Mammalian secretory phospholipase A2s (sPLA2s) are classified into several groups according to molecular structure and the localization of intramolecular disulfide bridges. Among them, group IIA sPLA2 has been thought to be one of the key enzymes in the pathogenesis of inflammatory diseases owing to its augmented expression under various inflammatory conditions. However, in a number of inbred mouse strains, the group IIA sPLA2 gene is naturally disrupted by a frame-shift mutation. Here, we report the cloning of a cDNA encoding a novel sPLA2 expressed in the spleen of group IIA sPLA2-deficient mice. We also cloned its human homolog and mapped its gene location on chromosome 1p36.12 near the loci of group IIA and V sPLA2 genes. The human mature sPLA2 protein consists of 125 amino acids (Mr = 14,500) preceded by a 20-residue prepeptide and is most similar to group IIA sPLA2 with respect to the number and positions of cysteine residues as well as overall identity (48%). Based on these structural properties, the novel sPLA2 should be categorized into group II, called group IID to follow the already identified IIA to IIC sPLA2s. When the cDNA was expressed in COS-7 cells, PLA2 activity preferentially accumulated in the culture medium. It is maximally active at neutral to alkaline pH and with 2 mM Ca2+. In assays with individual substrates, 1-o-1-palmitoyl-2-linoleoyl phosphatidylethanolamine was more efficiently hydrolyzed than the other phospholipids examined. An RNA blot hybridized with the cDNA exhibited two transcripts (2.0 and 1.0 kb) in human spleen, thymus, and colon. The expression of a novel sPLA2 mRNA was elevated in the thymus after treatment with endotoxin in rats as well as in group IIA sPLA2-deficient mice, suggesting its functional role in the progression of the inflammatory process.

Phospholipase A2 (PLA2)1 comprises a diverse family of lipolytic enzymes that hydrolyze the sn-2 fatty acid ester bond of glycerolphospholipids to produce free fatty acid and lysophospholipids (1, 2). PLA2s participate in a wide variety of physiological processes, including phospholipid digestion, remodeling of cell membranes, and host defense, and also take part in pathophysiological processes by producing precursors of various types of biologically active lipid mediators, such as prostaglandins, leukotrienes, thromboxanes, and platelet-activating factor (3). Over the past two decades along with advances in molecular biology, numerous PLA2s have been identified and characterized (4–13). According to their biochemical features such as cellular localization, requirement of Ca2+, substrate specificity, and the primary structure, these PLA2s are classified into several families, including low molecular weight secretory PLA2 (sPLA2), Ca2+-sensitive arachidonoyl-specific 85-kDa cytosolic PLA2, Ca2+-independent PLAs2, and platelet-activating factor-acetylhydrolase (14).

