP through activation of the DP_1 receptor. This could be related

to differences in the concentration-response relationships for the DP_1 and DP_2 receptors, as well as the greater expression levels of DP_2 receptors compared to DP_1 receptors (Yoshimura-Uchiyama, et al., 2004). Furthermore, it is possible that BWA868C may have modest antagonist activity towards the DP_2 receptor, which could possibly counterbalance its DP_1-mediated effect at low concentrations of PGD_2. Overall, these results suggest that the DP_1 receptor could serve to limit the response of basophils to high levels of PGD_2, as might occur following mast cell activation.

Like PGD_2, PGE_2 has the potential to both activate basophils through EP_1 or EP_3 receptors, and to inhibit activation through the G_s-coupled EP_2 or EP_4 receptors. However, we found no evidence for a stimulatory effect of PGE_2 on these cells (Fig. 2A), but instead found that it is a potent inhibitor of agonist induced CD203c expression. This response appeared to be biphasic, with concentrations of PGE_2 as low as 100 pM resulting in about 20% inhibition of the response to 15R-methyl-PGD_2, but with complete inhibition occurring only at the highest concentration tested (10 μM). The effect of low concentrations of PGE_2 may be due to the activation of EP_4 receptors, as it was not observed with the highly selective EP_2 receptor agonist CAY10399, whereas the effect of higher concentrations may be due to activation of EP_2 receptors, as it occurred with both PGE_2 and CAY10399. This would be consistent with the higher affinity of EP_4 receptors for PGE_2 (Abramovitz, et al., 2000). PGE_2 has previously been reported to stimulate adenylyl cyclase in basophils (Peachell, et al., 1988) but to have relatively little effect (Peachell, et al., 1988) or a modest inhibitory effect on histamine release (Virgolini, et al., 1992) (Peters, et al., 1984). The PGE_1 analog misoprostol, which activates EP_1, EP_3, and EP_4 receptors was reported to inhibit histamine release from basophils (Babakhin, et al., 2000). Thus PGE_2 or

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selective EP_2 or EP_4 receptor agonists may be useful in the treatment of allergic diseases such as asthma. Indeed. PGE_2 has been shown to be a potent inhibitor of airway responses and inflammation in both humans (Gauvreau, et al., 1999) and animal models (Martin, et al., 2002).

There is considerable evidence that PGD_2 may be an important mediator in asthma and other allergic diseases. Large amounts of PGD_2 are released into the airways following allergen challenge of human asthmatic subjects (Murray, et al., 1986). Transgenic mice overexpressing lipocalin-type PGD synthase exhibit enhanced pulmonary inflammation following antigen challenge (Fujitani, et al., 2002). PGD_2 induces migration of eosinophils, basophils, and Th2 cells through the DP_2 receptor (Hirai, et al., 2001; Monneret, et al., 2001). The present results are consistent with a role for PGD_2 in allergic diseases and suggest that it is a key endogenous mediator of basophil activation. As the DP_1 receptor can serve to attenuate DP_2 receptor-mediated basophil activation, the relative numbers of these two receptors could determine the degree of response of these cells to PGD_2. It will be interesting to determine whether the relative expression of these receptors is altered in allergic diseases such as asthma.

ACKNOWLEDGEMENTS

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FOOTNOTES

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LEGENDS TO FIGURES

Figure 1. Measurement of surface expression of CD203c on basophils by flow cytometry following treatment with PGD_2. **A,** Dot plot showing the flow cytometric analysis of mixed blood cells stained for FITC-anti-IgE and PE-anti-CD203c. The *abscissa* shows the degree of forward scatter, whereas the *ordinate* shows the fluorescence intensity due to FITC, indicative of anti-IgE staining. Basophils were first selected on the basis of forward scatter and high levels of IgE (black cells), and then on the basis of side scatter and IgE (black cells in inset). **B**, Histograms showing CD203c expression by basophils following treatment of blood cells with either vehicle (dotted line, filled with gray) or PGD_2 (100 nM; solid line).

Figure 2. Effects of eicosanoids on CD203c expression by basophils. Washed blood cells were incubated with various eicosanoids or vehicle for 10 min at 37^o C and CD203c expression was measured in basophils as shown in Fig. 2. **A,** The effects of various prostanoids, including the selective DP_2 receptor agonist 15R-methyl-PGD_2 (15M, ○, n = 10), $PGF_{2\alpha}$ (▲, n = 3), the selective TP receptor agonist U46619 (∇, n = 3), and PGE_2 (E_2, ■, n = 3) were compared to those of PGD_2 (●, n = 11). **B,** The effects of fMLP (□, n = 6) and the 5-lipoxygenase products LTB_4 (▼, n = 5), LTD_4 (■, n = 3), and 5-oxo-ETE (5o, Δ, n = 6) were compared to those of PGD_2 (●, n = 10). The values are means ± SE of data from the numbers of different subjects shown in brackets.

Figure 3. Effects of PGD_2 and 5-oxo-ETE on CD11b expression by basophils and eosinophils. CD11b was measured in washed whole blood cells by flow cytometry as described in Materials and Methods. Expression levels in both basophils (**A**) and eosinophils (**B**) were measured in each

of the samples following incubation with either PGD_2 (●, n = 8) or 5-oxo-ETE (5oETE, ○, n = 5). The values are means ± SE of data from the numbers of different subjects shown in brackets.

Figure 4. Effects of the selective DP_1 receptor agonist BW245C on basophil activation. **A**, Washed blood cells were preincubated for 5 min at 37° C with either vehicle (●) or BW245C (1 μM; ○), followed by incubation for 10 min with different concentrations of the selective DP_2 receptor agonist 15R-methyl-PGD_2. CD203c expression was measured as shown in Fig. 1. The Y-axis shows the degree of stimulation of CD203c expression in the presence and absence of BW245C (n = 4). **B**, Effects of different concentrations of BW245C on CD203c expression induced by either 15R-methyl-PGD_2 (15Me, 10 nM, ●, n = 5) or fMLP (10 nM, ○, n = 3). Blood cells were preincubated with BW245C for 5 min, at 37° C followed by incubation with agonists for a further 10 min.

