Detection of secretory phospholipase A_2s related but not identical to type IIA isozyme in cultured mast cells

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Abstract We previously reported that BALB/cJ mouse-derived bone marrow-derived mast cells (BMMC) exhibited two sequential phases of prostaglandin D_2 (PGD_2) generation in response to Fc_ε receptor I ($Fc_\varepsilon RI$) crosslinking and cytokine stimulation, the late phase of which was suppressed by an antibody raised against type IIA secretory phospholipase A_2 ($sPLA_2$). Here we report that BMMC derived from C57BL/6J mice, which are genetically deficient in type IIA $sPLA_2$, display both immediate and delayed PGD_2 generation normally. Lysates of C57BL/6J-derived BMMC contained a Ca^{2+}-dependent PLA_2 that was absorbed to a column conjugated with anti-type IIA $sPLA_2$ antibody and had a similar molecular mass of 14 kDa, as assessed by immunoblotting. Therefore we speculate that a $sPLA_2$ similar to, but distinct from, type IIA $sPLA_2$ would compensate for type IIA $sPLA_2$ deficiency in C57BL/6J-derived BMMC. We found that the two type IIA-related $sPLA_2$ family members, type V and type IIC $sPLA_2$s, were expressed in BMMC as well as in rat mastocytoma RBL-2H3 cells.

Key words: Phospholipase A_2; Mast cell; Prostaglandin D_2; Cytokine (mouse)

1. Introduction

We have previously reported that BALB/cJ mouse-derived bone marrow-derived mast cells (BMMC) stimulated by $Fc_\varepsilon RI$ crosslinking via IgE and antigen in the presence of specific accessory cytokines show two sequential prostaglandin D_2 (PGD_2) biosynthetic responses over time [1–6]. Immediate PGD_2 generation, occurring within a few minutes in response to a transient increase in cytoplasmic Ca^{2+} concentration, is associated with transient perinuclear translocation and phosphorylation of cytosolic phospholipase A_2 ($cPLA_2$) [3,7,8]. The constitutively expressed cyclooxygenase (COX) isoform, COX-1, is the dominant enzyme involved in this rapid response, which converts arachidonic acid to PGH_2, which in turn is metabolized to PGD_2 via glutathione-dependent PGD_2 synthase [1,2,4,5]. Delayed PGD_2 generation, occurring over several hours of culture, is associated with the de novo induction and function of COX-2 [1,4,5]. The segregated processing of arachidonic acid implies a distinct supply or different intracellular conditions for the metabolism of arachidonic acid by the alternative COX isoforms. Based upon observations that delayed PGD_2 generation is accompanied by the induction of type IIA secretory PLA_2 ($sPLA_2$) transcript and is suppressed efficiently by an antibody against this enzyme, we speculated that type IIA $sPLA_2$ is functionally linked to COX-2-dependent delayed PGD_2 generation [6,9]. Moreover, earlier findings that histamine release from activated mast cells was sensitive to several inhibitors directed against type IIA $sPLA_2$ led us to suggest that type IIA $sPLA_2$ is also involved in exocytosis [10].

However, the recent finding that several inbred mouse strains with natural disruption of the type IIA $sPLA_2$ gene do not exhibit any apparent abnormality [11,12] has raised confusion about the role played by type IIA $sPLA_2$ in mast cell activation. Here we show that BMMC derived from the C57BL/6J strain, possessing a disrupted type IIA $sPLA_2$ gene, have the ability to elicit immediate and delayed PGD_2 generation as well as exocytosis to a level comparable with that of BMMC derived from BALB/cJ strain, which expresses normal type IIA $sPLA_2$. Importantly, lysates of C57BL/6J-derived BMMC contained $sPLA_2$ immunoreactive with the anti-type IIA $sPLA_2$ antibody, suggesting that $sPLA_2$ related, but not identical, to type IIA $sPLA_2$ would compensate for the function of type IIA $sPLA_2$ in mast cells. RT-PCR analysis have revealed that cultured mast cells also express type V and IIC $sPLA_2$s.

2. Materials and methods

2.1. Materials

Recombinant mouse interleukin (IL)-1β was purchased from Genzyme. Recombinant mouse c-*kit* ligand (KL), IL-10 and type IIA $sPLA_2$ were obtained by expression in a baculovirus system as described previously [1]. A rabbit polyclonal antibody against mouse type II $sPLA_2$ was prepared as described previously [6]. A rabbit polyclonal antibody against mouse COX-2 was purchased from Cayman Chemical. Monoclonal IgE anti-trinitrophenyl (TNP) and TNP-conjugated bovine serum albumin as an antigen were provided by Dr. J.P. Arm and H. Katz (Harvard Medical School, Boston, MA). Aspirin was purchased from Sigma.

