Role of cytosolic phospholipase A_2 in the production of lipid mediators and histamine release in mouse bone-marrow-derived mast cells

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Cytosolic phospholipase A_2 ($cPLA_2$) plays a critical role in mast-cell-related allergic responses [Uozumi, Kume, Nagase, Nakatani, Ishii, Tashiro, Komagata, Maki, Ikuta, Ouchi et al. (1997) Nature (London) **390**, 618–622]. Bone-marrow-derived mast cells from mice lacking $cPLA_2$ ($cPLA_2^{-/-}$ mice) were used in order to better define the role of $cPLA_2$ in the maturation and degranulation of such cells. Cross-linking of high-affinity receptors for IgE (FcεRI) on cells from $cPLA_2^{-/-}$ mice led to the release of negligible amounts of arachidonic acid or its metabolites, the cysteinyl leukotrienes and prostaglandin D_2, indicating an essential role for $cPLA_2$ in the production of these allergic and pro-inflammatory lipid mediators. In addition, the histamine content of the mast cells and its release from the cells were reduced to 60%. While these results are in agreement with a reduced anaphylactic phenotype of $cPLA_2^{-/-}$ mice, the ratios of release of histamine and β-hexosaminidase were, paradoxically, significantly higher for cells from $cPLA_2^{-/-}$ mice than for those from wild-type mice. Consistently, IgE-induced calcium influx in mast cells was greater and more prolonged in cells from $cPLA_2^{-/-}$ mice than in those from wild-type mice. Thus the loss of $cPLA_2$ not only diminishes the release of lipid mediators, but also alters degranulation. While the overall effect is still a decrease in the release of mast cell mediators, explaining the *in vivo* findings, the present study proposes a novel link between $cPLA_2$ and the degranulation machinery.

Key words: anaphylaxis, exocytosis, leukotrienes, prostaglandins.

INTRODUCTION

Phospholipase A_2 (PLA_2) liberates fatty acids from the *sn*-2 position of glycerophospholipids, with the concomitant production of lysophospholipids. The arachidonic acid (AA) and lysophospholipids thus released are potential precursors of various types of eicosanoids and of platelet-activating factor (PAF) respectively. PLA_2 consists of a superfamily of more than 10 distinct molecular species of enzymes [1–3]. Among these, cytosolic PLA_2 ($cPLA_2$) is pre-eminent, because of its preference for AA. $cPLA_2$ is activated by submicromolar concentrations of calcium ions and by phosphorylation by mitogen-activated protein kinase [4–6]. To understand the roles of the enzyme *in vivo*, mice lacking $cPLA_2$ ($cPLA_2^{-/-}$ mice) have been established by gene targeting [7,8]. The mice mature normally, but when sensitized and challenged they have lowered anaphylactic responses to ovalbumin and diminished bronchial hyperreactivity compared with wild-type animals [7].

Antigen-specific activation through IgE on mast cells is considered to be responsible for allergic responses [9,10]. The cross-linking of a high-affinity IgE receptor (FcεRI) expressed on the cell surface initiates the secretion of pro-inflammatory mediators that are pre-stored in granules (such as histamine, 5-hydroxytryptamine, heparin and vasoactive peptides), whereas lipid mediators such as prostaglandins, leukotrienes and PAF are synthesized *de novo* [10,11]. It is now believed that the mast cells enter the circulation from the bone marrow as mononuclear cell precursors and migrate into tissues under the influence of local microenvironmental factors, where they undergo the final steps of differentiation and maturation into recognizable mast cells [10].

Bone-marrow-derived mast cells (BMMC) obtained under special culture conditions represent a relatively immature population of mast cells that have been used widely as a model of mast cells [12–18]. We used BMMC obtained from $cPLA_2^{+/+}$ and $cPLA_2^{-/-}$ mice, and measured the secretion of mediators from the cells after stimulation with an antigen. With cells from $cPLA_2^{-/-}$ mice, the amount of mediators was significantly reduced. A paradoxical finding was that BMMC from $cPLA_2^{-/-}$ mice showed augmented exocytosis of histamine and lysosomal enzymes upon IgE stimulation, but, because they contained lower levels of histamine, the absolute amount of histamine released was less than from wild-type cells. These results may prove useful in the development of $cPLA_2$ inhibitors, and in interpreting the therapeutic effects of such inhibitors in allergic and inflammatory disorders.