Figure 5. Effects of the selective DP_1 receptor antagonist BWA868C on PGD_2-induced CD203c expression by basophils. Washed blood cells were preincubated for 5 min at 37° C with either vehicle (●) or BWA868C (1 μM, ○), followed by incubation for 10 min with different concentrations of either PGD_2 (**A**, n = 8) or the selective DP_2 receptor agonist 15-methyl-PGD_2 (**B**, n = 4). CD203c expression on basophils was measured by flow cytometry as shown in Fig. 1. All values are the means ± SE of determinations on blood samples of the numbers of different individuals indicated in brackets. *, $P < 0.05$; **, $P < 0.01$.

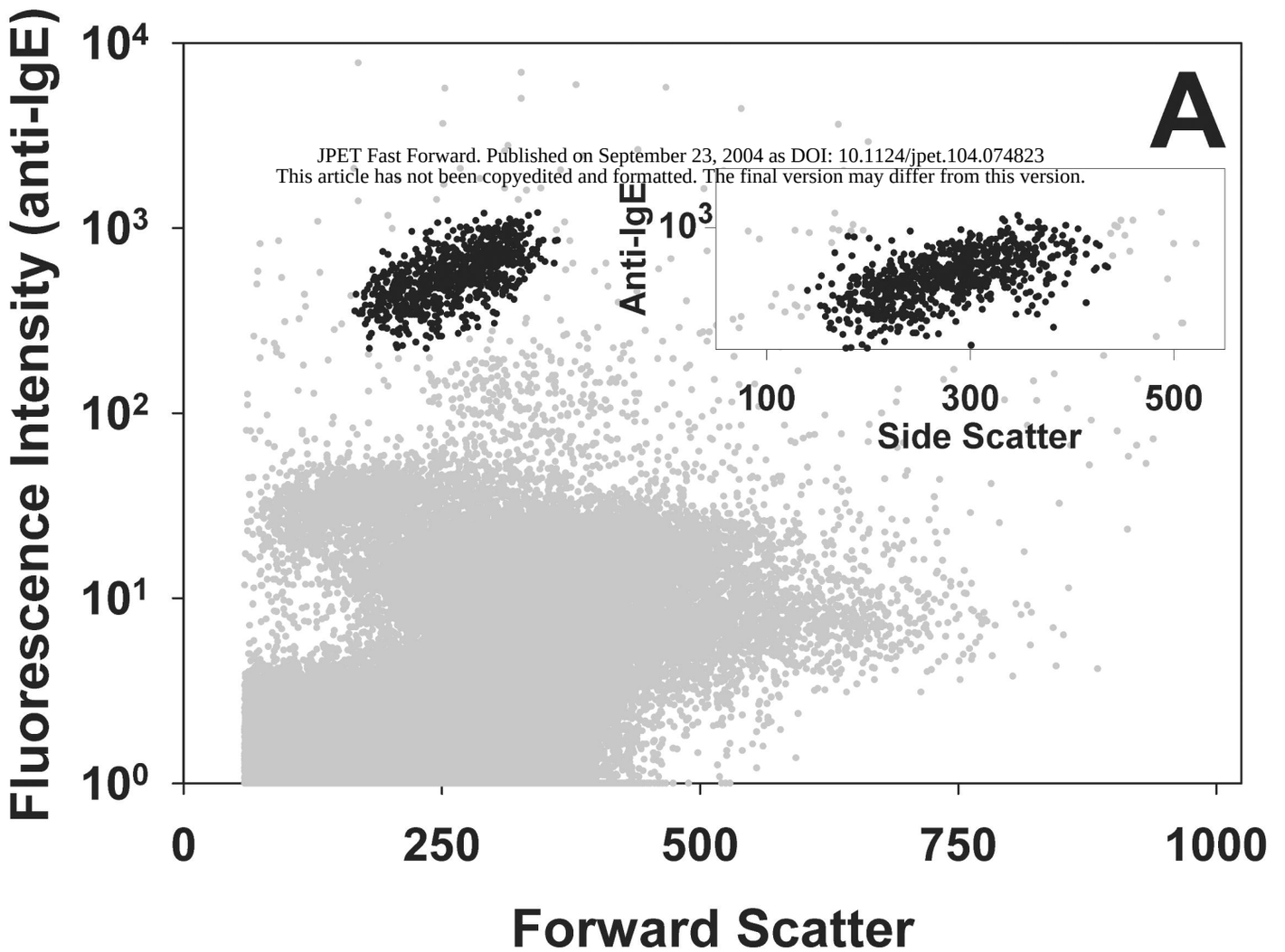
Figure 6. Effects of DP_1 and DP_2 receptor agonists and 5-oxo-ETE on degranulation of basophils. **A**, histamine was measured by radioimmunoassay as described in Materials and

Methods following incubation of blood for 30 min with various agonists. **B**, The degranulation marker CD63 was measured by flow cytometry as described in Materials and Methods following incubation of blood for 10 min with different agonists. The agonists used were as follows: PGD_2 (●, n = 5), fMLP (□, n = 5), the selective DP_1 receptor agonist BW245C (▲, n = 3), the selective DP_2 receptor agonist 13,14-dihydro-15-keto-PGD_2 (dhk-PGD_2, n = 5), and 5-oxo-ETE (Δ, n = 2). The values are means ± SE of data from the number of different subjects shown in brackets (except for 5-oxo-ETE, which are means ± range).