2.2. Preparation and activation of BMMC

BMMC were obtained from either BALB/cJ or C57BL/6J mouse bone marrow cells by culturing with 50% (v/v) WEHI-conditioned medium as a source of IL-3, as described previously [1]. Cells (10^7 cells/ml) were sensitized with 10 μg/ml IgE anti-TNP for 30 min at 37°C, washed, and suspended in enriched medium [6] containing 100 units/ml IL-10, 5 ng/ml IL-1β, 100 ng/ml KL and 20 ng/ml antigen at 37°C. After various incubation periods, the supernatants were assayed for PGD_2 and PGE_2 using a PGD_2 radioimmunoassay kit (Amersham) and a PGE_2 enzyme immunoassay kit (Cayman), respectively, and for β-hexosaminidase as a marker of exocytosis [2]. The cells were analyzed for protein expression. To assess only the delayed response, BMMC were pretreated with 10 μg/ml aspirin for 2 h to inactivate COX-1, which is essential for immediate PGD_2 generation, washed, and then activated as described previously [4].

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Abbreviations: BMMC, bone marrow-derived mast cells; $cPLA_2$, cytosolic phospholipase A_2; $sPLA_2$, secretory phospholipase A_2; COX, cyclooxygenase; $Fc_\varepsilon RI$, Fc_ε receptor I; PGD_2, prostaglandin D_2; IL, interleukin; KL, c-*kit* ligand; TNP, trinitrophenyl

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2.3. Immunoaffinity column

BMMC were suspended in 10 mM Tris-HCl (pH 7.4) containing 1 M NaCl and 1 mM EDTA at 1×10^7 cells/ml, disrupted by sonication and centrifuged for 1 h at $100\,000\times g$ at 4°C. The pooled supernatants were applied to a formyl cellulofine (Seikagaku Kogyo) column that had been conjugated with the anti-mouse type IIA $sPLA_2$ antibody, as described previously [6]. The bound $sPLA_2$ was eluted with 50 mM glycine-HCl buffer (pH 2.2).

2.4. SDS-PAGE/Immunoblotting

Samples were separated by SDS-PAGE, electroblotted onto nitrocellulose membranes (Schleicher and Schuell) using a semi-dry blotter (MilliBlot-SDE system; Millipore), probed with antibodies against type IIA $sPLA_2$ or COX-2, and visualized using an enhanced chemiluminescence Western blot analysis system (Amersham), as described previously [1,13].

2.5. $sPLA_2$ assay

The standard reaction mixture consisted of 100 mM Tris-HCl, pH 9.0, 4 mM $CaCl_2$, 2 μM 1-palmitoyl-2-[^{14}C]linoleoyl-phosphatidylethanolamine (Amersham) and an aliquot of the samples. After incubation for 30 min at 37°C, the released linoleic acid was extracted as described [13] and counted by a β-scintillation counter.

2.6. Recombinant type IIC $sPLA_2$

The cDNA for type IIC $sPLA_2$, obtained by RT-PCR from testis as described previously [6,14], was subcloned into pVL1393 (Pharmingen) and transfected into Sf9 cells (Invitrogen) using a baculovirus system [1]. One week after the third transfection, the supernatants were collected and used as recombinant type IIC $sPLA_2$.

2.7. RT-PCR

Specific primers for PCR for three $sPLA_2$s were synthesized (Greiner Japan). Type IIA $sPLA_2$ primers used were 5′-ATG AAG GTC CTC CTC CTG CTA G-3′ and 5′-TCA GCA TTT GGG CTT CTT CC-3′ [13], type IIC $sPLA_2$ primers were 5′-ATG GAC CTC CTG GTC TCC TCA GG-3′ and 5′-CTA GCA ATG AGT TTG TCC CTG C-3′ [6], and type V $sPLA_2$ primers were 5′-CAG GGG GCT TGC TAG AAC TCA A-3′ and 5′-AAG AGG GTT GTA AGT CCA GAG G-3′ [15]. The RT-PCR was carried out using a RNA PCR kit (AMV) version-2 (TaKaRa), according to the manufacturer's instructions, using 1 μg of total RNA from BMMC or rat mastocytoma RBL-2H3 as a template. Equal amounts of each RT product were PCR-amplified with *ex Taq* polymerase (TaKaRa) for 30 cycles consisting of 30 s at 94°C, 30 s at 55°C and 30 s at 72°C. The amplified cDNA fragments were resolved on 1.5% (w/v) agarose gels and visualyzed by ethidium bromide.

