

Arachidonate Release and Eicosanoid Generation by Group IIE Phospholipase A₂

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The heparin-binding group II subfamily of secretory phospholipase A₂s (sPLA₂s), such as sPLA₂-IIA and -IID, augments stimulus-induced arachidonic acid (AA) release through the cellular heparan sulfate proteoglycan (HSPG)-dependent pathway when transfected into HEK293 cells. Here we show that the closest homolog, sPLA₂-IIE, also promotes stimulus-induced AA release and prostaglandin (PG) production similar to those elicited by HSPG-dependent sPLA₂s. Confocal laser microscopic analysis demonstrates the location of sPLA₂-IIE in cytoplasmic punctate compartments. sPLA₂-IIE also enhances leukotriene (LT) production and granule exocytosis by RBL-2H3 mastocytoma cells. Expression of sPLA₂-IIE was highly upregulated in mice injected with lipopolysaccharide (LPS) and in mice with experimental atopic dermatitis. These observations suggest that this enzyme plays a role in the inflammatory process, as proposed for other group II subfamily sPLA₂s. © 2002 Elsevier Science (USA)

The sPLA₂ family in mammals comprises 10 isozymes, all of which have a conserved Ca²⁺-binding loop and a catalytic site with a His/Asp catalytic dyad (1, 2). The genes for 6 enzymes (IIA, IIC, IID, IIE, IIF, and V), which are often referred to as group II subfamily sPLA₂s, are mapped to the same chromosomal locus. Accumulating evidence suggests that several sPLA₂s play a crucial role in the regulation of arachi-

Abbreviations used: sPLA₂, secretory phospholipase A₂; AA, arachidonic acid; OA, oleic acid; PG, prostaglandin; LT, leukotriene; COX, cyclooxygenase; mPGES, microsomal PGE₂ synthase; PC, phosphatidylcholine; PE, phosphatidylethanolamine; HSPG, heparan sulfate proteoglycan; LPS, lipopolysaccharide; DNFB, dinitrofluorobenzene; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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donic acid (AA) metabolism in an autocrine, paracrine or juxtacrine manner (3–18).

sPLA₂-IIA is abundantly present at various inflamed sites and is upregulated in various cells and tissues in response to proinflammatory stimuli (9–12). In addition to its potent bactericidal activity (19–21) and potential anti-tumorigenic role (22), this enzyme promotes stimulus-induced release of AA, which is in turn supplied to cyclooxygenase (COX) enzymes for augmented PG generation (3–12). sPLA₂-IIA can elicit AA release from *activated* cells through the HSPG-shuttling pathway (4, 7), in which the enzyme binds to the HSPG glypican and is then sorted into cytoplasmic punctate and perinuclear compartments that are assumed to be rich in anionic or oxidized phospholipids (3–10). Phospholipids in these compartments may also be enriched in AA, since the enzyme often causes AA-selective release (4, 7). Other heparin-binding sPLA₂s such as sPLA₂-IID (8, 23, 24) and -V (4–8), which are also stimulus-inducible (24, 25), also utilize this HSPG-shuttling pathway. In contrast, sPLA₂-X (7, 8, 17, 18), as well as sPLA₂-V in certain situations (8, 13–16), is capable of releasing AA even from *resting* cells by acting on the phosphatidylcholine (PC)-rich external plasma membrane with no dependence on HSPG. Elevated expression of sPLA₂-X in some colorectal tumors implicates this enzyme in the development of cancer (26).

sPLA₂-IIE is a recently identified group II subfamily isozyme that shows the highest homology with sPLA₂-IIA and -IID (27, 28). The *in vitro* enzymatic properties (e.g., substrate specificity, optimal pH, and sPLA₂ inhibitor sensitivity) of human sPLA₂-IIE are reported to be similar to human sPLA₂-IIA and -IID (27, 28). In this study, the AA-releasing and PGE₂-biosynthetic functions of human sPLA₂-IIE were investigated in mammalian cells. The enzyme was found to promote