EXPERIMENTAL

Materials

Partial fragments of cDNAs for mouse mast cell protease-1 (MMCP-1), MMCP-4 and MMCP-5 were obtained from a

Abbreviations used: PLA_2, phospholipase A_2; $cPLA_2$, cytosolic PLA_2; $sPLA_2$, secretory PLA_2; AA, arachidonic acid; LTC_4, leukotriene C_4; PGD_2, prostaglandin D_2; PAF, platelet-activating factor; BMMC, bone-marrow-derived mast cells; MMCP, mouse mast cell protease; HDC, L-histidine decarboxylase; Dnp, dinitrophenyl; Dnp–HSA, Dnp conjugated to human serum albumin; $[Ca^{2+}]_i$, intracellular free calcium concentration; fura 2/AM, fura 2 acetoxymethyl ester.

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murine BMMC cDNA library [19–21]. WEHI-3 cells were obtained from A.T.C.C. FITC-labelled IgE and allophycocyanin-labelled anti-c-Kit antibody were generously donated by Dr C. Ra (Juntendo University, Tokyo, Japan). The following materials were purchased from the sources indicated: monoclonal anti-dinitrophenyl (Dnp) IgE antibody, Dnp-conjugated human serum albumin (Dnp–HSA) and *p*-nitrophenyl *N*-acetyl-β-D-glucosaminide from Sigma (St. Louis, MO, U.S.A.); RPMI 1640 from Nipro (Osaka, Japan); MEM non-essential amino acid solution from Life Technologies (Rockville, MD, U.S.A.); leukotriene C_4 (LTC_4)/D_4/E_4 enzyme immunoassay system and prostaglandin D_2 (PGD_2) ^3H assay system from Amersham (Little Chalfont, Bucks., U.K.); 5-hydroxytryptamine (serotonin) ELISA and histamine ELISA from ICN Pharmaceuticals (Costa Mesa, CA, U.S.A.); SKF96365 from Calbiochem (San Diego, CA, U.S.A.).

Mouse BMMC culture

Bone marrow cells from $cPLA_2^{+/+}$ and $cPLA_2^{-/-}$ F2 littermates (6–7-week-old females) were cultured for 4–8 weeks in 50% WEHI-3 cell conditioned medium/50% RPMI 1640 supplemented with 10% (v/v) fetal calf serum, 0.1 mM non-essential amino acids, 100 units/ml penicillin and 100 mg/ml streptomycin [16].

Light microscopy of BMMC

The cultured mast cells were centrifuged with Cytospin 3 (Shandon, Astmoor, U.K.) at 28 ***g*** for 5 min, and stained with safranin (0.1%, w/v) in 0.3% acetic acid solution and Alcian Blue (0.5%, w/v) in 0.1% acetic acid solution.

Electron microscopy of BMMC

Electron microscopy analyses were carried out following fixation of BMMC in 5% (v/v) glutaraldehyde, post-fixing in 1% (w/v) osmium tetroxide, embedding in epoxy resin and slicing to 60–90 nm thickness. Samples were stained with lead citrate and uranium acetate, and viewed with a transmission electron microscope (Hitachi-H600; Hitachi, Tokyo, Japan).

Flow cytometry of BMMC

Expression of FcεRI and c-Kit was assessed by FACS, with FITC-labelled IgE and allophycocyanin-labelled monoclonal anti-mouse c-Kit antibody respectively. Background staining was measured with propidium iodide.

Stimulation of BMMC

BMMC were suspended at a density of $(0.5–1) \times 10^7$ cells/ml in an enriched medium containing 50% (v/v) WEHI-3 conditioned medium, and sensitized with 2.5–5 mg/ml anti-Dnp IgE for 30 min at 37 °C. After being washed twice, cells were resuspended in enriched medium at a density of $(1–5) \times 10^6$ cells/ml and incubated at 37 °C with various concentrations of Dnp–HSA. Cells were placed on ice to terminate the reaction, and then centrifuged at 3000 ***g*** for 5 min at 4 °C to obtain supernatants. The supernatants were assayed for PGD_2 by RIA, and for cysteinyl-leukotrienes, histamine and 5-hydroxytryptamine by enzyme immunoassay. The production of PAF was determined as described [22]. For measurement of released AA, cells at a density of 1×10^6 cells/ml were prelabelled with [^{3}H]AA (5 μCi/well) overnight, and were sensitized with anti-Dnp IgE for 30 min. After washing, the cells were incubated with 50 ng/ml Dnp–HSA at 37 °C, and the radioactivity in the medium was counted with a scintillation counter. The activity of β-hexosaminidase was quantified in the supernatants and cell pellets by spectrophotometric analysis using *p*-nitrophenyl *N*-acetyl-β-D-glucosaminide as a substrate [16]. The percentage of β-hexosaminidase released was calculated from the formula $S/(S+P) \times 100$, where S and P are the β-hexosaminidase contents of equal portions of each supernatant and cell pellet respectively.

