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Group V Secretory Phospholipase A₂ Amplifies the Induction of Cyclooxygenase 2 and Delayed Prostaglandin D₂ Generation in Mouse Bone Marrow Culture-Derived Mast Cells in a Strain-Dependent Manner.

Bruno L. Diaz^{a,b,1}, Yoshiyuki Satake^b, Eriya Kikawada^b, Barbara Balestrieri^b, and Jonathan P. Arm^{b,c,*}

a Department of Medicine Harvard Medical School and the Division of Rheumatology Immunology and Allergy, Brigham and Women's Hospital, Boston, MA;

b Divisão de Biologia Celular, Coordenação de Pesquisa, Instituto Nacional de Câncer, Rio de Janeiro, Brazil 20231-050;

c Partners Asthma Center, Brigham and Women's Hospital, Boston, MA.

Abstract

Activation of bone marrow-derived mast cells (BMMC) with stem cell factor (SCF) or IgE and antigen elicits exocytosis and an immediate phase of prostaglandin (PG) D₂ and leukotriene (LT) C₄ generation. Activation of BMMC by SCF, IL-1 β and IL-10 elicits a delayed phase of PGD₂ generation dependent on cyclooxygenase (COX) 2 induction. Cytosolic phospholipase A₂ α provides arachidonic acid in both phases and amplifies COX-2 induction. Pharmacological experiments implicate an amplifying role for secretory (s) PLA₂. We used mice lacking the gene encoding group V sPLA₂ (*Pla2g5* $-/-$) to definitively test its role in eicosanoid generation by BMMC. *Pla2g5* $-/-$ BMMC on a C57BL/6 genetic background showed a modest reduction in exocytosis and immediate PGD₂ generation after activation with SCF or with IgE and antigen, while LTC₄ generation was not modified. Delayed-phase PGD₂ generation and COX-2 induction were reduced ~35% in C57BL/6 *Pla2g5* $-/-$ BMMC and were restored by exogenous PGE₂. There was no deficit in either phase of eicosanoid generation by *Pla2g5* $-/-$ BMMC on a BALB/c background. Thus, group V sPLA₂ amplifies COX-2 expression and delayed phase PGD₂ generation in a strain-dependent manner; it has at best a limited role in immediate eicosanoid generation by BMMC.

Keywords

Phospholipase A₂; secretory phospholipase A₂; mast cells; leukotriene C₄; prostaglandin D₂; cyclooxygenase

Keywords

Abbreviations: BMMC, bone marrow-derived mast cells; cPLA₂, cytosolic phospholipase A₂; COX, cyclooxygenase; HBA, Hanks' balanced salt solution without Mg²⁺ or Ca²⁺ containing 0.1% BSA; LT, leukotriene; PG, prostaglandin; SCF, stem cell factor; sPLA₂, secretory phospholipase A₂

*Corresponding Author. Smith Research Building, Room 638B 1, Jimmy Fund Way Boston, MA 02115 Tel (617) 525-1305; Fax (617) 525-1310 E-Mail: jarm@rics.bwh.harvard.edu.

¹Present address: Programa de Imunobiologia, Instituto de Biofísica, Universidade Federal do Rio de Janeiro, Brazil 21949-900

1. Introduction

The release of esterified arachidonic acid from the *sn*-2 position of cell membrane phospholipids by the action of phospholipase A₂ (PLA₂) is the first and essential step in its metabolism by 5-lipoxygenase and cyclooxygenase to leukotrienes and prostaglandins, respectively [1,2]. These eicosanoids are prominently implicated in a range of physiological and pathological processes including hemostasis, gastric mucosal integrity, parturition, regulation of renal tubular flow, inflammation, carcinogenesis, and asthma [3,4]. Other products of arachidonic acid include hydroxyeicosatetraenoic acids, lipoxins, epoxyeicosatrienic acids, and isoprostanes that also may contribute to and modulate inflammatory responses [5].