Low molecular mass sPLA2s (13–18 kDa) have several features distinct from other PLA2 families, such as a high disulfide bond content, a requirement for millimolar concentration of Ca2+ for catalysis, and a broad specificity for phospholipids with different polar head groups and fatty acyl chains (15). At present, mammalian sPLA2s are classified into five different groups (groups IB, IIA, IIC, V, and X), depending on the primary structure characterized by the number and positions of cysteine residues (12, 14). Among them, group IIA sPLA2 has been a focus of attention as a potent mediator of the inflammatory process, because its local and systemic levels are elevated in numerous inflammatory diseases, including sepsis, Crohn’s disease, and acute pancreatitis (16, 17), and correlate well with disease severity in rheumatoid arthritis (18). Furthermore, the expression of group IIA sPLA2 is enhanced by inflammatory cytokines such as interleukin 1β and tumor necrosis factor-α as well as lipopolysaccharide (LPS) in various cell types (19–22). In some inbred mouse strains, however, the group IIA sPLA2 gene is spontaneously inactivated by a point mutation (23, 24). These deficient mice are susceptible to arthritis in antigen-induced models (25, 26), and the mast cells derived from these mice exhibit normal prostaglandin production to ligand activation (27). The transgenic mice expressing the human group IIA sPLA2 gene do not develop any overt inflammatory conditions (28). These findings point to the need to reassess the contribution of group IIA sPLA2 in inflammatory diseases and suggest that other types of the sPLA2 isoform play a pivotal role in place of or in concert with the group IIA sPLA2. For example, group V sPLA2, one of the newly identified sPLA2 isoforms (7), has been reported to be involved in the production of lipid mediators in P388D1 murine macrophages and bone marrow-derived mast cells based on antiense experiments (27, 29). The most recently identified group X sPLA2 (12) is another candidate. The involvement of group X sPLA2 in inflammatory responses is suggested by its restricted expression in immune tissues such as the spleen and thymus, although there is no direct evidence for its commitment to the pathological conditions. A possibility of the involvement of the most classical
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sPLA₂ group IB sPLA₂, in the inflammatory response is also worth considering. This sPLA₂ has been thought to act as a digestive enzyme, given its abundance in digestive organs including the pancreas (30). However, a series of our studies have revealed group IB sPLA₂-induced various biological responses, such as cell proliferation, smooth muscle contraction, and lipid mediator release, through the binding to its specific receptor, the PLA₂ receptor (31–36). Furthermore, recent studies with mice deficient for both PLA₂ receptor and group IIA sPLA₂ demonstrated a potential role of group IB sPLA₂/PLA₂ receptor-mediated responses in the progression of endotoxin shock, because the knock-out mice exhibit resistance to endotoxin-induced lethality with reduced plasma levels of inflammatory cytokines (37). Besides previously identified sPLA₂s, other low molecular weight sPLA₂s have been detected in various tissues including the brain and lung (38), suggesting the presence of novel sPLA₂s that might play a compensatory role in the deficiency in group IIA sPLA₂ or an independently functional role in the inflammatory processes.

During a survey of the DNA data base, we encountered an expressed sequence tag (EST) that could represent part of a new sPLA₂ isoform. Here, we report the cloning of a cDNA encoding a novel sPLA₂ expressed in the spleen of group IIA sPLA₂-deficient mice. We also describe the cloning of its human homolog, the characterization of recombinant protein and its expression profile in humans, as well as in endotoxin-treated rats and group IIA sPLA₂-deficient mice.

**Experimental Procedures**

**Materials**—All oligonucleotides were purchased from Kojuski Shiyaku KK (Kobe, Japan). LPS (Escherichia coli: 055:B5) was purchased from Sigma, LPS (Salmonella typhosa 0901) was from Difco Laboratories. DMSO (dimethyl sulfoxide) was obtained from Life Technologies, Inc. Recombinant human sPLA₂ (GeneBridge4, Research Genetics). 1 μl of each DNA aliquot was subjected to PCR according to the manufacturer's protocol with primers (5'-aagaggcttgtggtagacg-3' and 5'-ccgacgtagctgcttcgct-3') which amplified the 108-bp PL A₂ encoding genomic DNA fragment. The PCR was initiated at 94°C for 2 min and then followed by 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min for 30 cycles with ExTaq and TaqStart™ Antibody (CLONTECH) using the hot start technique. The PCR products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. The presence or absence of the product in each of the hybrid clones was scored. With the screening result, mapping was performed on the server computer at the Whitehead Genome Research Center.