Measurement of intracellular free calcium concentration ($[Ca^{2+}]_i$)

BMMC [$(1–3) \times 10^6$ cells/ml] sensitized with anti-Dnp IgE were loaded with 3 mM fura 2 acetoxymethyl ester (fura 2/AM) (Dojin, Kumamoto, Japan) in RPMI 1640 at 37 °C for 1 h. The cells were then washed and resuspended in Hepes/Tyrode's buffer at 1×10^6 cells/ml in a stirred cuvette. The fura 2/AM-loaded cells were stimulated with Dnp–HSA at 37 °C. The fluorescence of the cell suspension was monitored continuously using CAF-100, a calcium analyser (JASCO, Tokyo, Japan), with excitation at 340 nm and 380 nm, and emission at 500 nm.

RESULTS

Bone marrow cells from $cPLA_2^{-/-}$ mice grow normally into BMMC

Bone marrow cells were prepared from 6–7-week-old F2 littermates. After 5 weeks of culture they were evaluated by staining with Alcian Blue/safranin (Figure 1A). Heterogeneous staining was observed in cells from both genotypes, which reflects differential expression of the various granule proteoglycans [19]. As shown in Figures 1(A) and 1(B), the cells from the two genotypes were morphologically similar. The secretory granules of mast cells contain a family of serine proteases termed MMCP-1–MMCP-9 [23–26]. Northern blot analyses showed

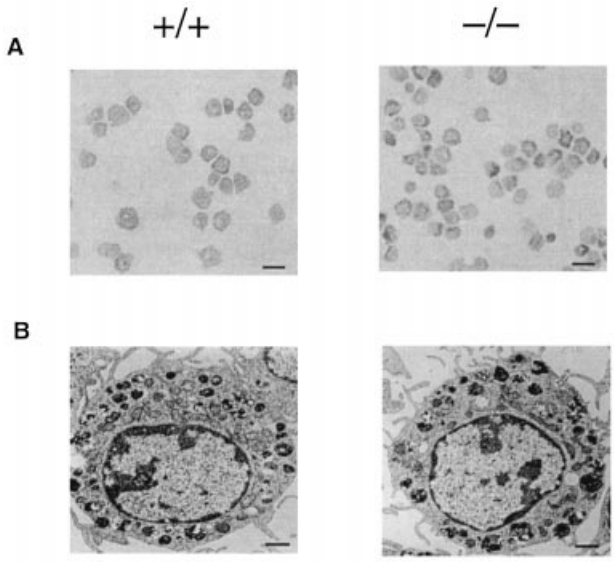

Figure 1 Light and electron microscopy of BMMC from $cPLA_2^{+/+}$ and $cPLA_2^{-/-}$ mice

(**A**) Mast cells cultured for 4 weeks were stained with Alcian Blue and safranin after a cytospin procedure. (**B**) Electron microscopy of mast cells cultured for 4 weeks. Bars: 10 μm (**A**) and 1 μm (**B**).

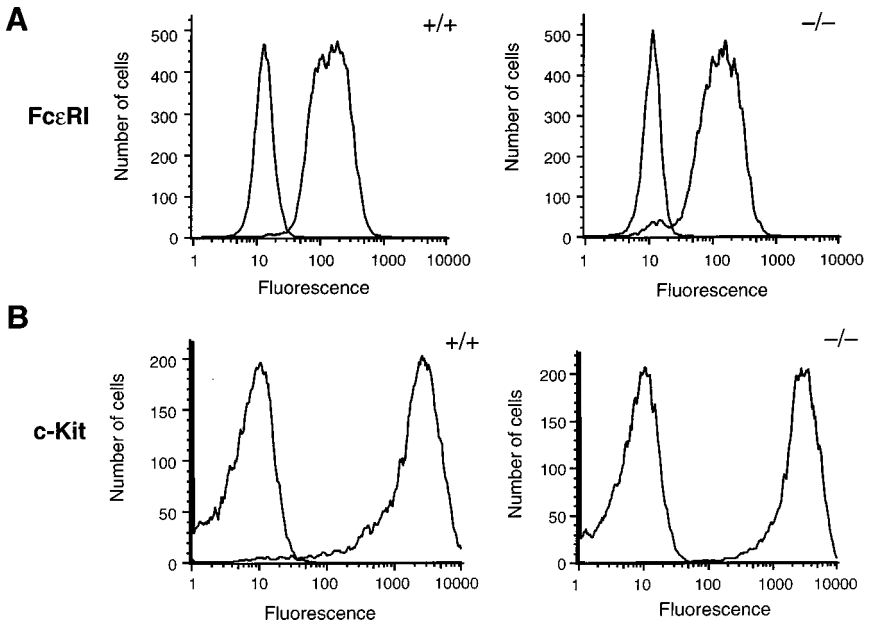

Figure 2 Expression of FcεRI and c-Kit on the cell surface of BMMC from $cPLA_2^{+/+}$ and $cPLA_2^{-/-}$ mice

Cells were stained with FITC-labelled IgE and allophycocyanin-labelled anti-c-Kit antibody, and assessed by FACS. Background staining was with propidium iodide.