PLA₂ enzymes fall into four broad categories. Cytosolic PLA₂ (cPLA₂)- α is an 85-kDa cytosolic enzyme that utilizes a catalytic serine residue and preferentially cleaves arachidonic acid from cell membrane phospholipids [6]. It is activated by Ca²⁺-dependent translocation from the cytosol to the nuclear envelope [7], which is a prominent site of eicosanoid biosynthesis. Five paralogues of cPLA₂- α have been described [8,9,10]. There are 10 mammalian low molecular weight, secretory PLA₂ (sPLA₂) enzymes that are characterized by a conserved motif containing a catalytic histidine residue, by their relatively small size of ~14 kDa, and by their highly disulfide-linked tertiary structures [11,12,13]. They are distinguished from one another by certain aspects of their structure, their biochemical properties, and their tissue distribution. Calcium-independent PLA₂ enzymes have been described in myocardium and in leukocytes. They have been implicated in membrane remodeling [14], regulation of store operated calcium channels [15], apoptosis, and release of arachidonic acid. The fourth group of PLA₂ enzymes comprises the acetyl hydrolases of platelet activating factor.

Mast cells are effector cells of innate and adaptive immunity [16]. In addition to their established role in asthma and allergic disease, they have been shown to be critical for innate immune responses to bacterial infections [17] and to be critical effector cells in autoimmune diseases such as rheumatoid arthritis [18] and bullous pemphigoid [19,20]. Mast cells are the predominant source of PGD₂ and a major source of LTC₄, the parent cysteinyl leukotriene [21,22]. Our previous studies have demonstrated that mouse bone marrow culture-derived mast cells (BMMC) may be activated for secretory granule exocytosis and an immediate phase of LTC₄ and PGD₂ generation by cross-linking of Fc ϵ RI and by interaction of SCF with its receptor, *c-kit* [23]. These events are complete within 10 minutes. The immediate phase of PGD₂ generation requires the action of constitutively expressed COX-1 [23]. When BMMC are stimulated with SCF in combination with IL-1 β and IL-10 [24] or with antigen after sensitization with hapten-specific IgE [25,26], there is a delayed phase of PGD₂ generation that is characterized by the generation of PGD₂ in the absence of leukotrienes. Importantly and distinctively, delayed phase PGD₂ generation depends on the induced expression of COX-2 [24,23,27].

Both cPLA₂- α and a heparin-binding sPLA₂ have been implicated in the release of arachidonic acid for eicosanoid generation in mast cells. The contribution of a sPLA₂ to immediate eicosanoid generation by mouse mast cells was suggested by the capacity of sPLA₂ enzymes to elicit AA release and PGD₂ generation from BMMC [28], by the inhibition of immediate IgE-dependent PGD₂ generation by the sPLA₂ inhibitor SB203347 [29], and by the inhibition of immediate PGD₂ generation by antisense oligonucleotides specific for group V sPLA₂ in the MMC-34 mast cell line [29]. On the other hand, cPLA₂- α was implicated in the immediate phase by its phosphorylation within 2 min of activation of BMMC through Fc ϵ RI or *c-kit* [23], the inhibition of the immediate response by preincubation of BMMC with methyl arachidonyl fluorophosphate, an inhibitor of cPLA₂- α [27], and by translocation of cPLA₂- α from the cytosol to the nuclear envelope when RBL cells were activated through Fc ϵ RI [30].

In our own hands, 12-epi-scalaradial, an inhibitor of sPLA₂ enzymes, did not inhibit the immediate phase of either PGD₂ or LTC₄ generation [31]. By contrast, the delayed phase of PGD₂ generation was inhibited by heparin, which released a sPLA₂ activity from the cell into the culture medium, implying the participation of a heparin-binding sPLA₂ [31]. Both immediate and delayed PGD₂ generation were intact in BMMC derived from C57BL/6mice, in which the gene for group IIA sPLA₂ is disrupted, suggesting the participation of another heparin-binding sPLA₂ [31].