Figure 7. Effects of EP_2/EP_4 and IP receptor agonists on basophil activation. **A**, Washed blood cells were preincubated for 5 min with either vehicle, PGE_2 (●, n = 5), the selective EP_2 receptor agonist CAY10399 (CAY, ∇, n = 5), or the selective IP receptor agonist carbaprostacyclin (Carba, ▲, n = 3), followed by stimulation with 15R-methyl-PGD_2 (10 nM) for 10 min. All values are means ± SE of data from the numbers of different subjects shown in brackets.

Figure 8. Regulation of CD203c and CD11b by prostaglandins. PGD_2 and the selective DP_2 receptor agonist 15R-methyl-PGD_2 (15R-Me-D_2) stimulate the surface expression of CD203c and CD11b on basophils. Activation of the DP_1 receptor opposes this effect, limiting the maximal response to PGD_2 and reducing the response to selective activation of the DP_2 receptor with 15R-methyl-PGD_2. The selective DP_1 receptor antagonist BWA868C enhances CD203c expression in response to high concentrations of PGD_2 by blocking the response of the DP_1 receptor to PGD_2. Activation of EP_2 and possibly also EP_4 receptors also inhibit DP_2-mediated CD203c expression.

Fig. 1
#74823



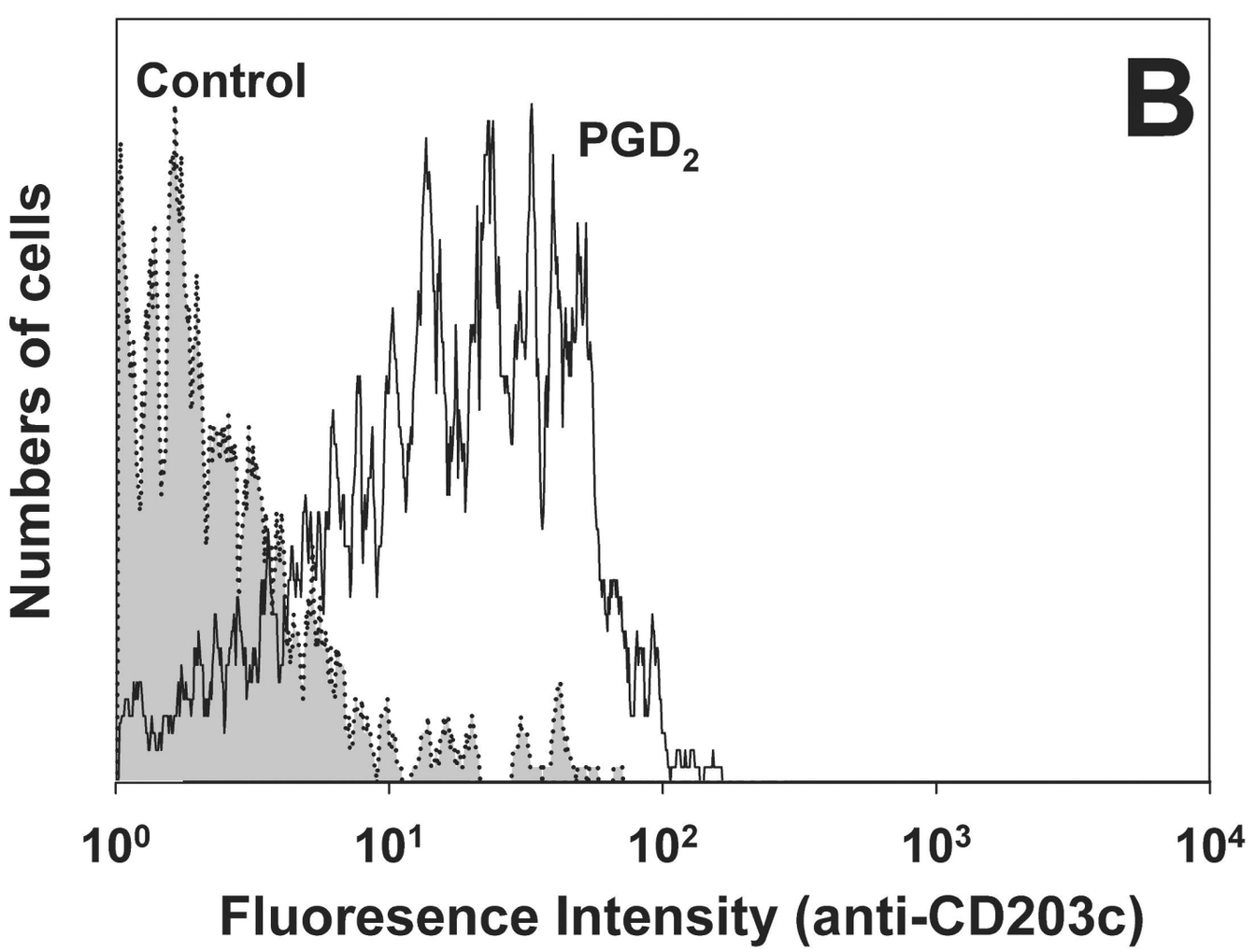


Fig. 2
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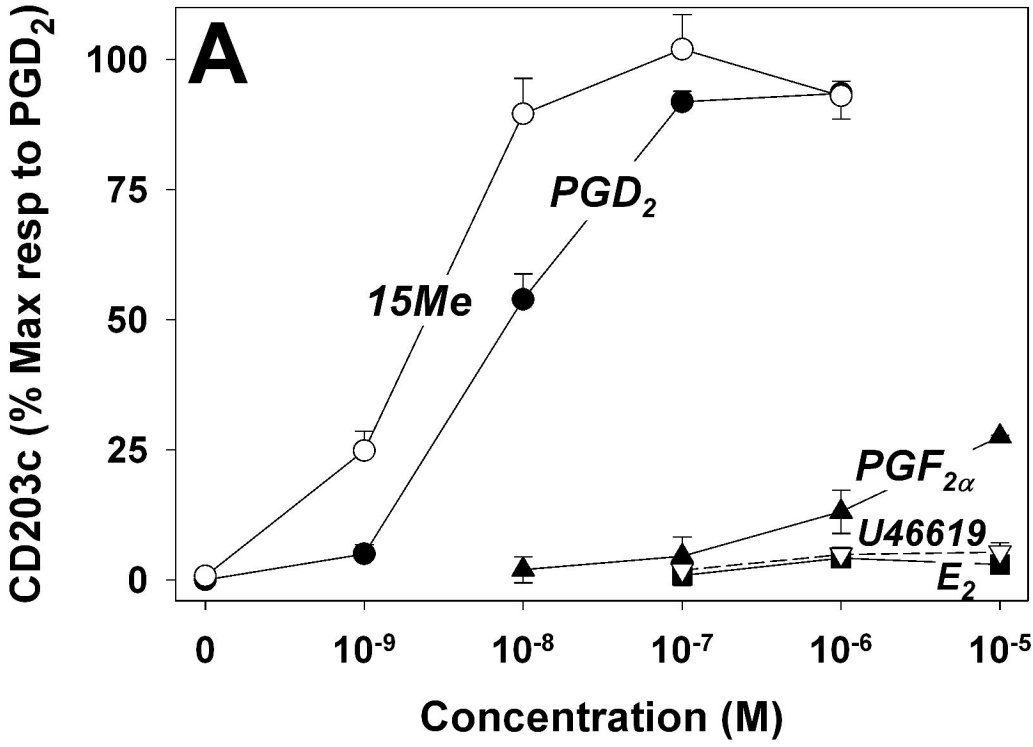

A
CD203c (% Max resp to PGD2)
100
75
50
25
0
PGD2
15Me
PGF2α
U46619
E2
0
10-9
10-8
10-7
10-6
10-5
Concentration (M)

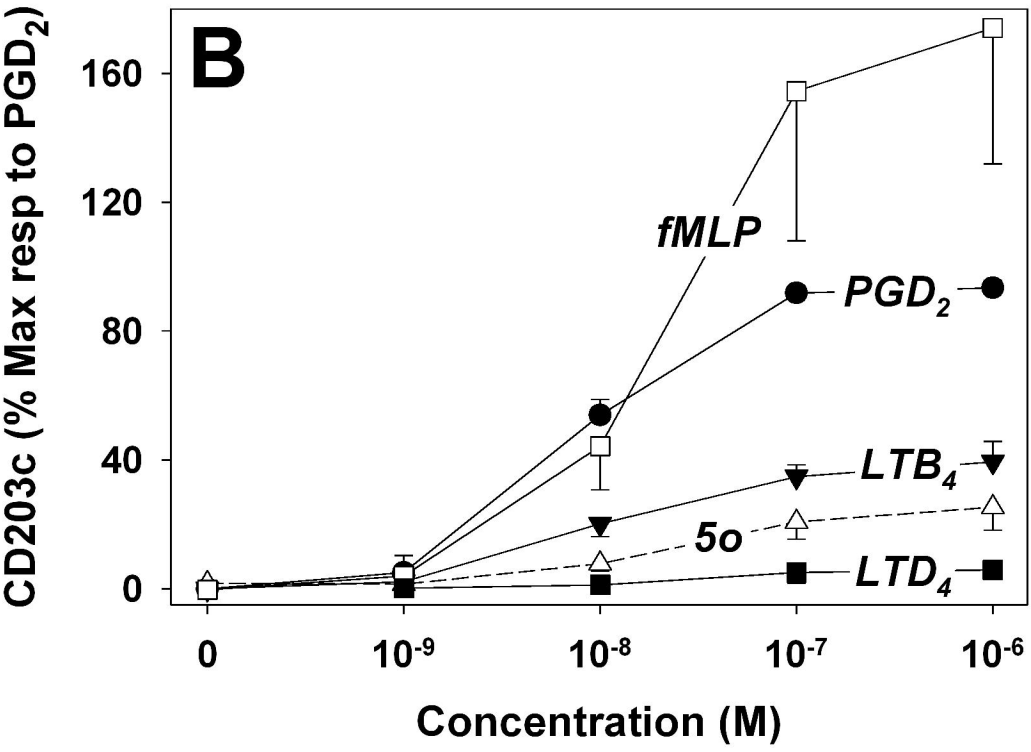
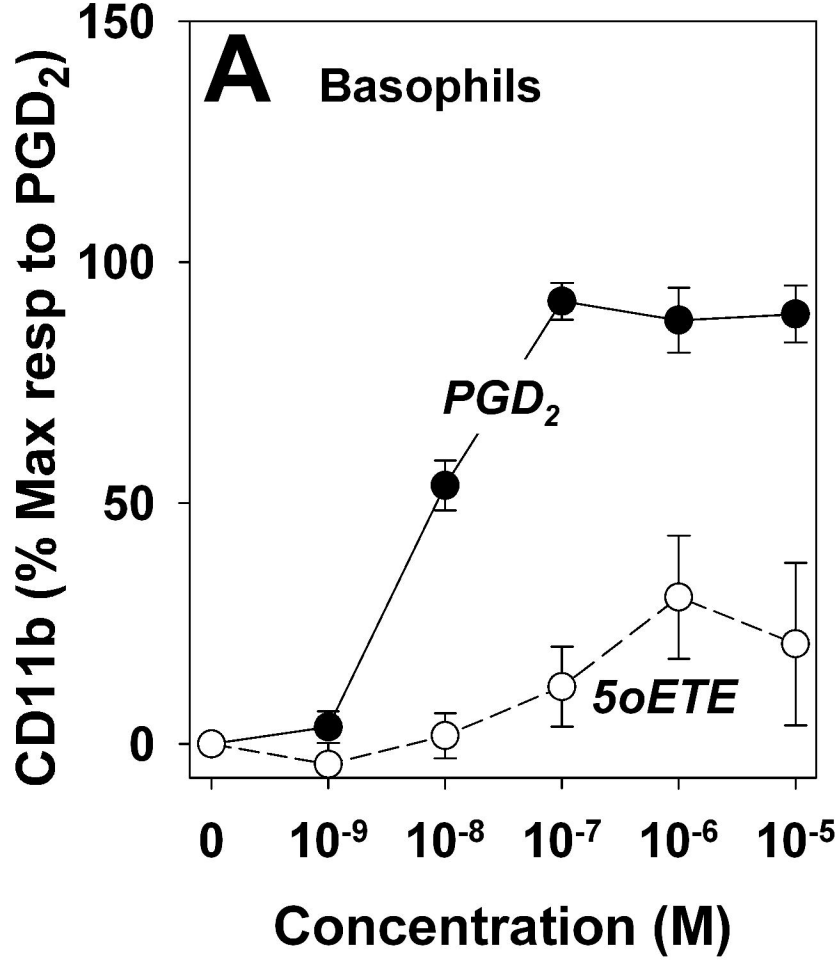