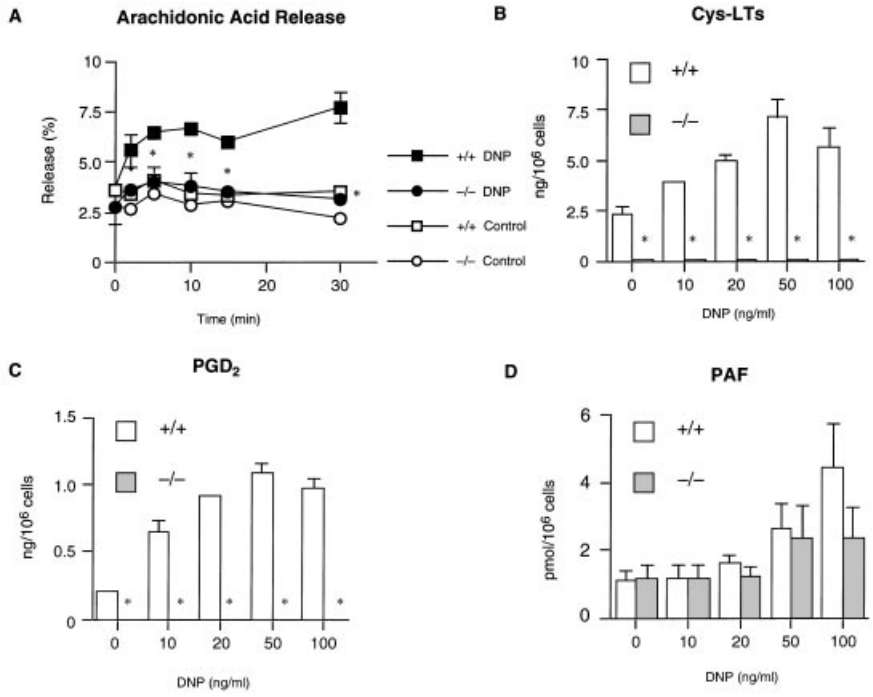

Figure 3 FcεRI-induced AA release and production of eicosanoids and PAF in $cPLA_2^{+/+}$ and $cPLA_2^{-/-}$ BMMC

Cells prelabelled (**A**) or not (**B**–**D**) with [^{3}H]AA were sensitized with 5 μg/ml anti-Dnp IgE for 30 min. The cells were resuspended in culture medium and incubated with 50 ng/ml Dnp–HSA for various times (**A**), or for 10 min (**B**), 30 min (**C**) or 2 min (**D**). The release of AA (**A**), cysteinyl-leukotrienes (Cys-LTs) (**B**), PGD_2 (**C**) and PAF (**D**) into the supernatants was measured. Values are means $\pm$ S.D. ($n = 3$); $^*P < 0.01$ compared with wild-type cells.

similar amounts of the 1 kb MMCP-5 mRNA in cells from $cPLA_2^{+/+}$ and $cPLA_2^{-/-}$ mice. A faint band of MMCP-1 was seen, while the MMCP-4 transcript was not detected in any cells (results not shown). More than 93 % of BMMC were double-positive for FcεRI and c-Kit, with a similar mean fluorescence as assessed by FACS (Figures 2A and 2B).

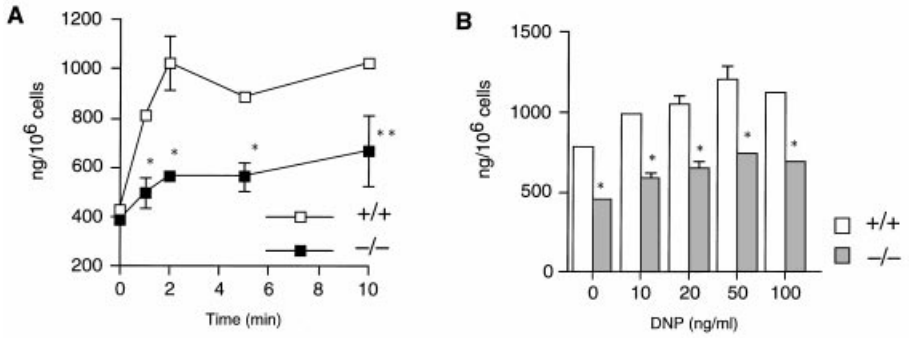

Figure 4 FcεRI-induced histamine release from cPLA$_2^{+/+}$ and cPLA$_2^{-/-}$ BMMC

Cells were sensitized with 5 μg/ml anti-Dnp IgE for 30 min. The cells were then resuspended in culture medium and stimulated with 50 ng/ml Dnp–HSA for various times (**A**) or with the indicated doses of Dnp–HSA for 5 min (**B**). Histamine release into the medium was measured by ELISA. Values are means ± S.D. ($n = 3$); $^*P < 0.01$ compared with wild-type cells.