To clarify the role of cPLA₂- α in the immediate and delayed phases of eicosanoid generation, we turned from pharmacological approaches to the analysis of BMMC derived from mice in which the gene for cPLA₂- α had been disrupted by homologous recombination (cPLA₂- α -/-) [32]. The immediate phase of both LTC₄ and PGD₂ generation, in response to SCF or IgE/antigen, was completely ablated in cPLA₂- α -/- BMMC. In addition, delayed phase PGD₂ generation in response to SCF + IL-10 + IL-1 β was undetectable in cPLA₂ -/- BMMC, and the induction of COX-2 was attenuated ~70%. Delayed phase PGD₂ generation was restored by supply of exogenous arachidonic acid, which led to a modest increase in the expression of COX-2 accompanied by a concentration-dependent restoration of delayed phase PGD₂ generation. Interestingly, the sPLA₂ inhibitor, 12-epi-scalaradial, caused a dose-dependent inhibition of the delayed phase of PGD₂ generation restored by exogenous AA in cPLA₂ -/- BMMC [33], consistent with a requirement for both cPLA₂- α and a sPLA₂.

Several lines of investigation suggest that sPLA₂ enzymes can amplify eicosanoid generation that is dependent upon cPLA₂- α [34,35,36]. The ability of the heparin-binding group V sPLA₂ to couple to eicosanoid generation both through binding to cell membrane proteoglycans and also through interfacial binding to phosphatidylcholine at the plasma membrane [37,38] and its expression in a perinuclear location in BMMC [39] make it the most likely candidate. Here we describe studies that definitively test the requirement for group V sPLA₂ in immediate and delayed phases of eicosanoid generation in mouse BMMC using mice in which the gene encoding the enzyme, *Pla2g5*, has been disrupted.

2. Materials and Methods

2.1 Targeted disruption of *Pla2g5*

We previously described mice with targeted disruption of *Pla2g5* [36]. Mice on a mixed C57BL/6 $\times$ 129 background, heterozygous for the disrupted *Pla2g5* allele, were crossed for 3 to 11 generations to C57BL/6 mice and to BALB/c mice. Heterozygous mice on a C57BL/6 or BALB/c background were crossed to obtain *Pla2g5*-null mice and wild-type breeding pairs.

2.2 Culture and activation of BMMC

BMMC were derived in 50% WEHI-3 cell-conditioned medium as a source of IL-3, as previously described [24]. After 4 weeks, greater than 99% of the cells were mast cells with characteristic metachromatic granules as assessed by toluidine blue staining.

For immediate eicosanoid generation [23], *Pla2g5* +/+ and -/- BMMC were resuspended at 2×10^7 cells/ml in 50% WEHI-3 cell-conditioned medium and incubated with a 1:300 dilution of monoclonal IgE derived from ascites for 1 hour at 37°C. This dilution of ascites was that which, in dose-ranging experiments, provided optimal secretory granule exocytosis in response to 1 to 100 ng/ml TNP-BSA. After washing, BMMC were incubated with TNP-BSA (0–100 ng/ml) at 5×10^6 cells/ml in Tyrode's Gelatin Buffer for 15 min at 37°C. Alternatively, BMMC were incubated with SCF (0–100 ng/ml, R&D, Minneapolis, MN) at 10^7 cells/ml for 15 min at 37 °C. Cells were pelleted at $200 \times g$, and supernatants were analyzed for mediator release.

For delayed-phase PGD₂ generation [24,40], *Pla2g5*^{+/+} and *Pla2g5*^{-/-} BMMC were cultured in 50% WEHI-3 cell-conditioned medium with 1 to 100 ng/ml SCF (R&D), 10 U/ml IL-10, and 5 ng/ml IL-1 β (R&D). After 1 h, cells were washed to remove the products of the immediate phase of eicosanoid generation, and fresh medium with cytokines was added. 7 h later, cells were pelleted, the concentration of PGD₂ was measured in the supernatants, and COX-2 expression was determined in cell pellets by Western blotting as described [24,40]. To measure the expression of COX-2, Western blots were scanned using a Hewlett Packard Scanjet 4890, and the intensity of protein bands was quantified using Image J (National Institutes of Health). Comparisons between COX-2 expression in WT and *Pla2g5*-null BMMC were made on samples run on the same day either on the same blot or on blots processed at the same time in an identical manner. In selected experiments, 0.1 to 10 μ g/ml PGE₂ (Cayman Chemical, Ann Arbor, MI) was added to the culture medium during incubation with cytokines.