**Recombinant Expression of the sPLA₂**—Two primers, 5'-agtgttgaggcagccaccaagtgaagcagttg-3' and 5'-taaagttttctaagctagctgaggtctctt-3', were used for PCR amplification of the coding region of the mouse sPLA₂ cDNA. Those for the human sPLA₂ were 5'-agtgttgaggcagccaccaagtgaagcagttg-3' and 5'-taaagttttctaagctagctgaggtctctt-3'. Upstream primers have a NotI recognition site and XbaI sequence (italic). Downstream primers are with the XbaI recognition site. The sPLA₂ cDNA was amplified by PCR from mouse or human spleen cDNA followed by digestion with NotI and XbaI and inserted into pcDNA3.1(+)(Invitrogen) to construct mouse and human sPLA₂ expression plasmids. After sequencing confirmation, 5 μg of recombinant plasmid was transfected into 50% confluent COS-7 cells per well in 12-well plates. The resulting recombinant plasmid was isolated and sequenced. The 5'- and 3'-ends of the human sPLA₂ was determined using the radiation hybrid mapping panel (Genebridge4, Research Genetics). 1 μl of each DNA aliquot was subjected to PCR according to the manufacturer's protocol with primers (5'-aagaggcttgtggtagacg-3' and 5'-ccgacgtagctgcttcgct-3') which amplified the 108-bp PL A₂ encoding genomic DNA fragment. The PCR was initiated at 94°C for 2 min and then followed by 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min for 30 cycles with ExTaq and TaqStart™ Antibody (CLONTECH) using the hot start technique. The PCR products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. The presence or absence of the product in each of the hybrid clones was scored. With the screening result, mapping was performed on the server computer at the Whitehead Genome Research Center.

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**Experimental Procedures**

**Cloning of the Mouse sPLA₂**—BLASTn search of the GenBank™ Database was performed (39) using an PE amino acid sequence (DRC-CVTDDCC) around the catalytic center of the mouse group IIA sPLA₂ (24). A cDNA fragment corresponding to the identified EST sequence was amplified by polymerase chain reaction (PCR). Primers for amplification were 5'-ctctgaaactctagagctgtc-3' and 5'-ctgaaactctagagctgtc-3' (sense) and 5'-taggtgaggccgagcagagctgtc-3' and 5'-taggtgaggccgagcagagctgtc-3' (antisense). Two rounds of amplifications (nested PCR) were carried out with these primers and ExTaq (Takara, Japan). Reverse transcribed cDNAs from various mouse tissues were used as templates. Amplification conditions were 94°C for 1 min, 55°C for 1 min, and 72°C for 3 min for 30 cycles. The PCR products were separated on agarose gel, and the DNA of the expected size was isolated. The recombinant plasmid was then constructed with PCR cloning vector (Invitrogen), purified with GFX Micro Plasmid Prep Kit (Amersham Pharmacia Biotech), and sequenced with Applied Biosystems PRISM 310 genetic analyzer. From the determined DNA sequence, four primers were designed for the isolation of 5' and 3' portions of the cDNA. The cloning of these remaining parts was carried out with rapid amplification of the cDNA ends protocol using mouse spleen marathon-ready cDNA (CLONTECH) according to the manufacturer's manual. After a single modification in the PCR, KlenTaq polymerase was used instead of KlenTaq polymerase. The full-length cDNA was isolated by PCR with primers, 5'-ataaggggctgtggtagacg-3' and 5'-gaagttgatttagagccgcttc-3'. In each cloning step, sequences were determined with more than 10 individual clones to rule out the possibility of misincorporation during the PCR.

**Cloning of Human sPLA₂**—Based on the mouse sPLA₂ cDNA sequence, four primers (5'-acaggtgtcagctgcttc-3', 5'-cataggtgtcagctgcttc-3', 5'-acaggtgtcagctgcttc-3', and 5'-ttcaggtgtcagctgcttc-3') were prepared for amplification of the middle part of the human homolog cDNA. Using human spleen marathon-ready cDNA (CLONTECH) as a template, two consecutive rounds of PCR were performed with two pairs of primers. In the nested manner of the PCR, the PCR conditions were 94°C for 1 min, 55°C for 1 min, 45°C for 1 min, and 72°C for 3 min for 30 cycles with ExTaq. The amplified product was separated on agarose gel, and the DNA of the expected size (117 base pairs (bp)) was isolated and sequenced. The 5' and 3'-rapid amplification of cDNA ends were performed basically as described above using human sPLA₂-specific primers and spleen cDNA. The radiation hybrid mapping panel (Genebridge4, Research Genetics). 1 μl of each DNA aliquot was subjected to PCR according to the manufacturer's protocol with primers (5'-aagaggcttgtggtagacg-3' and 5'-ccgacgtagctgcttcgct-3') which amplified the 108-bp PL A₂ encoding genomic DNA fragment. The PCR was initiated at 94°C for 2 min and then followed by 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min for 30 cycles with ExTaq and TaqStart™ Antibody (CLONTECH) using the hot start technique. The PCR products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. The presence or absence of the product in each of the hybrid clones was scored. With the screening result, mapping was performed on the server computer at the Whitehead Genome Research Center.
RESULTS