Production of lipid mediators is decreased in BMMC from cPLA$_2^{-/-}$ mice

BMMC sensitized with anti-Dnp IgE were stimulated with various doses of Dnp–HSA, and the percentage release of AA and the amounts of cysteinyl-leukotrienes, PGD_2 and PAF in the supernatant were measured (Figure 3). AA release from cPLA$_2^{-/-}$ cells was markedly reduced to the basal level, in contrast with that from cPLA$_2^{+/+}$ cells (Figure 3A). Although cPLA$_2^{+/+}$ cells produced cysteinyl-leukotrienes and PGD_2 in a dose-dependent manner, production of these mediators in cPLA$_2^{-/-}$ cells was negligible (Figures 3B and 3C). On the other hand, PAF production was only slightly decreased in cPLA$_2^{-/-}$ cells (Figure 3D).

Histamine release and content are decreased in BMMC from cPLA$_2^{-/-}$ mice

Histamine is one of the mediators involved in the immediate phase of allergic responses, such as constriction of bronchial and intestinal smooth muscles, increased vasopermeability and vaso-

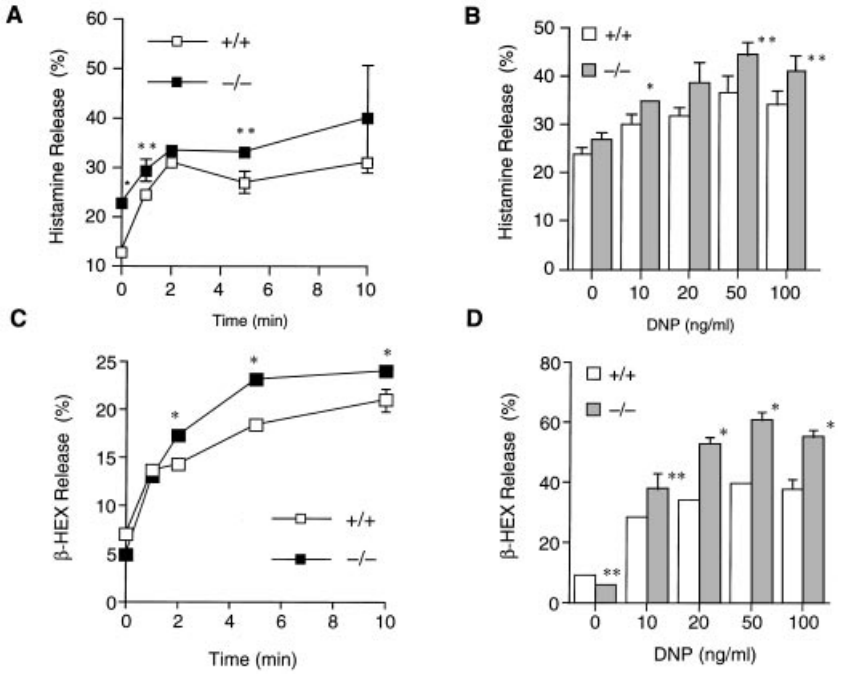

Figure 5 Percentage secretion of histamine and β-hexosaminidase from cPLA$_2^{+/+}$ and cPLA$_2^{-/-}$ BMMC

Cells sensitized with 5 μg/ml anti-Dnp IgE for 30 min were resuspended in culture medium. The cells were stimulated with 50 ng/ml Dnp–HSA for indicated times (**A** and **C**) or with the indicated doses of Dnp–HSA for 5 min (**B** and **D**). Histamine released into the medium and histamine content in cell pellets was measured by ELISA (**A** and **B**). β-Hexosaminidase (β-HEX) activity (**C** and **D**) in the supernatants and in cell pellets was quantified using a spectrophotometer (405 nm), and release was calculated using the formula $S/(S+P) \times 100$, where S and P are the β-hexosaminidase activities of equal portions of each supernatant and cell pellet respectively. Values are means ± S.D. ($n = 3$); $^*P < 0.01$, $^{**}P < 0.05$ compared with wild-type cells.