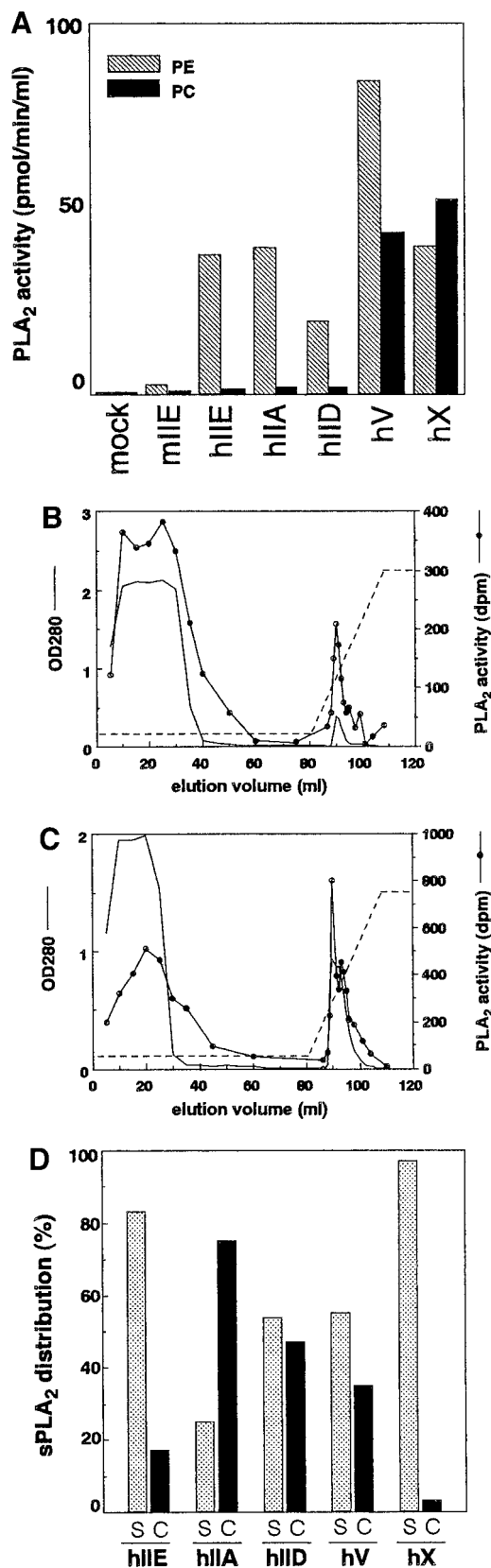


FIG. 1. *In vitro* activity and heparanoid binding capacity of sPLA₂-IIE. (A) *In vitro* enzymatic activities of various human (h) and mouse (m) sPLA₂s. Culture supernatants for HEK293 cells trans-

stimulus-induced AA release and subsequent eicosanoid generation in a manner similar to those of other related heparin-binding sPLA₂ isozymes. Moreover, sPLA₂-IIE was found to be an inducible enzyme in response to proinflammatory stimuli.

MATERIALS AND METHODS

Materials. Establishment, culture and activation of HEK293 (Human Science Research Resources Bank) and RBL-2H3 (Riken Cell Bank) transfectants were performed as described previously (4–8). Construction of the synthetic human sPLA₂-IIE cDNA (28) will be described elsewhere.

Heparin binding. Binding of human sPLA₂-IIE to heparin-Sepharose (Amersham Pharmacia Biotech) was assessed as described previously (3, 4). Briefly, approximately 25 ml of culture supernatants of HEK293 cells transfected with human sPLA₂-IIE were applied to a heparin-Sepharose column (1 × 5 cm) preequilibrated with 10 mM Tris-HCl, pH 7.4, containing 150 mM NaCl (TBS) at a flow rate of 20 ml/h. After extensive washing with TBS, the bound proteins were eluted using 10 mM Tris-HCl, pH 7.4, with a 0.15–1 M NaCl gradient. PLA₂ activity in each fraction was measured as described above. Sulfate-Cellulofine column (Seikagaku Kogyo) was performed in a similar way.

RT-PCR/Southern blotting. Synthesis of cDNA was performed using 1 μg of total RNA from mouse tissues and AMV reverse transcriptase, according to the manufacturer's instructions supplied with the RNA PCR kit (Takara Biomedicals). Subsequent amplification of the cDNA fragments for mouse sPLA₂-IIE was performed using 1 μl of the reverse-transcribed mixture as a template with specific oligonucleotide primers (Greiner) as follows: mIIE-5' primer 5'-ATG AAA CCT CCC ATT GCG CTG-3' and mIIE-3' primer 5'-TCA GCA GGG TGG GGT GGG CCC AG-3'. The PCR condition was 94°C for 30 s and then 35 cycles of amplification at 94°C for 5 s and 68°C for 4 min, using the Advantage cDNA polymerase mix (Clontech). Expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was assessed by 25 cycles of PCR amplification using specific primers (Clontech). The PCR products were analyzed by 1% agarose gel electrophoresis with ethidium bromide. The gels were further subjected to Southern blot hybridization using mouse sPLA₂-IIE cDNA as a probe, as described previously (29).