3. Results

3.1. Mediator release from C57BL/6J-derived BMMC

To assess the immediate response, BMMC derived from C57BL/6J and BALB/cJ mice (type IIA sPLA2 (−) and (+), respectively) were each sensitized with IgE and activated with antigen for 10 min. C57BL/6J- and BALB/cJ-derived BMMC generated 4.5 ± 1.5 and 3.1 ± 0.7 ng of PGD2 per 10^6 cells and released 33 ± 8% and 28 ± 5% of β-hexosaminidase, respectively ($n=2$, mean ± S.D.).

To assess the delayed response, these BMMC were sensitized with IgE for 2 h in the presence of aspirin to irreversibly inactivate preexisting COX-1 [4], and then activated with antigen in the presence of KL, IL-10 and IL-1β, thus eliciting maximal delayed PGD_2 generation without accompanying immediate PGD_2 generation. As shown in Fig. 1, BMMC from both strains produced PGD_2 over 2–10 h, reaching a maximal level of ~10 ng/10^6 cells at 10 h, in parallel with the induction of COX-2 expression. PGE_2 was produced only in minimal amounts by both strains. Thus, despite the lack of type IIA $sPLA_2$, C57BL/6J-derived BMMC were capable of both immediate and delayed PGD_2 generation and exocytosis to a level comparable with BALB/cJ-derived BMMC, which express type IIA $sPLA_2$ normally.

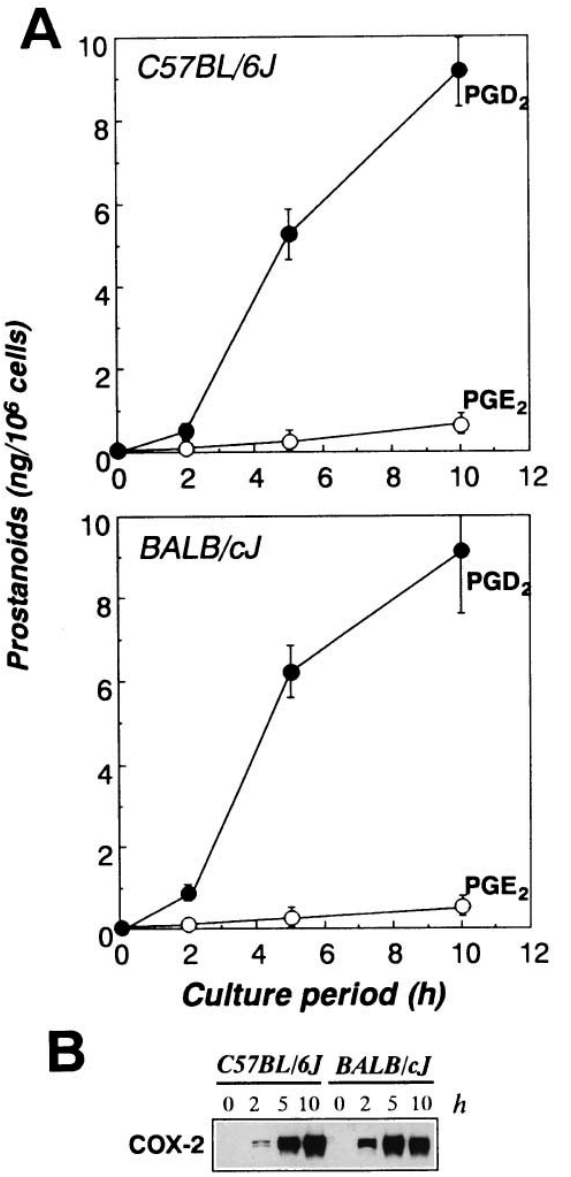


Fig. 1. Delayed PGD_2 and PGE_2 generation and COX-2 protein expression by BMMC derived from C57BL/6J (left) and BALB/cJ (right). BMMC (1×10^6 cells/ml) sensitized with IgE in the presence of aspirin were washed and then activated for the indicated periods with antigen in combination with the cytokine triad, KL, IL-10 and IL-1β. Supernatants and cells were taken for PGD_2 (closed symbols) and PGE_2 (open symbols) assays (mean ± S.D.; $n=2$) (A) and immunoblotting for COX-2 (B), respectively.