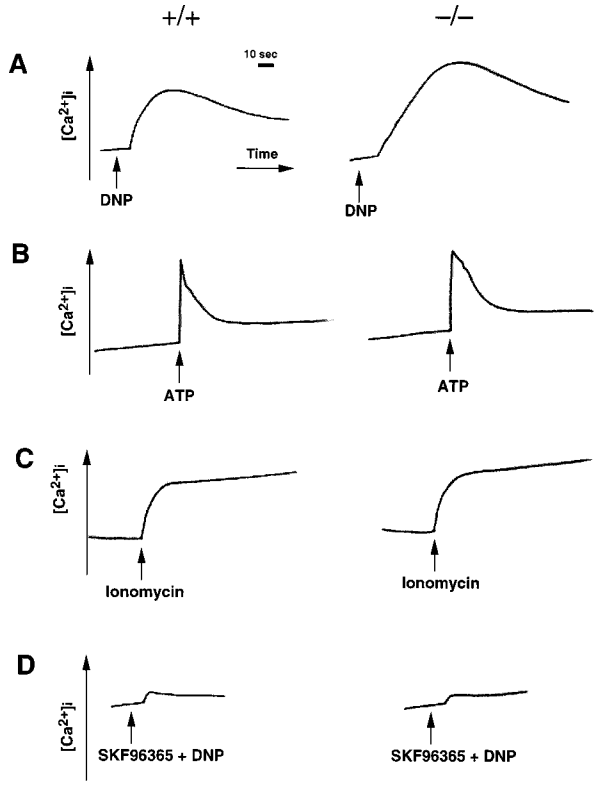

Figure 6 Measurement of $[Ca^{2+}]_i$ in $cPLA_2^{+/+}$ and $cPLA_2^{-/-}$ BMMC

BMMC sensitized with anti-Dnp IgE for 30 min were loaded with fura 2/AM for 1 h. The cells were resuspended in Hepes/Tyrode's buffer and stimulated with 50 ng/ml Dnp–HSA (**A**), 10 μM ATP (**B**) or 1 μM ionomycin (**C**). In addition, fura 2/AM-loaded cells were incubated with 50 μM SKF96365 at 37 °C for 2 min, and stimulated with 50 ng/ml Dnp–HSA (**D**). The arrows indicate the times when the stimulants were added.

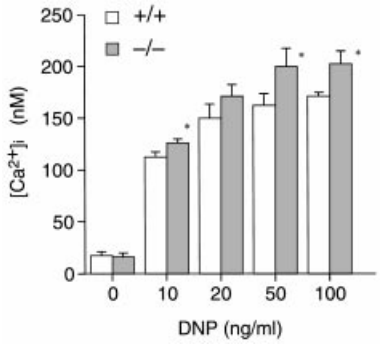

Figure 7 FcεRI-induced increases in $[Ca^{2+}]_i$ in $cPLA_2^{+/+}$ and $cPLA_2^{-/-}$ BMMC

BMMC were sensitized with anti-Dnp IgE for 30 min. The cells were loaded with fura 2/AM, and stimulated with the indicated concentrations of Dnp–HSA. The fluorescence ratio of the cell suspension was monitored with a CAF-100, with excitation at 340 nm and 380 nm, and emission at 500 nm. Values are means ± S.D. ($n = 3$); $^*P < 0.05$ compared with wild-type cells.

dilatation. $cPLA_2^{+/+}$ cells released histamine rapidly, reaching a maximum 2 min after stimulation with the antigen (Figure 4A). The amount of histamine secreted from $cPLA_2^{-/-}$ cells at 2 min was approx. 50% of that observed with $cPLA_2^{+/+}$ cells (Figure 4A). Histamine release from $cPLA_2^{-/-}$ cells was decreased significantly at all doses of Dnp–HSA tested (Figure 4B). The total histamine contents in cultures were 2602 ± 189 ng/10^6 cells ($cPLA_2^{+/+}$) and 1513 ± 60 ng/10^6 cells ($cPLA_2^{-/-}$) (means ± S.D., $n = 3$, $P < 0.01$).

The percentage release of histamine and β-hexosaminidase is augmented in BMMC from $cPLA_2^{-/-}$ mice

The percentage histamine release from $cPLA_2^{-/-}$ cells was found to be higher than that from $cPLA_2^{+/+}$ cells (Figures 5A and 5B). Similar results were obtained for 5-hydroxytryptamine, another important preformed mediator (results not shown). To confirm the enhancement of degranulation in $cPLA_2^{-/-}$ cells, we measured β-hexosaminidase, a mast cell lysosomal enzyme. As with histamine and 5-hydroxytryptamine, the percentage of β-hexosaminidase released from $cPLA_2^{-/-}$ cells was higher than that from $cPLA_2^{+/+}$ cells (Figures 5C and 5D). In contrast with histamine, however, the total content of β-hexosaminidase in the resting cells did not vary with genotype (results not shown).