B
CD203c (% Max resp to PGD2)
160
120
80
40
0
fMLP
PGD2
LTB4
5o
LTD4
0
10-9
10-8
10-7
10-6
Concentration (M)

Fig. 3
#74823



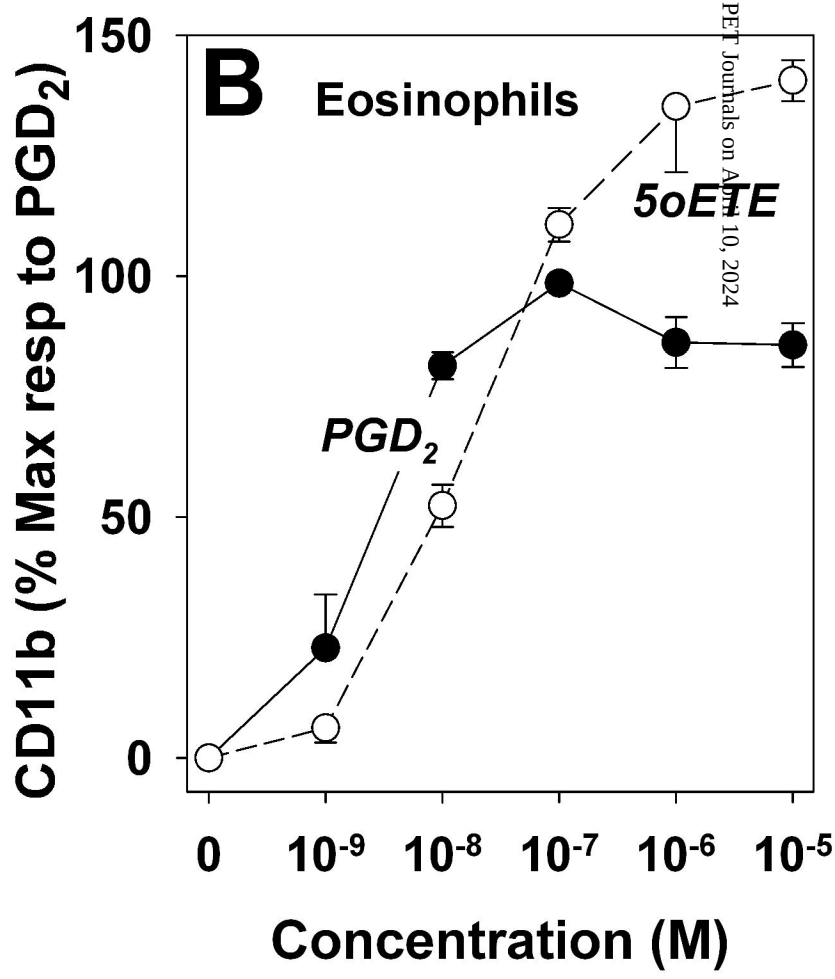
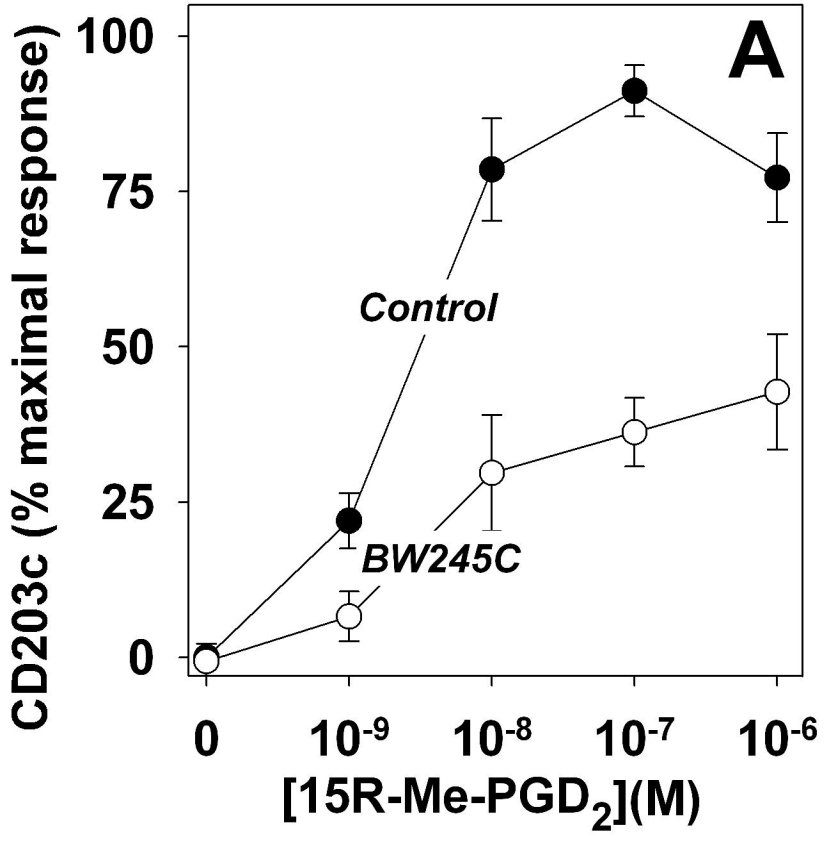