Confocal laser microscopy. Cells grown on collagen-coated cover glasses (Iwaki Glass) were fixed with 3% paraformaldehyde for 30 min in phosphate-buffered saline (PBS). After three washes with PBS, the fixed cells were sequentially treated with 1% bovine serum albumin (for blocking) and 1% saponin (for permeabilization) in PBS for 1 h, with anti-human or mouse sPLA₂-IIE antibody (1:500 dilution) (30) for 1 h, and then with FITC-goat anti-rabbit IgG (1:500

fectected with various sPLA₂s were taken for the PLA₂ assay using PE (striped) and PC (solid), both of which have [¹⁴C]AA at their *sn*-2 positions, as substrates. (B and C) Heparin-Sepharose (B) and sulfate-Cellulofine (C) column chromatographies. A pooled culture supernatant of human sPLA₂-IIE-expressing HEK293 cells was applied to heparin-Sepharose (B) or sulfate-Cellulofine (C) column, and bound proteins were eluted with a gradient of NaCl from 0.15 to 1 M (dashed lines). An equal portion of each fraction was subjected to the PLA₂ assay. (D) Cell surface-binding properties of various human sPLA₂s. After collecting the culture supernatants, the cells were incubated with medium containing 1 M NaCl for 30 min to solubilize the cell surface HSPG-bound form of the enzyme. PLA₂ activities in the secreted (S) and cell membrane-associated (i.e., 1 M NaCl-solubilized) (C) fractions were quantified.

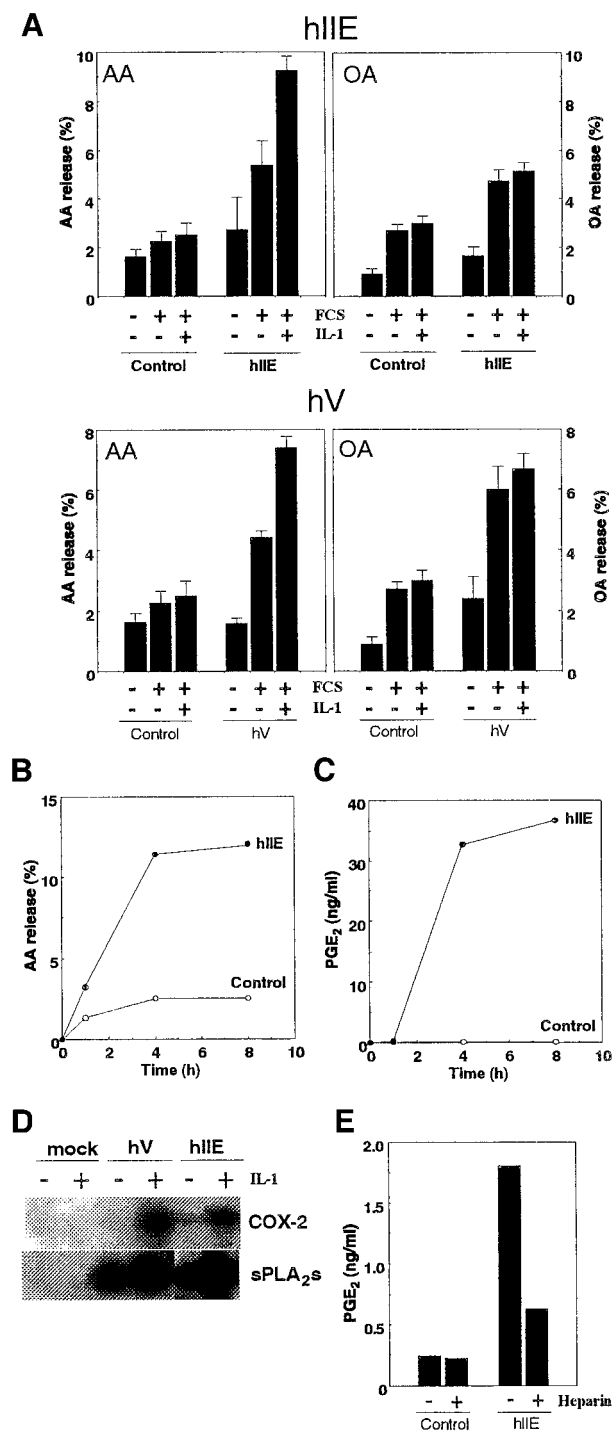


FIG. 2. AA release and PGE₂ generation by sPLA₂-IIE and other human sPLA₂s in HEK293 cells. (A) Delayed fatty acid release by various human sPLA₂s. Parental HEK293 cells and cells expressing human sPLA₂-IIE (hIIE), sPLA₂-IIA (hIIA), sPLA₂-V (hV), or sPLA₂-X (hX) were prelabeled with [³H]AA or [³H]OA, washed, and then cultured for 4 h with 1% FCS (-FCS), 10% FCS (+FCS) or 10% FCS plus 1 ng/ml IL-1 β to assess [³H]AA or [³H]OA release (mean \pm SE, $n = 4$). (B and C) Time course of AA release (B) and PGE₂ generation (C) by control and hIIE-transfected cells stimulated for the indicated periods with 10% FCS plus 1 ng/ml IL-1 β . (D) Induction of COX-2. RNAs obtained from control and hIIE-transfected

dilution; Zymed) for 1 h. After six washes with PBS, the cells were mounted on glass slides using Perma Fluor (Shandon), and the sPLA₂ signal was visualized using a laser scanning confocal microscope (IX70; Olympus), as described previously (6).