3.2. Detection of immunoreactive $sPLA_2$ in C57BL/6J-derived BMMC

In order to search for the $sPLA_2$-like activity in BMMC, $100\,000\times g$ supernatants of cell lysates were prepared from both strains and applied to an immunoaffinity column conjugated with anti-mouse type IIA $sPLA_2$ antibody. Although the PLA_2 activity hydrolyzing 2-linoleoyl-phosphatidylethanolamine was undetectable in the flow-through fractions (data not shown), significant PLA_2 activity, which was comparable to that in BALB/cJ-derived BMMC, was recovered

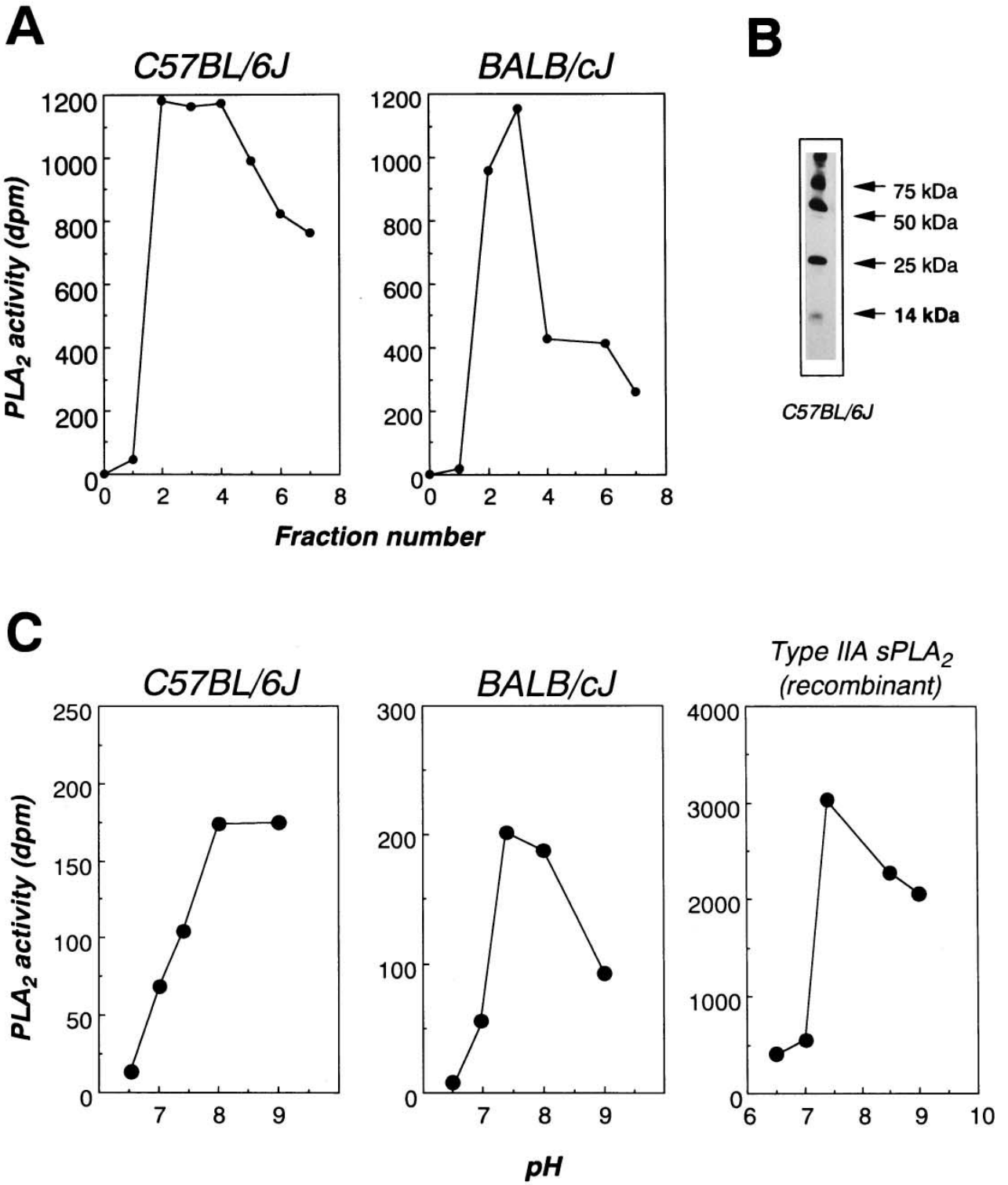
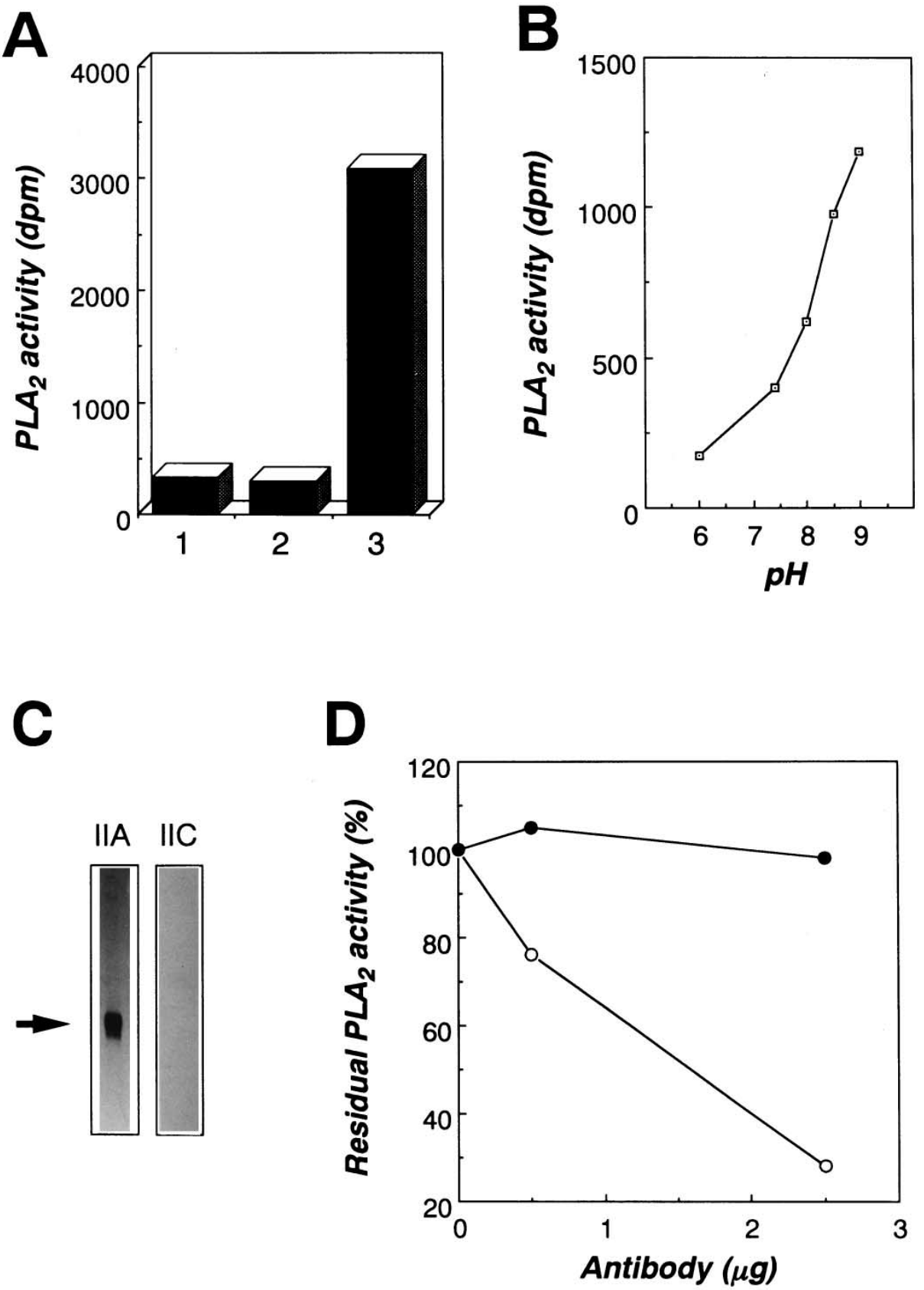