Fig. 4
#74823



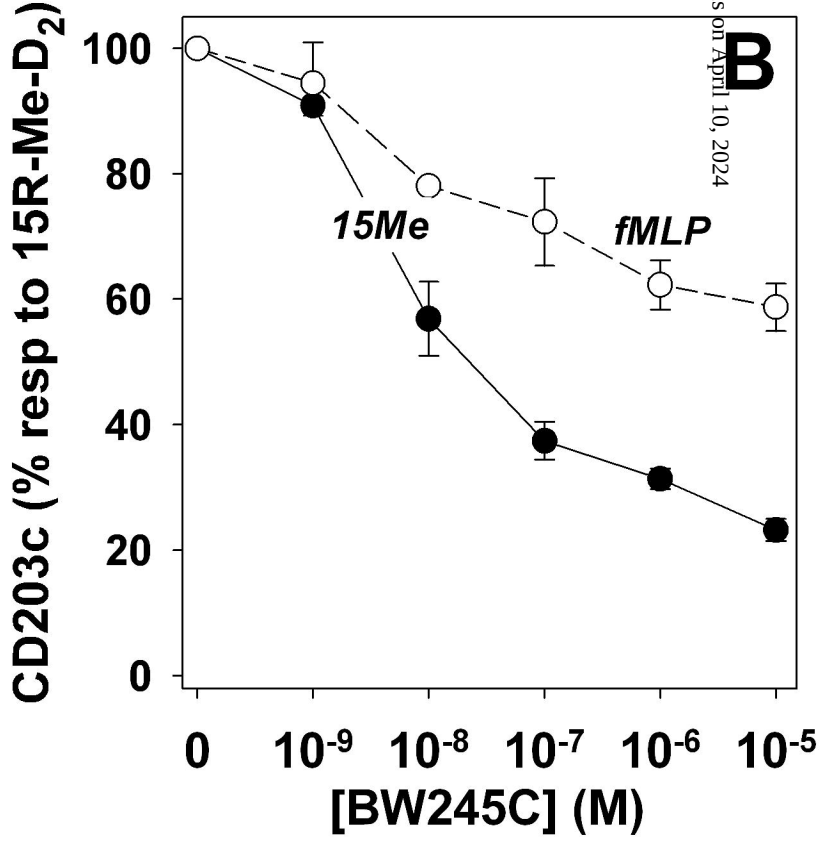


Fig. 5
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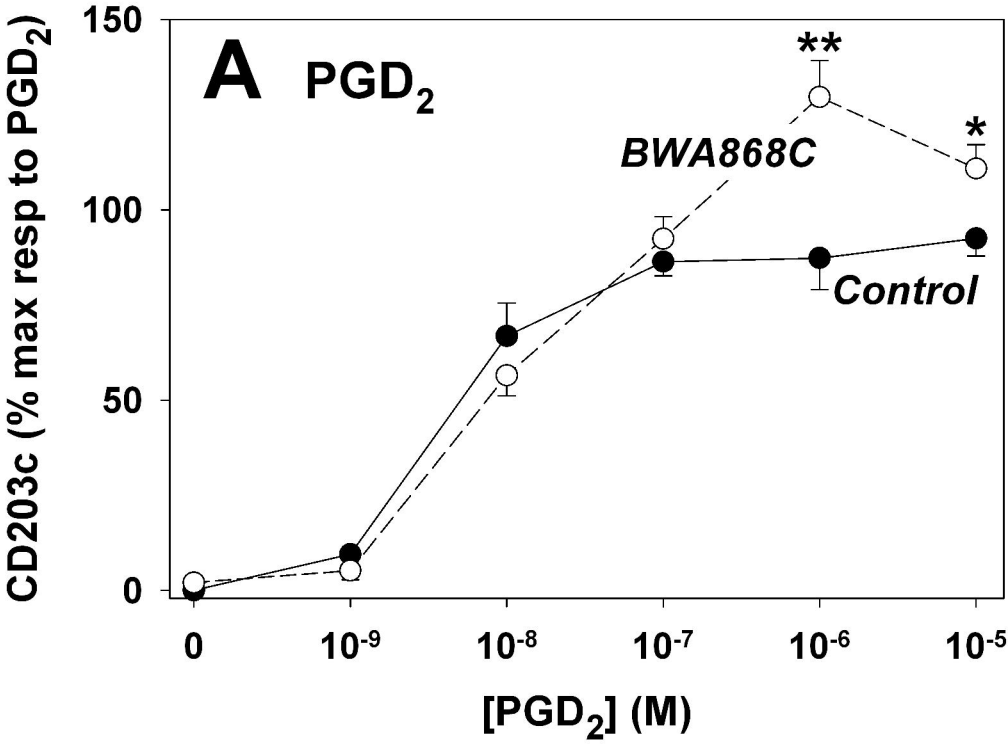

A
PGD2
**
*
BWA868C
Control
150
100
50
0
CD203c (% max resp to PGD2)
0
10-9
10-8
10-7
10-6
10-5
[PGD2] (M)