LPS treatment of mice. LPS (*Salmonella minnesota* Re 595; Sigma) (5 mg/kg) was injected intraperitoneally to 4-week-old male C57BL/6 mice (Nippon Bio-Supply Center). After 24 h, mice were sacrificed by bleeding, their organs were removed, and RNA was extracted by homogenization in TRIZOL reagent (Life Technologies) using 10 strokes of a Potter homogenizer at 1000 rpm.

Mouse ear topical dermatitis. Five repeated topical applications of 2,4-dinitrobenzene (DNFB) to the ears of BALB/c, but not C57BL/6, mice result in contact hypersensitivity of the ears as well as significant elevation of serum IgE level, accompanied by the increased T_{H1} response for the onset of skin dermatitis and the T_{H2} response in the lymph node (31). Briefly, the ears of BALB/c mice (Nippon Bio-Supply Center, Tokyo, Japan) were painted with 25 μ l of 0.15% (w/v) DNFB or vehicle (acetone:olive oil 3:1) once a week. The ears were removed 24 h after the fifth painting and subjected to RNA extraction. Replicate ear sections were fixed by formalin, embedded in paraffin and stained with hematoxylin and eosin to verify the progress of inflammation. All the procedures and analyses of other parameters are detailed elsewhere (31).

Other procedures. Northern blotting, Western blotting, and *in vitro* sPLA₂ assay were performed as described previously (4-8). Data were analyzed by Student's *t* test. Results are expressed as the mean \pm SE, with $P = 0.05$ as the limit of significance.

RESULTS

Enzymatic properties of human sPLA₂-IIE. cDNA for human sPLA₂-IIE was transfected into HEK293 cells to establish genetisin-resistant stable transfectants. The expression of the enzyme in the transfectants was assessed by enzyme activity (Fig. 1A) and Northern blotting (see Fig. 2D). PLA₂ activity on phosphatidylethanolamine (PE) in the culture supernatant of cells transfected with human sPLA₂-IIE was greatly elevated to a level comparable to that of cells transfected with several other human sPLA₂s (Fig. 1B). Mouse sPLA₂-IIE exhibited very poor PLA₂ activity under the same assay condition (Fig. 1B), in line with previous observations (27, 28). This species difference is noteworthy because the enzymatic activity of other sPLA₂s, including IB, IIA, IIC, V, and X, did not show such a species-related difference (4-8). Human sPLA₂-IIE hydrolyzed PE in marked preference to PC, as did human sPLA₂-IIA and -IID, whereas human sPLA₂-V and -X hydrolyzed both substrates efficiently (Fig. 1A).

When the pooled culture supernatant of human sPLA₂-IIE-expressing cells was applied to heparin-Sepharose (Fig. 1B) and sulfate-Cellulofine (Fig. 1C) columns, approximately 20 and 50%, respectively, of

cells, which were stimulated for 4 h in medium containing 10% FCS with or without 1 ng/ml IL-1 β , were subjected to RNA blotting using COX-2 and hIIE cDNAs as probes. (E) Effect of heparin. Control and hIIE-transfected cells were incubated with 400 μ g/ml heparin overnight, and PGE₂ generation 4 h after incubation with 10% FCS plus 1 ng/ml IL-1 β in the continued presence of heparin was examined.