Fig. 2. Characterization of immunoreactive $sPLA_2$s in BALB/cJ- and C57BL/6J-derived BMMC. (A) Immunoaffinity column chromatography. The $100\,000 \times g$ supernatants of lysates of C57BL/6J (left) or BALB/cJ (right) BMMC (5×10^7 cell equivalents) were applied to an anti-type IIA $sPLA_2$ antibody-conjugated cellulofine column, and $sPLA_2$ activity bound to the column was eluted with 50 mM glycine-HCl buffer, pH 2.2. PLA_2 activity after elution with glycine-HCl buffer is shown. (B) Immunoblotting. Fraction 13 in panel (A) of C57BL/6J BMMC was neutralized and incubated with anti-type IIA $sPLA_2$ antibody-conjugated beads for 1 h at room temperature. The immunoprecipitate was subjected to SDS-PAGE/immunoblotting using anti-type IIA $sPLA_2$ antibody. Visualized bands with molecular masses of approximately 25, 50, and 75 kDa correspond to IgG light chain, heavy chain and their complex, respectively. (C) pH dependence of immunoreactive $sPLA_2$ activities. $sPLA_2$ activities of C57BL/6J and BALB/cJ BMMC [fraction 12 in panel (A)] were assayed under different pH conditions. A representative result of three independent experiments is shown.



from C57BL/6J-derived BMMC after elution of the bound proteins with an acidic buffer (Fig. 2A). The PLA_2 immunoreactive with the anti-type IIA $sPLA_2$ antibody in C57BL/6J-derived BMMC (Fig. 2B) showed a molecular mass of approximately 14 kDa as visualized by immunoblotting. Both immunoreactive PLA_2 activities in BALB/cJ- and C57BL/6J-