2.3 Measurement of mediator release

PGD₂ and LTC₄ generation were assayed in the cell-free supernatants by EIA (Cayman Chemical, and GE Healthcare, Piscataway, NJ, respectively) according to the manufacturer's instructions. Secretory granule exocytosis was determined by measuring the release of β -hexosaminidase in the supernatant and lysed cell pellets as described [41,23].

2.4 Immunofluorescence analysis of group V sPLA₂ expression

BMMC in suspension were fixed with 2% paraformaldehyde (Sigma, St. Louis, MO) in PBS for 10 min at room temperature, washed once in Hanks' balanced salt solution without Mg²⁺ or Ca²⁺ containing 0.1% BSA (HBA), and resuspended in a mixture of 50% HBA with 50% FBS. 3.3×10^5 BMMC were pelleted (5min, 500RPM) onto 12-mm circular glass coverslips (Fisher Scientific, Pittsburg, PA) with a Thermo Shandon cytocentrifuge and were placed in wells of a 24-well tissue culture plate. BMMC were then permeabilized with 0.025% saponin (Sigma) in PBS for 10 min at room temperature and washed twice with HBA. Alternatively BMMC were fixed with 2% paraformaldehyde in PBS for 10 min at 4°C, washed once, cytospun onto glass cover slips, and permeabilized in 100% methanol at -20°C for 20 min. After fixation and permeabilization, BMMC were blocked in HBA containing 5% normal donkey serum for 1 h at room temperature. Cells were incubated with 5 μ g/ml rabbit anti-mouse group V sPLA₂ [39] in blocking buffer for 1 h at room temperature, washed extensively with HBA, and incubated with Texas Red-conjugated donkey anti-rabbit IgG heavy and light chains (Jackson Immunosearch, West Grove, PA) diluted 1:2000 and 0.5 μ g/ml Hoechst dye for 1h at room temperature. Cells were washed in HBA and mounted in Vectashield™ mounting medium (Vector Laboratories, Burlingame, CA). Specimens were imaged using a Nikon Eclipse 800 microscope coupled with a spot RT digital camera and analyzed using Adobe Photoshop software.

2.5 Statistical Analyses

We performed 2-way analysis of variance using the Prism 4 software to compare mediator release between populations of BMMC derived from gene-disrupted mice and their wild-type controls.

3. Results

3.1 Expression of group V sPLA₂ in mouse BMMC

BMMC derived from *Pla2g5*^{-/-} mice in the presence of WEHI cell-conditioned medium showed no difference in numbers, purity, or maturation as judged by toluidine blue staining when compared to age-matched BMMC derived from wild type littermates (data not shown), indicating that group V sPLA₂ has no fundamental role in mast cell growth and development.

We previously generated rabbit anti-peptide antibodies to mouse group V sPLA₂. As described before [39], immunostaining for group V sPLA₂ in wild-type BMMC showed a punctate cytoplasmic pattern and prominent perinuclear labeling in cells permeabilized with saponin (Figure 1A), and prominent plasma membrane staining of cells permeabilized with methanol (Figure 1B). Immunostaining of *Pla2g5*^{-/-} BMMC performed in the same conditions was negative (Figure 1C&D) confirming that gene disruption completely abrogated group V sPLA₂ expression in BMMC.