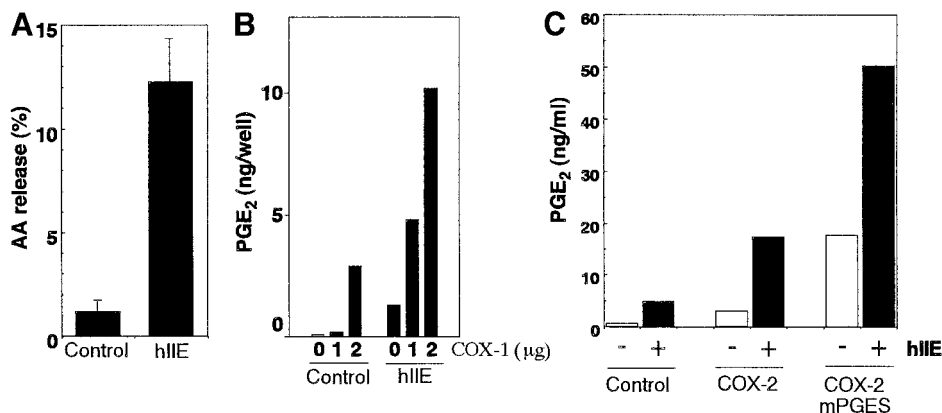


FIG. 3. Immediate PGE₂ production and transcellular PGE₂ biosynthesis by sPLA₂-IIE in HEK293 cells. (A) Immediate AA release. Control and human sPLA₂-IIE (hIIE)-transfected HEK293 cells prelabeled with [³H]AA were stimulated for 30 min with 10 μM A23187 to assess [³H]AA release. (B) COX-1-dependent immediate PGE₂ production. Control and hIIE-expressing HEK293 cells were transfected with the indicated amounts of COX-1 cDNA, and PGE₂ production in response to A23187 was examined. COX-1 expression was verified by immunoblotting (not shown). (C) Transcellular PGE₂ biosynthesis. Control, COX-2-expressing and COX-2/mPGES-coexpressing HEK293 cells were cocultured with control (-) or hIIE-expressing (+) cells for 4 days, and PGE₂ levels released into the supernatants 4 h after treatment with 10% FCS and IL-1β were quantified. Values are means ± SE of 3 independent experiments in A and representative results of 3 experiments are shown in B and C.

the enzyme, as assessed by its activity, bound to the columns under conditions where sPLA₂-IIA, -IID and -V bound entirely (3, 4, 8, 16). The bound enzyme

was eluted from these columns with a buffer containing 0.3-0.4 M NaCl (Figs. 1B and 1C). Applying the flowthrough fractions to the columns again resulted in partial absorption of the enzyme, which was eluted with the same concentrations of NaCl (data not shown). As shown in Fig. 1D, approximately 80% of the enzyme activity was detected in the supernatants and the remaining 20% in the membrane-bound fraction that was solubilized with 1 M NaCl. In comparison, approximately 80, 50, and 40% of human sPLA₂-IIA, IID, and V, respectively, were distributed in the NaCl-extractable fraction, whereas human sPLA₂-X was largely secreted into the medium (Fig. 1D). Given the fact that the membrane distribution of sPLA₂-IIA, -IID, and -V reflects their association with HSPG on cell surface (3-8), these results indicate that human sPLA₂-IIE has a significant affinity for heparin and cell surface HSPG, although it is weaker than that of sPLA₂-IIA, -IID, and -V.

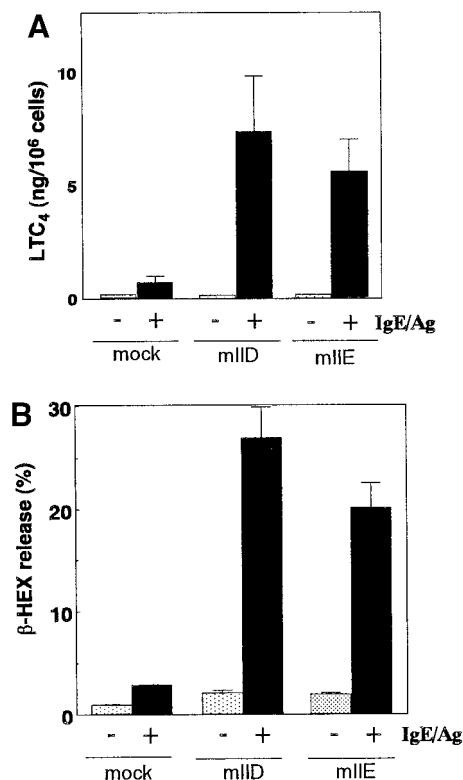


FIG. 4. Enhanced mast cell activation by sPLA₂-IIE. IgE/antigen-stimulated LTC₄ (A) and β-hexosaminidase (B) release by mock- and mouse sPLA₂-IID or sPLA₂-IIE-transfected RBL-2H3 cells. Cells were sensitized with IgE, washed, and then incubated with (+) or without (-) a suboptimal concentration of hapten-specific antigen. After 30 min, LTC₄ and β-hexosaminidase released into the supernatants were measured. Values are means ± SE of 3 independent experiments.