The antigen-dependent increase in $[Ca^{2+}]_i$ is greater in BMMC from $cPLA_2^{-/-}$ mice

To analyse the mechanism behind the increased degranulation, IgE-dependent calcium signalling was determined. Both cell types responded to the antigen with a delay of approx. 10 s, but the time taken to reach the maximum $[Ca^{2+}]_i$ was longer, and the peak value of $[Ca^{2+}]_i$ was higher, in $cPLA_2^{-/-}$ cells compared with $cPLA_2^{+/+}$ cells (Figures 6A and 7). On stimulation with ATP via G-protein-coupled receptors, $[Ca^{2+}]_i$ was increased transiently to an equal extent in the two cell types (Figure 6B). Ionomycin, a calcium ionophore, elevated $[Ca^{2+}]_i$ to an equivalent extent in both cell types, with a similar time course (Figure 6C). Addition of SKF96365, a calcium ion channel blocker, dramatically diminished the increase in $[Ca^{2+}]_i$ to the basal level (Figure 6D), suggesting that the antigen-induced increase in $[Ca^{2+}]_i$ is mostly due to the influx of extracellular calcium.

DISCUSSION

BMMC from $cPLA_2^{-/-}$ mice were successfully cultured to obtain populations of mast cells, as seen on staining with Alcian Blue/safranin (Figure 1A). Electron microscopy showed that cells from $cPLA_2^{-/-}$ mice were morphologically indistinguishable from those from $cPLA_2^{+/+}$ mice (Figure 1B). The two cell types expressed FcεRI and c-Kit on the cell surface to equal extents (Figure 2). MMCP-1 and MMCP-5 were expressed similarly in both cell types (results not shown). These findings indicate that $cPLA_2$ is not essential for the differentiation of mast cells *in vitro*, and that the events following the cross-linking of receptors on the membrane seem to occur equivalently in $cPLA_2^{+/+}$ and $cPLA_2^{-/-}$ cells (except for histamine content, as discussed below).

Mast cells contain at least three different types of PLA_2, i.e. $cPLA_2$, secretory PLA_2 ($sPLA_2$) (group IIA and group V) and calcium-independent PLA_2 [13,15,26]. BMMC of both genotypes used in the present study do not express group IIA PLA_2, because they are derived from F2 offspring of 129/Ola and C57BL/6J mice [27,28]. Bingham et al. [29] showed that C57BL/6J-derived BMMC produce considerable amounts of PGD_2 during delayed-phase responses, as seen with BALB/cJ-derived BMMC which have normal alleles for group IIA PLA_2.

On the other hand, the production of PGD_2 in 129/J-derived BMMC was minimal during the delayed-phase reaction. Since the cells used here were from mice of the F2 generation, which show the greatest variation compared with their grandparents, we did not investigate delayed-phase responses using these mice. Since immediate-phase responses are less influenced by genetic background, the focus of the present study was immediate-phase responses. When BMMC were stimulated with an antigen, release of AA into the medium was suppressed to background levels in $cPLA_2^{-/-}$ cells (Figure 3A). Production of cysteinyl-leukotrienes and PGD_2 was observed in $cPLA_2^{+/+}$ cells, but not in $cPLA_2^{-/-}$ cells (Figures 3B and 3C). Thus $cPLA_2$ supplies AA to cyclooxygenases and lipoxygenases in the immediate response of mast cell activation. While previous reports using inhibitors and antibodies have suggested a role for $sPLA_2$ in the immediate-phase production of eicosanoids in mouse MMC-34 mastcytoma cells [14], our present results suggest a dominant role for $cPLA_2$ in these responses. This difference might be due to the use of different types of mast cell (mastcytoma cells compared with BMMC), a different experimental protocol (inhibitors/antisense compared with gene targeting), or both. Fujishima et al. [30] also suggested an important role for $cPLA_2$ in immediate-phase eicosanoid production. With regard to PAF, its production was only partially decreased in $cPLA_2^{-/-}$ cells (Figure 3D), suggesting that PAF is also synthesized via $cPLA_2$-independent pathways, such as *de novo* synthesis or CoA-independent transacylation [31].

The histamine content and absolute amount of histamine released were decreased in $cPLA_2^{-/-}$ cells (Figure 4). To elucidate the mechanisms responsible for the decreased histamine content, we first measured the mRNA levels of L-histidine decarboxylase (HDC), which converts histidine into histamine. HDC transcripts are expressed equally in both genotypes of cells (N. Nakatani, unpublished work). As shown by metabolic labelling experiments using [^{35}S]methionine, the amount of HDC protein appears to be lower in $cPLA_2^{-/-}$ cells (N. Nakatani, unpublished work). HDC protein is synthesized as an inactive immature form in mast cells, is converted into a mature form by proteolytic processing, and is transported to vesicular compartments [32]. Although the molecular mechanism remains elusive, the $cPLA_2$ product may change the stability of the protein and/or the translational regulation of HDC. Taken together with the decreased amounts of lipid mediators, these data support the proposal that changes in the properties of mast cells are the cause of the reduced reactions of anaphylaxis that we described previously in $cPLA_2^{-/-}$ mice [7]. A recent study has shown that PGD_2 is a mediator of allergic responses [33].