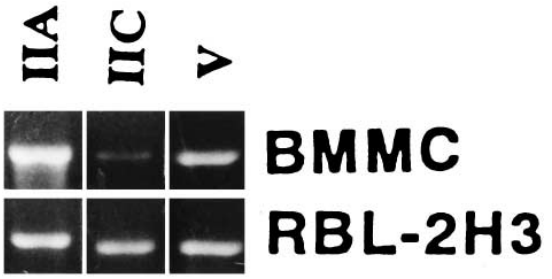


Fig. 4. Detection of transcripts for various $sPLA_2$s in cultured mast cells by RT-PCR. Expression of type IIA, IIC and V $sPLA_2$s in BALB/cJ-derived BMMC and RBL-2H3 cells were detected after 30 cycles of RT-PCR amplification. The fragment sizes (~430 bp) were consistent with the predicted sizes of each $sPLA_2$ cDNA. Hybridization with the specific cDNA probes confirmed that these bands indeed corresponded to the respective $sPLA_2$s (data not shown).

derived BMMC required millimolar order Ca^{2+} for enzymatic activity (data not shown). Thus, the ability to hydrolyze 2-linoleoyl-phosphatidylethanolamine, immunochemical property, molecular mass and mM Ca^{2+} requirement all suggest that the PLA_2 detected in C57BL/6J-derived BMMC is $sPLA_2$-like enzyme. The pH profiles of the $sPLA_2$ activities in both strains, in comparison to that of recombinant mouse type IIA $sPLA_2$, is shown in Fig. 2C. $sPLA_2$ detected in BALB/cJ-derived BMMC, as well as recombinant mouse type IIA $sPLA_2$, showed optimal activity at pH 7.4, whereas that in C57BL/6J-derived BMMC had an optimal pH of 8–9. This difference, together with the genetic evidence [11,12], implies that the immunoreactive $sPLA_2$ detected in C57BL/6J-derived BMMC is distinct from type IIA $sPLA_2$.

3.3. Comparison with type IIC $sPLA_2$

We have recently reported that BALB/cJ-derived BMMC express the type IIC $sPLA_2$ transcript, as assessed by RT-PCR [6]. In order to clarify whether the immunoreactive $sPLA_2$ detected in C57BL/6J-derived BMMC was identical to the type IIC enzyme, we prepared recombinant type IIC $sPLA_2$ using a baculovirus system (Fig. 3A) and compared its properties with those of C57BL/6J-derived BMMC $sPLA_2$. The activity of the baculovirus-expressed type IIC $sPLA_2$ was Ca^{2+}-dependent, requiring millimolar order Ca^{2+} (data not shown), and was optimal at pH 9 (Fig. 3B). The specific activity of this enzyme was about one eighth that of recombinant type IIA $sPLA_2$ (data not shown). However, the anti-type IIA $sPLA_2$ antibody did not crossreact with recombinant type IIC PLA_2, as demonstrated by immunoblotting (Fig. 3C) and immunoabsorption (Fig. 3D). Therefore most $sPLA_2$ detected in C57BL/6J-derived BMMC may differ from type IIC $sPLA_2$.

3.4. Detection of type V $sPLA_2$ in cultured mast cells

To assess the expression of type IIA-like $sPLA_2$s in BMMC, RT-PCR was carried out using specific primers for type IIA, IIC or V $sPLA_2$s. After 30 cycles of amplification, the expression of type IIA, IIC and V $sPLA_2$s was clearly detected in BALB/cJ-derived BMMC (Fig. 4). Similarly, type IIA, IIC and V $sPLA_2$s were detected in rat mastocytoma RBL-2H3 cells (Fig. 4), indicating that mast cells generally contain more than one $sPLA_2$. While this experiment was in progress, the expression of type V, but not type IIC, $sPLA_2$ has been shown in C57BL/6J-derived BMMC [16]. We also observed the similar expression pattern of these $sPLA_2$s in C57BL/6J-derived BMMC (data not shown). Thus, the expression of each $sPLA_2$ appears to differ among mast cells obtained from different sources.

4. Discussion

We have previously reported the cloning of mouse type IIA $sPLA_2$ from cytokine-stimulated BALB/cJ BMMC and its possible participation in COX-2-dependent delayed PGD_2 generation on the basis of its marked suppression by an antibody against recombinant type IIA $sPLA_2$ [6]. However, our present finding that type IIA $sPLA_2$-deficient C57BL/6J-derived BMMC displayed PGD_2 generation to an extent comparable with BALB/cJ-derived BMMC argues against the role of type IIA $sPLA_2$ in mast cell activation. Bingham et al. [9] have also recently observed a similar phenomenon, and concluded that a $sPLA_2$ distinct from type IIA $sPLA_2$ may be responsible for delayed PGD_2 generation.