AA release and eicosanoid generation by sPLA₂-IIE. When [³H]AA- or [³H]OA-prelabeled HEK293 cells were incubated for 4 h in enriched medium, the release of [³H]AA and [³H]OA by human sPLA₂-IIE-expressing cells was increased modestly compared with that by replicate control cells; [³H]AA release by sPLA₂-IIE-expressing cells was augmented further in the presence of interleukin-1β (IL-1β) (Fig. 2A). This fatty acid-releasing profile of human sPLA₂-IIE was similar to that of sPLA₂-IIA and -IID, which increased IL-1β-dependent, [³H]AA-selective release (3-8), but was distinct from that of sPLA₂-X, which increased both [³H]AA and [³H]OA release in parallel without showing any dependence on IL-1β (). It also differed from human sPLA₂-V-mediated fatty acid release, in which both [³H]AA and [³H]OA release was markedly in-

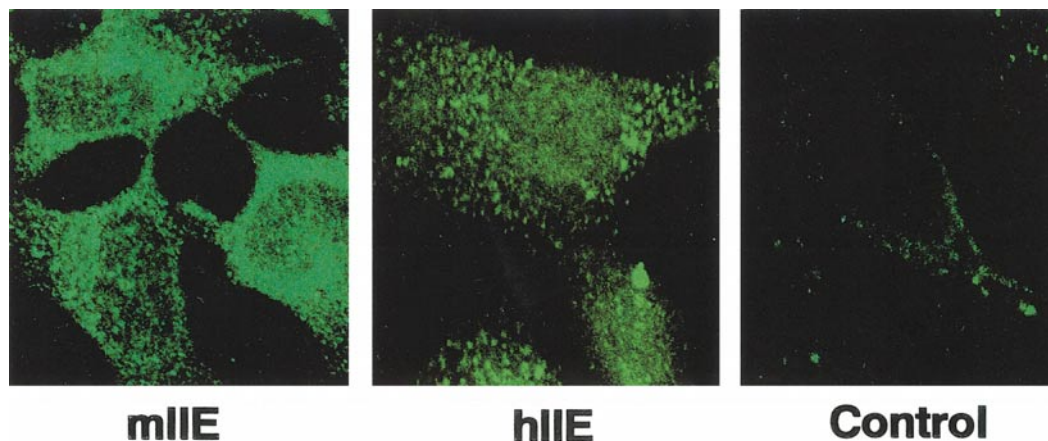


FIG. 5. Confocal microscopic immunostaining of sPLA₂-IIE in HEK293 cells. Cells transfected with mouse sPLA₂-IIE (mIIE) or human sPLA₂-IIE (hIIE) as well as parental cells were fixed, permeabilized, incubated sequentially with anti-mIIE or anti-hIIE antibody and with FITC-conjugated anti-mouse IgG, and then visualized by confocal laser microscopy.

creased independently of IL-1 β (like sPLA₂-X) and only [³H]AA release was further augmented by IL-1 β (like sPLA₂-IIA/IIE) (Fig. 2A).

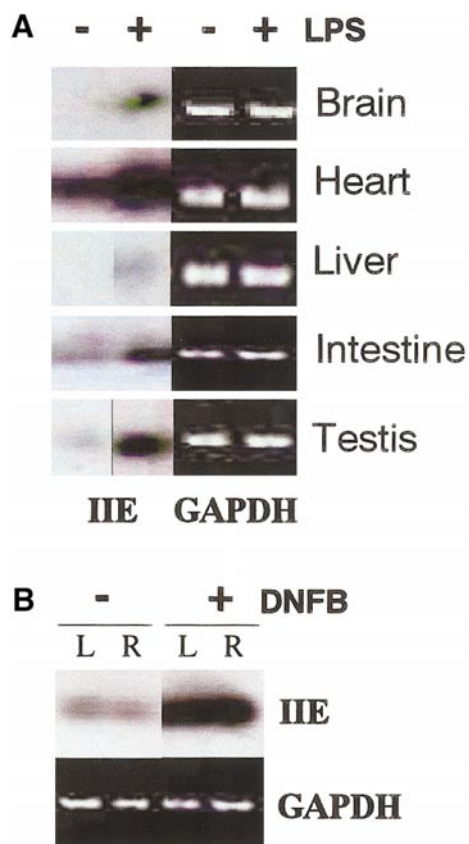


FIG. 6. Induction of sPLA₂-IIE expression in the mouse during inflammation. RNAs obtained from various tissues of mice with or without 24-h treatment with LPS (A) and from ears of mice with or without 5 repeated treatments with DNFB (B) were subjected to RT-PCR for mouse sPLA₂-IIE (35 cycles), which was visualized by Southern hybridization using mouse sPLA₂-IIE cDNA probe, and GAPDH (25 cycles), which was detected in agarose gels by ethidium bromide staining.