3.2 Immediate eicosanoid generation by BMMC

Mast cell activation through either the high affinity Fc receptor for (FcεRI) or *c-kit* leads to secretory granule exocytosis and *de novo* generation of LTC₄ in preference to PGD₂ [23,42]. To evaluate the role of group V sPLA₂ in the immediate phase of eicosanoid generation, *Pla2g5*^{-/-} and wild-type BMMC were stimulated for 15 minutes with SCF or were sensitized with IgE anti-TNP and activated for 15 minutes with TNP-BSA in a dose-dependent manner. To allow for the possibility of a strain-dependent contribution of group V sPLA₂ to eicosanoid generation we studied BMMC from mice bred to C57BL/6 and to BALB/c backgrounds for 3 to 11 generations.

There was no difference in secretory granule exocytosis, LTC₄ generation, or PGD₂ generation in response to SCF or to IgE and antigen between *Pla2g5*^{-/-} and wild type BMMC derived from mice on a BALB/c background (Figure 2).

For BMMC derived from mice on a C57BL/6 background (Figure 3), there was a modest attenuation of both β-hexosaminidase release and PGD₂ generation in response to stimulation with both IgE/antigen (p<0.01 and p<0.01, respectively) and SCF (p=0.14 and p<0.02, respectively), with no significant attenuation of LTC₄ generation. The attenuation of PGD₂ generation was modest (22% and 23% at 100 ng/ml in response to SCF and IgE/Ag, respectively) and was comparable to the decrement in secretory granule exocytosis (10% and 26%, respectively).

3.3 Delayed eicosanoid generation by BMMC

Our previous data indicated that a heparin-sensitive sPLA₂ participates in the delayed phase of COX-2-dependent PGD₂ generation in mouse BMMC [31]. The enzyme responsible was not group IIA sPLA₂ as delayed phase PGD₂ generation was intact in BMMC derived from C57BL/6 mice, which have a natural disruption of the gene encoding group IIA sPLA₂ [43]. To test the hypothesis that group V sPLA₂ is the responsible sPLA₂, we compared delayed phase PGD₂ generation in *Pla2g5*^{-/-} and WT BMMC derived from mice that had been bred to a BALB/c or to a C57BL/6 background for three generations (Figures 4 and 5, respectively).

Wild type BMMC showed the characteristic delayed-phase generation of PGD₂ accompanied by COX-2 induction that reached a plateau between 100 and 200 ng/ml of SCF. Delayed phase PGD₂ generation and COX-2 induction were not attenuated in *Pla2g5*^{-/-} BMMC derived from N3 BALB/c mice (Figure 4A & B).

In contrast, the delayed phase of PGD₂ generation evoked by cytokine stimulation was attenuated in *Pla2g5*^{-/-} BMMC derived from N3 C57BL/6 mice (p=0.002). The attenuation was ~35% compared to that observed in BMMC derived from the wild-type controls and was apparent from 1 to 100 ng/ml SCF (Figure 5 A). In wild-type BMMC the maximal response reached a plateau at 10 to 100 ng/ml of SCF whereas that in the *Pla2g5*^{-/-} C57BL/6 BMMC did not reach a plateau until 200 ng/ml SCF, suggesting that the deficiency in group V sPLA₂ lead to a decrease in the sensitivity of the cells to the stimulation without a reduction

in the maximal response. Furthermore, the attenuation of delayed phase PGD₂ generation was accompanied by attenuated induction of COX-2 in *Pla2g5*^{-/-} C57BL/6 BMMC (Figure 5B).