Molecular Cloning of Novel sPLA2 and Chromosomal Localization of Its Gene—In searching for novel sPLA2 in the rapidly expanding data base, we identified a cDNA fragment (GenBank accession number AA762051) by tBLASTn search using catalytically essential residues of sPLA2s as a query. This cDNA was an EST sequence originally cloned from thymus of C57BL/6J mice (one of the group IIA sPLA2-deficient strains (23, 24)) and theoretically could encode a portion of functional sPLA2s previously uncharacterized. We amplified the cDNA corresponding to this EST sequence from reverse transcribed RNA samples extracted from various mouse tissues including the spleen, liver, and small intestine. Using splicing cDNA fragment as a probe, an expression profile was examined by Northern analysis of multiple tissues originated from Balb/c mice, which detected two transcripts (1.2 and 2.4 kb) expressed most abundantly in the spleen among the tissues examined (data not shown). Using the spleen cDNA library as a source, the 5′ and 3′ surrounding regions were isolated using the PCR-based protocol. These separately isolated 5′, middle, and 3′ cDNAs were assembled to yield one open reading frame that potentially encodes a functional sPLA2. The full-length cDNA was cloned with the 5′- and 3′-most primers by PCR from spleen cDNA to confirm the existence of the consecutive transcript and its sequence. The 1233-bp cDNA thus identified encoded a novel sPLA2 consisting of 144 amino acids.

To clone its human homolog, we first attempted to amplify the middle portion of the cDNA by PCR using primers designed from the mouse sPLA2 sequence under the assumption that the catalytically and functionally essential residues are conserved between the two animals. Through intensive search for the human PLA2 cDNA fragments after PCR using several combinations of primers, one of the amplified cDNA fragments was found to have a nucleotide sequence 70% identical to that of the corresponding region of the mouse sPLA2 cDNA and encoded 25 amino acid residues with a characteristic of sPLA2. Using this sequence information, we were able to clone the full-length cDNA from a human spleen cDNA library with the rapid amplification of cDNA ends protocol. The human sPLA2 cDNA clone consists of 878 bp with one long open reading frame encoding 145 amino acids. The coding region has 79% nucleotide sequence identity with the mouse counterpart.

To assign the chromosome localization of the human novel sPLA2 gene, we performed radiation hybrid mapping. The PCR using two primers described under “Experimental Procedures” gave a 108-bp fragment from human genomic DNA as well as from the isolated sPLA2 cDNA, which means that the corresponding gene sequence is not separated by interrupting introns. With this pair of primers, the radiation hybrid mapping panel was screened. 25 of 93 DNA aliquots derived from human/hamster hybrid clones gave clear amplification of the genomic fragment. The PCR results statistically assigned the sPLA2 gene location to chromosome 1p36.12 at 3.77 centiRay relative to the sequence tagged site WI-5273.