IL-1 β -stimulated [³H]AA release by human sPLA₂-IIE-expressing cells reached a peak by 4 h (Fig. 2B), with concomitant production of PGE₂ (Fig. 2C). This PGE₂ generation was blunted by the COX-2-selective inhibitor, NS-398 (data not shown), indicating a functional linkage between sPLA₂-IIE and COX-2. As shown in Fig. 2D, human sPLA₂-IIE-expressing cells expressed more COX-2 than control cells after IL-1 β stimulation. Human sPLA₂-V (Fig. 2D) and -IIA (data not shown) also increased COX-2 expression (6, 7). Thus, like other HSPG-binding sPLA₂s (IIA, IID and V) (4–8), human sPLA₂-IIE is capable of augmenting IL-1 β -stimulated AA-selective release and attendant COX-2-mediated delayed PGE₂ generation. Treating the cells with heparin, which solubilizes the HSPG-bound form of sPLA₂ from cell surfaces (4, 6, 8, 9), markedly reduced PGE₂ generation by human sPLA₂-IIE (Fig. 2E), further implying that AA release by this enzyme occurs largely through the HSPG-dependent mechanism.

A23187-induced immediate [³H]AA release was also markedly elevated in human sPLA₂-IIE-transfected cells relative to replicate control cells (Fig. 3A). When COX-1 was transfected, A23187-induced immediate (Fig. 3B), but not IL-1 β -induced delayed (data not shown), PGE₂ production was markedly augmented in human sPLA₂-IIE-expressing cells compared with replicate control cells. Thus, human sPLA₂-IIE can supply the substrate AA to COX-1 for immediate PGE₂ production.

To assess whether human sPLA₂-IIE secreted from cells can influence PG generation by neighboring cells through the transcellular route (5), cells expressing human sPLA₂-IIE were cocultured with cells expressing the downstream enzymes COX-2 and microsomal PGE₂ synthase (mPGES), and IL-1 β -stimulated generation of PGE₂ was examined. As shown in Fig. 3C, PGE₂ production by coculture of human sPLA₂-IIE-

expressing cells and COX-2-expressing cells was several-fold higher than that produced by each type of cells alone. Introduction of mPGES, which lies downstream of COX-2 in the PGE₂-biosynthetic pathway (32), further increased this transcellular pathway-mediated PGE₂ production. Thus, human sPLA₂-IIE produced by one cell can act on another COX-2-expressing cell in a paracrine manner to augment PGE₂ production by the latter.

Mouse sPLA₂-IIE was unable to elicit these responses in HEK293 cells under any of the conditions tested (8). However, when it was transfected into RBL-2H3 cells, IgE/antigen-dependent production of LTC₄ was several-fold higher than that observed in replicate control cells, reaching a level comparable to that induced by replicate mouse sPLA₂-IID-transfected cells (Fig. 4A). Thus, mouse sPLA₂-IIE can be coupled with endogenous 5-lipoxygenase for LTC₄ biosynthesis in RBL-2H3 cells. Moreover, mouse sPLA₂-IIE, like sPLA₂-IID, enhanced IgE/Ag-induced β -hexosaminidase (β -HEX) exocytosis by RBL-2H3 transfectants (Fig. 4B).

Subcellular localization of sPLA₂-IIE. We next performed confocal laser microscopy in order to assess the subcellular distribution of sPLA₂-IIE in HEK293 transfectants. As shown in Fig. 5, both mouse and human sPLA₂-IIEs were distributed in punctate compartments throughout the cytoplasm. This punctate staining pattern was also observed with cells expressing other HSPG-binding sPLA₂s, such as sPLA₂-IIA and IID, but not with cells expressing sPLA₂-X, a HSPG-nonbinding enzyme (6, 8, 10).

Induction of sPLA₂-IIE during inflammation. As examined by RT-PCR/Southern blotting, the expression of sPLA₂-IIE in several mouse tissues, which was low under basal conditions, was markedly increased 24 h after intraperitoneal administration of LPS (Fig. 6A). sPLA₂-IIE expression was also markedly increased in the ears of mice after five repeated treatments with DNFB (Fig. 6B), an experimental atopic dermatitis model.

DISCUSSION

Current evidence suggests that the AA-releasing function of various sPLA₂s in mammalian cells are crucially influenced by the ability of the enzymes to interact with PC-rich membrane (8, 13–18), HSPG (3–8), and the sPLA₂ receptor (33, 34). Several group II subfamily sPLA₂ enzymes (IIA, IID and V) have been implicated in the inflammatory and immune responses, since they are expressed in immune regulatory cells and are up-regulated by various cytokines and immunological stimuli (9–13, 24, 25). However, the regulatory functions of more recently cloned sPLA₂s (IIE, IIF, III and XII) remain unknown. sPLA₂-IIE, a new member of the group II subfamily sPLA₂s