The modest attenuation of delayed phase PGD₂ generation in *Pla2g5*^{-/-} BMMC derived from N3 C57BL/6 mice could be attributable to a role of group V sPLA₂ in amplifying the supply of arachidonic acid by cPLA₂ α or to the attenuation of COX-2 induction. The quantity of free arachidonic acid released from BMMC during the delayed phase of PGD₂ generation is very small, barely twice background [31], making it difficult to assess the contribution of group V sPLA₂ to the release of arachidonic acid. Nevertheless, the expression of cPLA₂ α was comparable between wild-type and *Pla2g5*-null BMMC (data not shown). Analysis of individual experiments indicated that delayed phase PGD₂ generation was comparable between wild type and *Pla2g5*^{-/-} BMMC for a given level of COX-2 induction, suggesting that the primary role of group V sPLA₂ in the delayed phase is amplification of COX-2 expression (Figure 5C). To investigate this further, BMMC were incubated with PGE₂ at the time of cytokine stimulation to amplify COX-2 expression [40,32](Figure 6). For each dose of PGE₂, COX-2 expression and delayed phase PGD₂ generation were amplified in parallel in both wild-type and *Pla2g5*^{-/-} BMMC without restoring delayed phase PGD₂ generation in *Pla2g5*^{-/-} BMMC to the level observed for wild-type BMMC at any dose of PGE₂. PGD₂ generation reflected the induction of COX-2. Thus, the expression of COX-2 and delayed phase PGD₂ generation in wild-type BMMC incubated with 0.1 μ g/ml PGE₂ were equivalent to those observed in *Pla2g5*^{-/-} BMMC incubated with 10 μ g/ml PGE₂ (Figure 6C). These data therefore support a primary role for group V sPLA₂ in amplifying delayed phase PGD₂ generation by amplifying COX-2 induction.

4. Discussion

Experiments using pharmacologic inhibitors and antisense DNA constructs have provided conflicting results as to the participation of individual PLA₂ enzymes in eicosanoid generation by mouse mast cells [31,29,27]. Use of pharmacologic inhibitors and antisense DNA in the MMC 34 mast cell line suggested that group V sPLA₂ and cPLA₂ α were required for immediate and delayed PGD₂ generation, respectively [27,29]. In contrast, the sPLA₂ inhibitor 12-epi-scalaradial inhibited delayed, but not immediate, eicosanoid generation from BMMC [31]. Furthermore, heparin, which has been used to sequester heparin-binding sPLA₂ enzymes in the culture medium and abrogate their action in the cell, inhibited delayed phase PGD₂ generation by BMMC [31]. The role of cPLA₂ α in both immediate and delayed phases of eicosanoid generation was definitively demonstrated using BMMC from mice in which the gene encoding cPLA₂ α had been disrupted [32]. These data not only demonstrated the role of cPLA₂ α in eicosanoid generation by mast cells, but also suggested that the conclusions drawn using inhibitors and antisense constructs were not reliable or informative. Therefore to address the role of group V sPLA₂ we adopted a gene disruption strategy, generating the first *Pla2g5*-null mice [36].

Our results definitively demonstrate that group V sPLA₂ is not required for immediate LTC₄ generation by BMMC in response to either SCF or to IgE and antigen. This was observed in BMMC derived from mice bred to a C57BL/6 or BALB/c background (Figure 2). The data for PGD₂ generation indicate at most a modest (~20%) reduction in the immediate response to SCF or to IgE and antigen, accompanied by a similar decrement in secretory granule exocytosis (Figure 3). This is consistent with the capacity of group V sPLA₂ to augment Fc ϵ RI-dependent granule exocytosis and PGD₂ generation when transfected into RBL-2H3 cells, an effect that required both the catalytic and the heparin-binding domains of the enzyme [44]. However, group V sPLA₂ also augmented Fc ϵ RI-dependent LTC₄ generation when transfected into RBL cells [38] and we did not see any decrement in LTC₄ biosynthesis in *Pla2g5*-null BMMC of either strain of mouse (Figures 2 & 3). An alternative explanation is that the modest decrement

in PGD₂ generation that we observed in *Pla2g5*-null C57BL/6 BMMC is simply a reflection of the normal biological variation in the capacity of different cultures of BMMC to respond to exogenous stimuli, as reflected in the similar decrement in secretory granule exocytosis (Figure 3). Whatever the explanation, immediate generation of PGD₂ and LTC₄ generation is robust in *Pla2g5*-null BMMC from 2 different strains of mice in response to stimulation through both FcεRI and *c-kit*. Any effect of group V sPLA₂ in immediate eicosanoid generation is modest at best.