Detailed analysis of the PLA_2 activity in BMMC lysates revealed that C57BL/6J-derived, type IIA $sPLA_2$-deficient BMMC contained a $sPLA_2$ immunochemically close to type IIA $sPLA_2$ (Fig. 2A and B). The level of this enzyme activity was comparable with that of immunoreactive $sPLA_2$ activity detected in BALB/cJ-derived BMMC (Fig. 2A), consistent with the fact that BMMC derived from both strains exhibited PGD_2 generation to a similar extent (Fig. 1). Although $sPLA_2$s in C57BL/6J- and BALB/cJ-derived BMMC were immunochemically indistinguishable, they differed significantly in their pH-dependence; the former preferred alkaline pH and the latter was most active at neutral pH (Fig. 2C). Neutral pH preference was also observed in recombinant type IIA $sPLA_2$, consistent with its expression in BALB/cJ-derived BMMC. We previously reported that rat mastocytoma RBL-2H3 cells expressed at least three PLA_2 enzymes, including $cPLA_2$, type IIA $sPLA_2$ and an unknown PLA_2 with chromatographic behaviors similar to type IIA $sPLA_2$ [17]. Fonteh et al. [18] also demonstrated the presence of two type IIA $sPLA_2$-like enzymes in BMMC derived from CBA/J mice. Collectively, these results suggest that C57BL/6J-derived BMMC express the $sPLA_2$-like enzyme, which can substitute for type IIA $sPLA_2$ to regulate mast cell function, thereby reflecting the redundancy of $sPLA_2$ family members. Alternatively, the $sPLA_2$-like enzyme, rather than type IIA

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Fig. 3. Characteristics of recombinant type IIC $sPLA_2$ expressed by Sf9 cells. (A) Release of type IIC $sPLA_2$ activity from baculovirus-infected Sf9 cells. Columns 1, 2, and 3 correspond to cells without transfection, cells transfected with control vector, and cells transfected with type IIC $sPLA_2$, respectively. The supernatants were collected and assayed for PLA_2 activity. (B) pH profile of type IIC $sPLA_2$. (C) Supernatants of Sf9 cells expressing type IIA (lane 1) and type IIC (lane 2) $sPLA_2$s were immunoblotted with the anti-type IIA $sPLA_2$ antibody. (D) Supernatants of Sf9 cells expressing type IIA (open circles) and type IIC (closed circles) $sPLA_2$s were incubated with the indicated amounts of anti-type IIA $sPLA_2$ antibody for 1 h. After precipitation with protein-A Sepharose (Pharmacia), PLA_2 activities remaining in the supernatants were measured. A representative result of three independent experiments is shown.

$sPLA_2$, may be a true effector of arachidonate metabolism even in BMMC expressing type IIA $sPLA_2$.

Type IIA, IIC and V $sPLA_2$s are closely related to one another and map to the same chromosome locus [14,19], implying their functional redundancy. In view of our previous finding that BALB/cJ-derived BMMC express mRNA for type IIC $sPLA_2$ [6] (Fig. 4), we examined the identity of C57BL/6J-derived BMMC $sPLA_2$ with the recombinant type IIC enzyme. Although type IIC $sPLA_2$ showed Ca^{2+} and pH dependence similar to that of C57BL/6J-derived BMMC $sPLA_2$, no crossreactivity of the anti-type IIA $sPLA_2$ antibody with type IIC $sPLA_2$ was observed (Fig. 3), indicating that C57BL/6J-derived BMMC $sPLA_2$ may be distinct from type IIC $sPLA_2$. This conclusion is further supported by the observation that type IIC $sPLA_2$ transcript was detectable only minimally in C57BL/6J-derived BMMC (data not shown) [16] and its expression in BALB/cJ-derived BMMC was not correlated with PGD_2 generation [6].