Structural Features of Novel sPLA2 and Comparison with Other Mammalian sPLA2s—Amino acid sequences of novel mouse and human sPLA2s are shown in Fig. 1 aligned with those of other sPLA2s.2 The sequence similarity and hydropathy profiles (data not shown) suggest that the N-terminal 19 (mouse) and 20 (human) residues are signal peptides. Judging from the length of the predicted signal peptide and absence of basic amino acids preceding the N terminus of the mature protein, this sPLA2 does not have a propeptide. The calculated molecular masses of mouse and human novel sPLA2 are 14.3 and 14.5 kDa, respectively. There is one potential N-glycosylation site in each sPLA2: Asn99 (mouse) and Asn89 (human). As shown in Fig. 1, the novel sPLA2s have about 40% identity with other isoforms and show preferential homology with group IIA sPLA2 (47 and 48% in mouse and human, respectively). All of the previously published sPLA2s contain 12–16 cysteine residues to form 6–8 intramolecular disulfide bonds by which each isoform is taxonomically characterized (15). In the mature portion of the novel sPLA2, mouse and human enzymes share identical distribution of 14 cysteine residues. Compared with the characteristic cysteine residues found in the known sPLA2 sequences, the novel sPLA2 possesses 50–137 pairs, which is typical of group IIA sPLA2, but does not have the 11–77 and 86–92 sets, which are characteristic of group IB and IIC sPLA2, respectively. In addition, novel sPLA2 has an amino acid C-terminal extension, which is found in group IIA, IIC, and X sPLA2s, whereas it does not contain the pancreatic loop, a feature characteristic of the group IB sPLA2. Taken together, the novel sPLA2 is most similar to group IIA sPLA2 and should be categorized into group II based on the traditional grouping criteria proposed by Heinrikson (43). The sequence comparison clearly established the molecular identity of novel sPLA2 distinguished from the PLA2s thus far cloned (groups I–X (12, 14)). Therefore, we propose to assign the name of the novel sPLA2 as group IID following so far identified IIA–IIC sPLA2s. Langlais et al. (44) identified PLA2 activity in human spermatogonia and determined its 19 N-terminal amino acids, which lacks Cys11. The novel sPLA2 is distinct from the spermatic PLA2 in its sequence.

Recombinant Expression of Novel sPLA2 and Characterization of sPLA2 Activity—The deduced amino acid sequences from both mouse and human novel sPLA2 cDNA contain all of the amino acids that are absolutely conserved in all functional sPLA2s including His48 and Asp49. Therefore, they were expected to possess enzymatic activities, which should be exported extracellularly after cleavage from the presumed signal peptide. To confirm this, the mouse and human novel sPLA2 cDNAs were subcloned into the eukaryotic expression plasmid and then transfected into COS-7 cells. As shown in Fig. 2A, PLA2 activity was detected in supernatant of the culture medium of mouse and human sPLA2 recombinant cells, whereas cells transfected with the parent vector did not show enzymatic activity. Only 2–4% of the total PLA2 activity was detected in the cell-associated fraction, indicating that novel sPLA2s were actively secreted from COS-7 cells. Similar results were obtained when human embryonic kidney 293 cells were used as a recombinant host (data not shown). As shown in Fig. 2B, sPLA2 activity was completely dependent on Ca2+ and required 2 mM Ca2+ for the maximal level. Recombinant sPLA2
was optimally active over a broad range of pH 6–10 (Fig. 2C), whereas the activity of human group IIA sPLA₂ was optimal within pH 6–9 and declined at pH 10 (data not shown). The Ca²⁺ dependence and optimal pH profile were compatible with common features of sPLA₂s (15).

The substrate preference of novel human sPLA₂ was determined individually with 13 types of commercially available phospholipids that possess palmitic acid at the sn-1 position and have different fatty acids at the sn-2 position as well as polar head groups. For this experiment, recombinant human sPLA₂ was partially purified by heparin affinity chromatography from the culture medium of Chinese hamster ovary cells and have different fatty acids at the sn-1 position as well as polar head groups. For this experiment, recombinant human sPLA₂ was partially purified by heparin affinity chromatography from the culture medium of Chinese hamster ovary cells.