We can not rule out a role for group V sPLA₂ in regulating the immediate phase of eicosanoid generation from BMMC in response to other stimuli. Furthermore, we have demonstrated a role for group V sPLA₂ in augmenting the cPLA₂α-dependent generation of LTC₄ and PGE₂ in mouse macrophages in response to zymosan stimulation [36]. We therefore consider it likely that the role of group V sPLA₂ in regulating eicosanoid generation depends on the cell type and the nature of the stimulus.

Despite the lack of a clear role for group V sPLA₂ in the immediate phase of eicosanoid generation in BMMC, we found a consistent diminution of delayed phase PGD₂ generation and COX-2 induction in BMMC from N3 C57BL/6 *Pla2g5*^{-/-} mice (Figures 5 & 6). We have previously demonstrated a role for cPLA₂α both in providing arachidonic acid and in regulating COX-2 induction in this delayed phase of PGD₂ generation [32]. The release of arachidonic acid during the delayed phase of PGD₂ generation is small, barely above the background release in cells maintained in IL-3 alone [31]. We were, therefore, unable to directly address the role of group V sPLA₂ in amplifying delayed phase release of arachidonic acid. Nevertheless, the attenuation of delayed phase PGD₂ generation in BMMC from N3 C57BL/6 *Pla2g5*^{-/-} mice was accompanied by attenuated induction of COX-2 (Figure 6B). In both *Pla2g5*^{-/-} and wild-type BMMC, PGE₂ amplified the cytokine-dependent COX-2 induction and delayed phase PGD₂ generation. In a limited number of experiments in which we analyzed both delayed phase PGD₂ generation and COX-2 induction, we found that PGD₂ generation closely paralleled the induction of COX-2 in both wild type and *Pla2g5*-null BMMC (Figures 5C and 6C). It seems likely, therefore, that group V sPLA₂ amplifies delayed phase PGD₂ generation through its effect on COX-2 expression, though we can not rule out a modest effect on provision of arachidonic acid. This is consistent with data implicating endogenous group V sPLA₂ in amplifying COX-2 induction in the P388D1 macrophage cell line in response to LPS and PAF [45]. In the MAB subclone of the P388D1 cell line, delayed phase, LPS-stimulated COX-2 induction was also dependent upon induction of group V sPLA₂ expression [46]. Various sPLA₂ enzymes, including group V sPLA₂, amplified COX-2 induction when transfected into HEK 293 cells [47].

The strain-dependent requirement for group V sPLA₂ in the delayed phase of PGD₂ generation contrasts to the data with 12-episcalaradial and heparin, which inhibited delayed phase PGD₂ generation by BMMC derived from both C57BL/6 and BALB/c mice [31]. Due to concerns about specificity it is unwise to infer a role for a sPLA₂ based solely on pharmacologic inhibition. Furthermore, heparin is pleotropic in its effects and its use to imply a heparin-binding sPLA₂ in eicosanoid generation may be misleading. One might argue that the strain dependent function of group V sPLA₂ relates to the lack of expression of heparin-binding group IIA sPLA₂ in C57BL/6 BMMC and that group IIA sPLA₂ expression in BALB/c BMMC may compensate for deletion of group V sPLA₂. However, the *Pla2g5*^{-/-} mice were derived from 129 ES cells that harbor a natural disruption of *Pla2g2a*, which is tightly linked to *Pla2g5* on mouse chromosome 4 [48]. Thus, *Pla2g5*^{-/-} BALB/c BMMC also lack expression of group IIA sPLA₂ indicating that other epigenetic effects likely regulate the strain-dependent function of group V sPLA₂.

The large number of low molecular weight enzymes raises the issue of redundancy of function. The different biochemical characteristics of the enzymes [38,12], their differing tissue distribution [49], and the different subcellular locations of group IIA sPLA₂ (in granules) and group V sPLA₂ (at plasma membrane, Golgi, and nuclear envelope) in BMMC [39] argue against this. Furthermore, while transcripts for several sPLA₂ enzymes are expressed at low levels in BMMC [38] most have not been detected as protein. Despite the development of antibodies to the sPLA₂ enzymes, current methods of detection have limited sensitivity. In our own hands immunofluorescence staining is the most sensitive method for detection of group V sPLA₂ and group IIA sPLA₂ in BMMC, but this is not a quantitative assay. Thus, we can not rule out compensatory overexpression of another sPLA₂ in group V sPLA₂-null BMMC.