We have found that type V $sPLA_2$ was expressed in BMMC (Fig. 4). During the course of this study, Reddy et al. [16] have also reported that type V $sPLA_2$ is expressed in various mouse mast cells, including those derived from type IIA $sPLA_2$-deficient C57BL/6J mice, and is crucial for the regulation of arachidonate metabolism. Although the contribution of type V $sPLA_2$ was confined to immediate, but not delayed, PGD_2 generation in the mast cell lines they used [16], it is at present unclear whether the delayed PGD_2 generation observed in primary BMMC, which Bingham et al. [9] and our group have studied, also depends on type V $sPLA_2$. Of note, whereas Reddy et al. [16] failed to detect the transcripts for type IIA and IIC $sPLA_2$s in their mast cells, these transcripts were readily detectable in our BALB/cJ-derived BMMC (Fig. 4). Moreover, these three $sPLA_2$s were also detected in rat mastocytoma RBL-2H3 (Fig. 4). The distinct profiles of $sPLA_2$ expression might reflect the heterogeneity of mast cells, which is highly affected by microenvironments during culture. Indeed, type IIA, but not IIC, $sPLA_2$ expression was greatly induced in BALB/cJ-derived BMMC when cultured with KL in combination with IL-10 and IL-1β [6,9]. Although these observations suggest that $sPLA_2$ detected in C57BL/6J-derived BMMC in this study is identical to type V $sPLA_2$, the final conclusion will have to be awaited until the enzymatic properties of recombinant type V $sPLA_2$, such as immunoreactivity and pH dependence, are compared with the present results. Also, it will be of interest to examine the cytokine regulation of type V $sPLA_2$ expression in BMMC. Nevertheless, type V $sPLA_2$ has also been implicated in PGE_2 generation in $P388D_1$ mouse macrophage-like cells, in which type IIA $sPLA_2$ expression is barely detectable [18], and therefore is emerging as a novel effector involved in arachidonate-mediated signal transduction.

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References

[1] Murakami, M., Matsumoto, R., Austen, K.F. and Arm, J.P. (1994) J. Biol. Chem. 269, 22269–22275.
[2] Murakami, M., Matsumoto, R., Urade, Y., Austen, K.F. and Arm, J.P. (1995) J. Biol. Chem. 270, 3239–3246.
[3] Murakami, M., Austen, K.F. and Arm, J.P. (1995) J. Exp. Med. 182, 197–206.
[4] Murakami, M., Bingham III, C.O., Matsumoto, R., Austen, K.F. and Arm, J.P. (1995) J. Immunol. 155, 4445–4453.
[5] Ashraf, M.M.D., Murakami, M. and Kudo, I. (1996) Biochem. J. 320, 965–973.
[6] Ashraf, M.M.D., Murakami, M., Shimbara, S., Amakasu, Y., Atsumi, G. and Kudo, I. (1996) Biochem. Biophys. Res. Commun. 229, 726–732.
[7] Nakatani, Y., Murakami, M., Kudo, I. and Inoue, K. (1994) J. Immunol. 153, 796–803.
[8] Glover, S., de Carvalho, M.S., Bayburt, T., Jonas, M., Chi, E., Leslie, C.C. and Gelb, M.H. (1995) J. Biol. Chem. 270, 15359–15367.
[9] Bingham III, C.O., Murakami, M., Fujishima, H., Hunt, J.E., Austen, K.F. and Arm, J.P. (1996) J. Biol. Chem. 271, 25936–25944.
[10] Murakami, M., Kudo, I., Suwa, Y. and Inoue, K. (1992) Eur. J. Biochem. 209, 257–265.
[11] MacPhee, M., Chepenik, K.P., Liddell, R., Nelson, K.K., Siracusa, L.D. and Buchberg, A.M. (1995) Cell 81, 957–966.
[12] Kennedy, B.P., Payette, P., Mudgett, J., Vadas, P., Pruzanski, W., Kwan, M., Tang, C., Rancourt, D.E. and Cromlish, W.A. (1995) J. Biol. Chem. 270, 22378–22385.
[13] Murakami, M., Nakatani, Y. and Kudo, I. (1996) J. Biol. Chem. 271, 30041–30051.
[14] Chen, J., Engle, S.J., Seilhamer, J.J. and Tischfield, J.A. (1994) J. Biol. Chem. 269, 23018–23024.
[15] Balboa, M.A., Balsinde, J., Winstead, M.V., Tischfield, J.A. and Dennis, E.A. (1996) J. Biol. Chem. 271, 32381–32384.
[16] Reddy, S.T., Winstead, M.V., Tischfield, J.A. and Herschman, H.R. (1997) J. Biol. Chem. 272, 13591–13596.
[17] Murakami, M., Kudo, I., Umeda, M., Matsuzawa, A., Takeda, M., Komada, M., Fujimori, Y., Takahashi, K. and Inoue, K. (1992) J. Biochem. (Tokyo) 111, 175–181.
[18] Fonteh, A.N., Samet, J.M. and Chilton, F.H. (1995) J. Clin. Invest. 96, 1432–1439.
[19] Tischfield, J.A., Xia, Y.R., Shih, D.M., Klisak, I., Chen, J., Engle, S.J., Siakotos, A.N., Winstead, M.V., Seilhammer, J.J., Allamond, V., Gyapay, G. and Lusis, A. (1996) Genomics 32, 328–333.