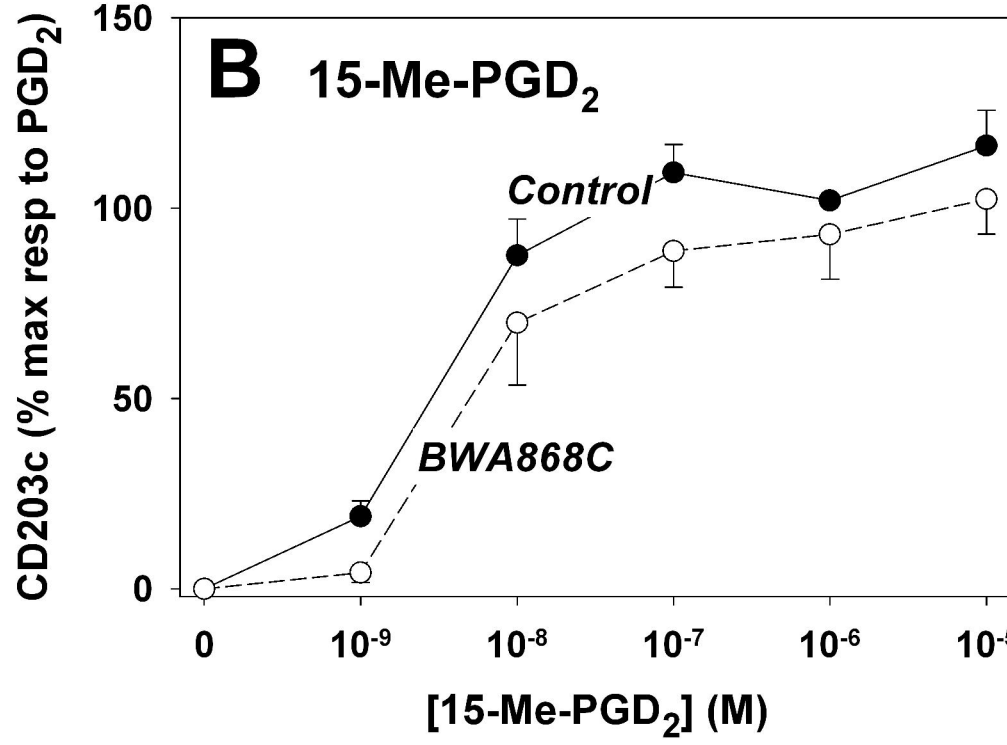
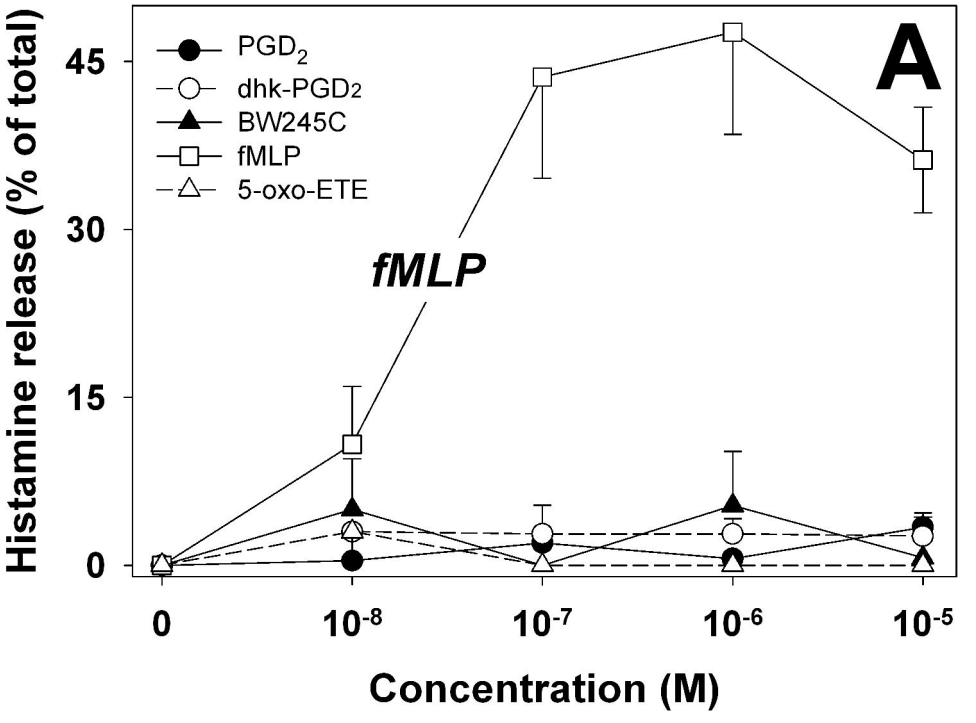

B
15-Me-PGD2
Control
BWA868C
150
100
50
0
CD203c (% max resp to PGD2)
0
10-9
10-8
10-7
10-6
10-5
[15-Me-PGD2] (M)

Fig. 6
#74823


A
PGD2
dhk-PGD2
BW245C
fMLP
5-oxo-ETE
fMLP
Histamine release (% of total)
45
30
15
0
0
10-8
10-7
10-6
10-5
Concentration (M)