In conclusion, our studies suggest that group V sPLA₂ has at best a limited role in immediate eicosanoid generation in the mast cell and that its role in amplifying delayed phase COX-2-dependent PGD₂ generation is strain-dependent.

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Fig. 1. Immunofluorescence staining group V sPLA₂ in BMMC. BMMC from wild type (A, B) or *Pla2g5*-null (C, D) mice were fixed in 2% Paraformaldehyde, cytopun onto coverslips, and permeabilized with 0.025% Saponin (A, C) or Methanol (B, D) prior to immunofluorescence staining for group V sPLA₂ as described in the Materials and Methods. Cells were visualized on Nikon Eclipse 800 microscope.

Fig. 2.

Immediate mediator release from wild type and *Pla2g5*-null BALB/c BMMC. BMMC from wild type (shaded bars) or *Pla2g5*-null (open bars) mice, bred for 3 generations to a BALB/c background, were stimulated with IgE and antigen (left panels) or with SCF (right panels). Supernatants were assayed for β -hexosaminidase, PGD₂ and LTC₄. n=3.

Fig. 3.

Immediate mediator release from wild type and *Pla2g5*-null C57BL/6 BMMC. BMMC from wild type (shaded bars) or *Pla2g5*-null (open bars) mice, bred for 3 to 11 generations to a C57BL/6 background, were stimulated with IgE and antigen (left panels) or with SCF (right panels). Supernatants were assayed for β -hexosaminidase, PGD₂ and LTC₄. n=9, except for SCF-dependent LTC₄ generation (n=3).

Fig. 4.

Delayed PGD₂ generation and COX-2 induction from wild type and *Pla2g5*-null BALB/c BMMC. BMMC from wild type (shaded bars) or *Pla2g5*-null (open bars) mice, bred for 3 generations to a BALB/c background, were stimulated in 50% WEHI-3 cell-conditioned medium, containing IL-10, IL-1 β , and increasing doses of SCF for 1 hour, washed, and incubated for a further 7 hours in fresh cytokines. (A) Supernatants were assayed for PGD₂ generation, n=6, and (B) COX-2 induction was assessed in cell pellets by Western blotting; a representative blot is depicted.

Fig. 5. Delayed PGD₂ generation and COX-2 induction from wild type and *Pla2g5*-null C57BL/6 BMMC. BMMC from wild type (shaded bars) or *Pla2g5*-null (open bars) mice, bred for 3 generations to a C57BL/6 background, were stimulated in 50% WEHI-3 cell-conditioned medium, containing IL-10, IL-1 β , and increasing doses of SCF for 1 hour, washed, and incubated for a further 7 hours in fresh cytokines. (A) Supernatants were assayed for PGD₂, n=11, and (B) COX-2 induction was assessed in cell pellets by Western blotting; a representative blot is depicted. (C) COX-2 expression, assessed by densitometry, is directly compared with PGD₂ generation in the same BMMC, mean data are shown for 2 experiments.

Fig. 6.

Amplification of delayed PGD₂ generation and COX-2 induction by PGE₂ in wild type and *Pla2g5*-null C57BL/6 BMDC. BMDC from wild type (shaded bars) or *Pla2g5*-null (open bars) mice, bred for 11 generations to a C57BL/6 background, were stimulated in WEHI-3 cell-conditioned medium alone or containing 100 ng/ml SCF, 10 U/ml IL-10, 5 ng/ml IL-1β and increasing concentrations of PGE₂ for 1 hour, washed, and incubated for a further 7 hours in fresh cytokines and PGE₂. (A) Supernatants were assayed for PGD₂, n=3, and (B) COX-2 induction was assessed in cell pellets by Western blotting; a representative blot is depicted. (C) COX-2 expression, assessed by densitometry, is compared with PGD₂ generation for the experiment depicted in panel B.