that is closely related structurally to sPLA₂-IIA and -IID, is reportedly expressed at relatively low levels in restricted tissues (27, 28). We herein show that this novel group II sPLA₂ is an additional inducible regulator of AA metabolism that may play a role in the inflammatory process. The modes of action of human sPLA₂-IIE resemble those of its closest relatives sPLA₂-IIA and -IID in several ways. First, the enzymatic properties of these three group II enzymes are similar (Fig. 1) (27, 28). Second, these enzymes bind anionic heparin- or sulfate-enriched resins (Figs. 1B and 1C), even though the heparin affinity of sPLA₂-IIE is lower than that of sPLA₂-IIA, -IID and -V (IIA > IID > V > IIE in order, consistent with their calculated *pI* values of ~9.4, 8.8, 8.5, and 8.0, respectively). Accordingly, a low, but significant, portion of human sPLA₂-IIE expressed in HEK293 cells is associated with HSPG on the cell surface (Fig. 1D). Third, human sPLA₂-IIE augments stimulus-induced, AA-selective release in HEK293 transfectants in a manner similar to sPLA₂-IIA and -IID (4, 7) but not to sPLA₂-X, which causes stimulus-independent, fatty acid-nonspecific release (7, 8). Fourth, human sPLA₂-IIE induces the expression of endogenous COX-2 and attendant delayed PGE₂ generation (Figs. 2C and 2D), an event peculiar to HSPG-binding group II subfamily sPLA₂s (6–8). Moreover, PGE₂ generation by human sPLA₂-IIE is markedly suppressed by exogenous heparin (Fig. 2E), which solubilizes the cell surface HSPG-bound form of sPLA₂s (3, 4, 6, 8). Although the mechanisms for COX-2 induction by HSPG-binding sPLA₂s are unclear, the requirement for their catalytic activity (7) suggests that certain reaction products spatiotemporally generated by sPLA₂ action in particular subcellular sites may in turn trigger the COX-2 induction machinery. Finally, sPLA₂-IIE is distributed in cytoplasmic punctate compartments (Fig. 4). Previous studies have demonstrated that other HSPG-bound sPLA₂s, such as sPLA₂-IIA and sPLA₂-IID, also display a similar punctate distribution (6, 8, 10).

Collectively, these results suggest that human sPLA₂-IIE, like sPLA₂-IIA and -IID (4–8), is capable of promoting AA release through the HSPG-shuttling pathway. Although the affinity of sPLA₂-IIE for heparanoids is weak, a small fraction of the enzyme that binds to cell surface HSPG (Fig. 1D) may be sufficient to elicit the cellular responses. Our previous failure to detect AA release by mouse sPLA₂-IIE in HEK293 cells (8) might be due to its intrinsically weak enzyme activity rather than to its defect in HSPG binding, since human and mouse sPLA₂-IIEs have well conserved cationic amino acid residues (27, 28) and display a similar subcellular distribution (Fig. 4). This does not necessarily mean that mouse sPLA₂-IIE is functionally a null enzyme in any cell type, since it is capable of augmenting IgE/antigen-dependent LTC₄ production (Fig. 4A) and degranulation (Fig. 4B) in rat

mastocytoma RBL-2H3 cells, even though the LTC₄-biosynthetic action is weaker than sPLA₂-V and -X (8). Enhanced degranulation of mast cells by sPLA₂-IIE is compatible with our previous observation that the heparin-binding enzymes, sPLA₂-IIA, -IID, and -V, exert this effect (29), probably by facilitating the spatio-temporal production of fusogenic lysophospholipids. Given these findings, mouse sPLA₂-IIE may require more restricted microdomain structures on target membranes to exert its proper enzymatic function, and such a membrane microdomain could be spatiotemporally formed in IgE/antigen-activated RBL-2H3 cells.

We have shown that the expression of sPLA₂-IIE is markedly increased in two mouse experimental inflammation models. Induction of sPLA₂-IIE in several tissues of LPS-treated mice (Fig. 6A) is consistent with the recent work by Suzuki *et al.* (28), which demonstrated, by means of *in situ* hybridization, that sPLA₂-IIE is expressed in macrophage-like cells in LPS-treated, but not in control, mice. The observation that the expression of sPLA₂-IIE is increased in mouse ears with atopic dermatitis (Fig. 6B), together with the facts that it is expressed endogenously in mouse cultured mast cells (29) and that it enhances LTC₄ production and degranulation in a rat mast cell line (Figs. 3C and 3D), suggests that sPLA₂-IIE may participate in the process of inflammation involving mast cell-directed allergic reactions.

Although our present results suggest that the three related group II subfamily sPLA₂s (IIA, IID and IIE) can be functionally redundant, it should be noted that the expression of sPLA₂-IIE is lower than other related sPLA₂ enzymes in all tissues (27, 28). It is now recognized that a main physiological function of sPLA₂-IIA is the destruction of infectious bacterial membranes (19–21), yet the bactericidal activity of sPLA₂-IIE is rather weaker than that of sPLA₂-IIA (21). Therefore, it still remains elusive if sPLA₂-IIE indeed plays a compensatory role in pathophysiological circumstances, which should be clarified by gene targeting in future.

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