The expression of the novel sPLA₂ in the spleen and thymus indicates its involvement in the regulation of the immune system and inflammation. Its expression levels in endotoxin-challenged rats and mice were also examined. In untreated rats, one transcript (2.1 kb) of novel sPLA₂ was detected in the spleen, thymus, and lung. At 24 h after LPS injection, the expression level of the sPLA₂ mRNA was elevated 6-fold in the thymus and lung (Fig. 4A). In the case of group IIA sPLA₂, a marked enhancement was observed in the thymus and lung in contrast to a slight decrease in the spleen. The elevation of group IIA sPLA₂ mRNA after LPS treatment was also detected in the small intestine, heart, kidney, pancreas, and liver, where the signal of novel sPLA₂ was not detected (data not shown). After challenge with LPS, the expression level of this sPLA₂ mRNA was elevated 3-fold in the thymus with no change in the spleen.

**DISCUSSION**

Rapid increase of DNA data, especially from EST projects, has led to discoveries of a number of genes that had not been known. Among the PLA₂ molecules, group X sPLA₂ and a novel paralog of the cytosolic PLA₂ were successful outcomes of these genomic approaches (12, 13). The initial retrieval of the EST sequence described in the present report is quite interesting, as
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Enzymatic activity of the novel and group IIA human sPLA₂ was measured as described under “Experimental Procedures.” The data were expressed as the percentages of hydrolyzed phospholipids within 30 min of incubation, ND, not detected.

| Substrate | sn-2 fatty acid | Substrate hydrolyzed % |
|-----------|----------------|------------------------|
| PC        | C16:0 (palmitic acid) | 2.81 | 3.37 |
|           | C18:1 (oleic acid)    | 1.87 | 1.09 |
|           | C18:2 (linoleic acid) | 2.13 | 1.10 |
|           | C20:4 (arachidonic acid) | 1.10 | 0.64 |
|           | C22:6 (docosahexaenoic acid) | 3.54 | 1.46 |
| PE        | C18:1 (oleic acid)    | 4.39 | 3.52 |
|           | C18:2 (linoleic acid) | 6.12 | 8.10 |
|           | C20:4 (arachidonic acid) | 0.98 | 0.87 |
| PS        | C18:1 (oleic acid)    | 0.23 | ND |
| PA        | C18:1 (oleic acid)    | 0.17 | ND |
|          | C20:4 (arachidonic acid) | 0.13 | ND |
| PG        | C16:0 (palmitic acid) | 1.47 | 0.97 |
|          | C18:1 (oleic acid)    | 4.63 | 28.97 |

Functional enzyme, because group IIC PLA₂ is thought to be a pseudogene in humans (15). Because the novel sPLA₂ is most similar to the group IIA sPLA₂ with respect to the number and positions of cysteine residues as well as overall identity, we propose to call the novel sPLA₂ as group IID following so far identified IIA to IIC sPLA₂s.

The group IIA sPLA₂ is thought to be one of the key enzymes critically important for the pathogenesis of inflammatory diseases, because its expression level is enhanced under various inflammatory conditions (19–22). The original EST corresponding to the novel mouse sPLA₂ has been cloned from the C57BL/6J strain in which the group IIA PLA₂ gene was naturally inactivated. Because these deficient mice are similar to group IIA sPLA₂ expressing mouse strains in their inflammatory responses (25, 26), the novel sPLA₂ might play a compensatory role for several functions of the group IIA sPLA₂. In the three mammals used in this study, the expression of novel sPLA₂ was detected commonly in the spleen and thymus, which is an expression profile similar to that of group X sPLA₂ in humans (12). Although the origin of cells producing this sPLA₂ is unknown at present, the tissue distribution pattern suggests its role in relation to the immune system and/or inflammation. Upon endotoxin challenge, the expression level of a novel sPLA₂ was elevated in the rat thymus along with the group IIA sPLA₂ transcript (Fig. 4). Enhanced expression of the novel sPLA₂ in the thymus was also observed in mice deficient for group IIA sPLA₂. During the progression of sepsis, thymic atrophy is induced via apoptosis (46). Because several reports suggest an involvement of PLA₂ in thymocyte apoptosis (47, 48), the novel sPLA₂ might play a role in this process. The expression of novel sPLA₂ mRNA was also changed in rat lung after LPS treatment, but its pattern was different from the case of group IIA sPLA₂. In humans, the distribution of the transcript of novel sPLA₂ contrasted to that of group IIA sPLA₂ (Fig. 3), suggesting distinct biological functions for these two related sPLA₂s in the physiological and pathological states. In addition to diverse tissue expression profiles, we observed a drastic difference between group IIA and novel sPLA₂s in susceptibility to one of the 1-oxamoylindolidine derivatives (49). This sPLA₂ inhibitor has a strong inhibitory potency for group IIA sPLA₂ (IC₅₀ = 1.2 nM), whereas more than 50% of the full activity of novel sPLA₂ remains even at 1000 nM. Moreover, an antibody that neutralized the human group IIA sPLA₂ activity