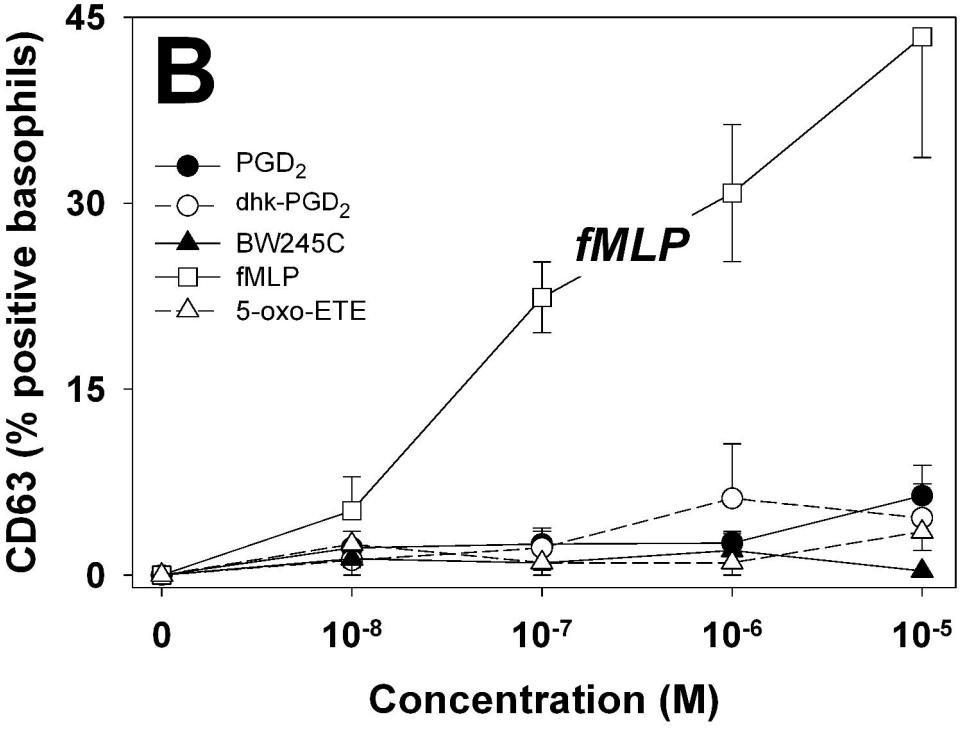

B
PGD2
dhk-PGD2
BW245C
fMLP
5-oxo-ETE
fMLP
CD63 (% positive basophils)
45
30
15
0
0
10-8
10-7
10-6
10-5
Concentration (M)

Fig. 7
#74823

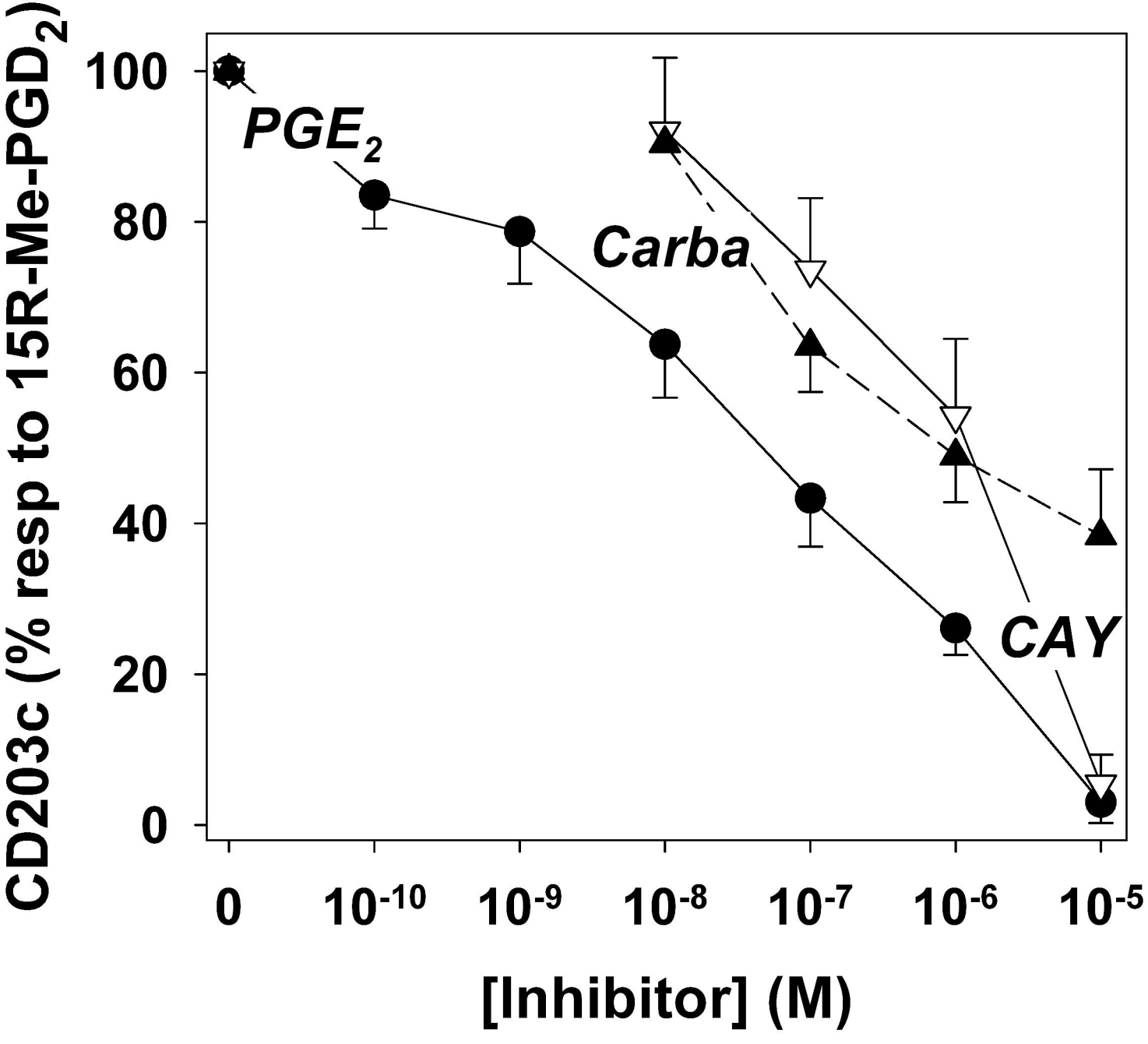

CD203c (% resp to 15R-Me-PGD2)
100
80
60
40
20
0
PGE2
Carba
CAY
0
10-10
10-9
10-8
10-7
10-6
10-5
[Inhibitor] (M)

Fig. 8
#74823