Unexpectedly, the percentage release of histamine was greater with $cPLA_2^{-/-}$ cells than with $cPLA_2^{+/+}$ cells (Figures 5A and 5B). Similarly, the release of β-hexosaminidase was increased in $cPLA_2^{-/-}$ cells (Figures 5C and 5D). In contrast with histamine, since the total cellular content of β-hexosaminidase was equivalent in two cell types, the absolute amount of enzyme released was increased in $cPLA_2^{-/-}$ cells. In an attempt to suppress the enhanced degranulation, we added a series of eicosanoids, or various lysophospholipids which may be produced by $cPLA_2$ in activated mast cells, to the culture medium, but none of these lipids suppressed the enhanced degranulation in $cPLA_2^{-/-}$ cells (results not shown). Degranulation is the consequence of two steps, i.e. tyrosine phosphorylation and calcium mobilization, in which a number of molecules are involved. FcεRI consists of one α-, one β- and two γ-subunits, and the β- and γ-subunits have an immunoreceptor tyrosine-based activation motif (ITAM) in their C-termini, which is phosphorylated and activated by Src-family kinases. The aggregation of FcεRI leads to the phosphorylation and/or activation of several protein tyrosine kinases (Lyn, Syk, Btk, etc.) [18,34–39]. Several investigators have suggested that the release of AA from a mast cell line is due to the activation of $cPLA_2$ by mitogen-activated protein kinase, while the release of β-hexosaminidase is regulated primarily by Ca^{2+} and protein kinase C [18,40]. The present study clearly shows that the two pathways are not completely independent, and that $cPLA_2$ or its products appear to be involved in the regulation of the exocytotic pathway by as yet unknown mechanisms. As shown in Figure 6(A), cells of both genotypes responded to Dnp–HSA with an elevation of $[Ca^{2+}]_i$. The increase in $[Ca^{2+}]_i$ was mostly due to calcium influx (Figure 6D). The $[Ca^{2+}]_i$ response was greater and more prolonged in $cPLA_2^{-/-}$ cells, which appears to parallel the increase in degranulation. Although the mechanisms behind prolonged $[Ca^{2+}]_i$ responses remain unclear, a similar pattern was observed in BMMC from SH2-containing inositol 5′-phosphatase (SHIP)-deficient mice [41,42], in which the elevation in $[Ca^{2+}]_i$ lasted much longer than normal. Further studies on signalling molecules between FcεRI and calcium influx would clarify the target of $cPLA_2$ products. In addition, there is a large body of evidence implicating protein kinase C, Rab-family kinases, SNAP (soluble *N*-ethylmaleimide-sensitive factor attachment protein) and phospholipase D as regulators of mast cell degranulation [43,44]. Either a $cPLA_2$ product or $cPLA_2$ itself might interact with and regulate the functions of these molecules. Our results, therefore, indicate a novel link between two different mast cell functions, i.e. degranulation and the production of lipid mediators. Enhanced degranulation of β-hexosaminidase in $cPLA_2^{-/-}$ BMMC was also reported by Fujishima et al. [30], although the mechanism was not described in detail.

In conclusion, BMMC from $cPLA_2^{-/-}$ mice showed decreases in AA release and in the production of eicosanoids and PAF. The cellular histamine content and, therefore, the amount of histamine released after stimulation with the antigen were decreased. Thus the reduced anaphylactic symptoms in these mice are due, at least in part, to this decreased mast cell function. In addition, our study suggests that the $cPLA_2$ pathway is also involved in degranulation. Further studies are required in order to demonstrate how $cPLA_2$ regulates IgE-dependent calcium influx and degranulation.

We are grateful to Dr C. Ra and Dr H. Matsuda (Juntendo University, Tokyo, Japan) for FITC-labelled IgE and anti-c-Kit, Dr T. Takahashi (Kanto Teishin Hospital, Tokyo, Japan) for electron microscopy, and Mrs K. Tada and M. Hisada (Showa University) for assistance in the culture of BMMC. We thank Dr S. Tanaka and Dr A. Ichikawa (Kyoto University, Kyoto, Japan), Dr H. Ohtsu and Dr T. Watanabe (Tohoku University, Miyagi, Japan), and Dr S. Ishii and Dr Y. Kita (The University of Tokyo) for discussions, and Mr Y. Suzuki and Miss K. Ishihara (The University of Tokyo) for technical assistance. We thank Dr D. Wong (The University of Tokyo) for critical reading of the manuscript. This work was supported by CREST of JST, Japan, a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan.

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Received 15 May 2000/7 August 2000; accepted 13 September 2000