![Table I](image_url)
relevant to their distinct functions. Apparently depends on a cluster of basic residues near the N terminus of the PLA₂ protein (53). In particular, the indispensability of Arg⁷ and Lys¹⁵ in human group IIA sPLA₂ was demonstrated by a site-directed mutagenesis experiment (54). The conservation of basic residues at these sites in novel sPLA₂ (Lys⁷ and Lys¹⁵) suggests its involvement in the antimicrobial activity. Its preferential hydrolysis of PE and PG (Table I) agrees well with this speculation, because they are major components of bacterial phospholipid.

Northern analysis detected an intense signal of the novel sPLA₂ transcript of relatively short size in the human pancreas, which displays abundant expression of group IB sPLA₂. Cross-hybridization of the novel sPLA₂ probe with group IB sPLA₂ mRNA is not likely, because the calculated identity between these two cDNAs is only 53%. One possible function of the novel sPLA₂ is the digestion of phospholipid in nutrition. Another possibility is the involvement of the PLA₂-specific receptor, which is known to be relevant to various biological reactions, because the pancreas is one of the tissues displaying ample expression of the PLA₂ receptor in humans (55). In this context, the receptor binding activity of the novel sPLA₂ should be evaluated in the future.

Radiation hybrid mapping analysis revealed the location of a human novel sPLA₂ gene on chromosome 1 in the vicinity of WI-5273. Around this region, group IIA, IIC, and V sPLA₂ genes have already been mapped (1p34-p36) (56), whereas group IB and X sPLA₂ genes are located on chromosome 12 and 16, respectively (12, 57). These findings indicate that three sPLA₂ genes (IIA, V, and the novel type) and one pseudogene (IIC) constitute a gene cluster that is likely to have emerged from ancient gene duplication events. Some mammalian genes such as globin and apolipoprotein genes are known to form a gene cluster that also includes pseudogenes (58, 59). It is interesting to note that some members of the apolipoprotein multigene family show similarities in the structural organization of their regulatory regions (60). A close linkage among sPLA₂ isofrom genes suggests the possibility that they are under similar or overlapping transcriptional control. Of special interest is the similar up-regulation of expression between the novel sPLA₂ and the group IIA sPLA₂ in the thymus in the response to LPS challenge (Fig. 4A). In the promoter region of human and rat group IIA sPLA₂ gene, a putative interleukin-6-responsive element that is found in several acute phase genes was identified (19). Analysis of the regulating region of the
novel sPLA2 gene should provide a clue to the functional signifi-
cance of this isoform.

In conclusion, we isolated novel mouse and human sPLA2s (group IID) and characterized the activities and expression. This sPLA2 shares common structural and catalytic features with previously known sPLA2 isoforms and is especially related to group IIA sPLA2. Further studies are required to establish the precise physiological functions of this new sPLA2 and to determine its role in disease states, especially in inflammatory conditions. Finally, the discovery of this novel sPLA2 should enable more precise assignment of distinct functions of each isoform and should also broaden our understanding of the biochemical properties of the sPLA2 family.

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