A Natural Disruption of the Secretary Group II Phospholipase A₂ Gene in Inbred Mouse Strains*

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The synovial fluid or group II secretory phospholipase A₂ (sPLA₂) has been implicated as an important agent involved in a number of inflammatory processes. In an attempt to determine the role of sPLA₂ in inflammation, we set out to generate sPLA₂-deficient mice. During this investigation, we observed that in a number of inbred mouse strains, the sPLA₂ gene was already disrupted by a frameshift mutation in exon 3. This mutation, a T insertion at position 166 from the ATG of the cDNA, terminates out of frame in exon 4, resulting in the disruption of the calcium binding domain in exon 3 and loss of both activity domains coded by exons 4 and 5. The mouse strains C57BL/6, 129/Sv, and B10.RII were found to be homozygous for the defective sPLA₂ gene, whereas outbred CD-1:SW mice had variable genotype at this locus. BALB/c, C3H/HE, DBA/2, NZB/BIN, and MRL Ipr/Ipr mice had a normal sPLA₂ genotype. The sPLA₂ mRNA was expressed at very high levels in the BALB/c mouse small intestine, whereas in the small intestine of the sPLA₂ mutant mouse strains, sPLA₂ mRNA was undetectable. In addition, PLA₂ activity in acid extracts of the small intestine were approximately 40 times higher in BALB/c than in the mutant mice. Transcription of the mutant sPLA₂ gene resulted in multiple transcripts due to exon skipping. None of the resulting mutant mRNAs encoded an active product. The identification of this mutation should not only help define the physiological role of sPLA₂ but also has important implications in mouse inflammatory models developed by targeted mutagenesis.

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The abbreviations used are: sPLA₂, synovial fluid or group II secretary phospholipase A₂; PLA₂, phospholipase A₂; bp, base pair(s); PCR, polymerase chain reaction; RT-PCR, reverse transcriptase-PCR; kb, kilobase pair(s); LPS, lipopolysaccharide; PBS, phosphate-buffered saline.

Secretory nonpancreatic phospholipase A₂ (sPLA₂) has been implicated as one of the important pathogenetic agents in both local and systemic inflammatory conditions (for reviews, see Refs. 1–3). Levels of circulating sPLA₂ in both adult (4) and juvenile (5) rheumatoid arthritis as well as in septic shock (6) and multigran failure (7) correlate positively with disease severity and poor prognosis. sPLA₂ gene expression has also been shown to be induced by various inflammatory stimuli, like endotoxin, IL-1β, TNF-α, and IL-6 (1, 2). Nevertheless, the exact mode of action of sPLA₂ and the role it plays in these diseases is not very well understood. sPLA₂ catalyzes the release of fatty acid from the sn-2 position of phospholipids producing free fatty acid and lysophospholipid. When the fatty acid is arachidonic acid, which is usually esterified at this position in phospholipids, it can lead to the production of the various leukotrienes and prostaglandin proinflammatory mediators. The other product of this reaction, lysophospholipid, can also be metabolized into a potent inflammatory mediator (platelet-activating factor). However, since sPLA₂ has very little preference for the type of fatty acid in the sn-2 position, it is not known to what extent the proinflammatory activity of sPLA₂ is mediated through the release of arachidonic acid and its subsequent metabolism into proinflammatory mediators.

One approach to determine the role of sPLA₂ in these various disease states would be to develop potent specific inhibitors of this enzyme. Recently, there have been a number of sPLA₂ inhibitors reported (8–13). However, none of these inhibitors have been able to convincingly determine the role sPLA₂ plays in inflammation. In an attempt to circumvent this problem and investigate the role of sPLA₂ in inflammation, we initiated studies to generate mice with a targeted disruption of the sPLA₂ gene. Provided, the mutation was not lethal, sPLA₂-deficient mice could be used in inflammatory models to determine the contribution of sPLA₂ in the inflammatory process.

During this investigation, we observed that the sPLA₂ gene, cloned from a 129/Sv-derived genomic library, already contained a natural mutation. This mutation causes a disruption in the reading frame of exon 3 resulting in an inactive gene product. This paper describes the nature of this mutation, its consequence, and its prevalence in a number of inbred mouse strains.

MATERIALS AND METHODS

Animals—Male mice, 20–25 g, were obtained from the following companies: CD-1 Swiss Webster, which is an outbred strain and BALB/c were from Charles River Laboratories (St. Constant, Quebec); 129/Sv were from Taconic (Germantown, NY); C57BL/6 were obtained from three suppliers, Jackson Laboratories (Bar Harbor, ME), Charles River Laboratories, and Taconic; and B10.RII and MRL Ipr/Ipr were from Jackson Laboratories.

Cloning of the Mouse sPLA₂ Gene—The mouse sPLA₂ gene was cloned from a CC1.2 Sau3A genomic library in XAD5H1 (Stratagene) using standard molecular biology methods (14). The probe used for...
screening was a random primed (Pharmacia Biotech) \textsuperscript{32}P-labeled rat sPLA\textsubscript{2} cDNA, a generous gift of Dr. H. Van den Bosch (15). When the library was initially screened with the full-length sPLA\textsubscript{2} cDNA, approximately 1 x 10\textsuperscript{9} phage were plated, and 30 positive clones were picked. These clones were all identical and were missing the 3'-end of the gene. The library was replated and screened using a 160-bp PvuII/HindIII fragment containing the 3' coding region of the rat sPLA\textsubscript{2} cDNA. This screening produced a single positive clone. The \( \lambda \) clones were mapped, and the appropriate DNA fragments containing the sPLA\textsubscript{2} gene were cloned and sequenced on both strands using an ABI 373A automated DNA sequencer.

Cloning of the Mouse sPLA\textsubscript{2} cDNA and COST7 Transfection—Primers derived from the 5' (5'-AGCTGAGCATGAGTCTTCC-3') and 3' (5'-TTCTGGGTAGAAGACAGAAGGGCC-3') ends of the coding region of the mouse sPLA\textsubscript{2} gene were used in RT-PCR reactions to clone the mouse sPLA\textsubscript{2} cDNA. These primers will also amplify the rat sPLA\textsubscript{2} cDNA. The RT-PCR reactions were carried out on 1 \( \mu \)g of total RNA using conditions described by the manufacturer (Perkin Elmer). The amplification conditions were 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s for 35 cycles. The PCR products were electrophoresed on a 1% low melt agarose gel, recovered, and cloned into the pCR1 cloning vector (Invitrogen). The cDNA clones were sequenced on both strands using an ABI 373A automated DNA sequencer. The sPLA\textsubscript{2} cDNA sequences were then cloned into the eukaryotic expression vector pSG5 (Stratagene). The various sPLA\textsubscript{2} cDNAs were then transfected into COS7 cells as described previously (16). The sPLA\textsubscript{2} activity released into the media 48 h after transfection was then determined.

Genomic Analysis—The DNA used in the genomic analyses were either prepared (14) or obtained from commercial sources (see the legends to Fig. 3 and Table I). DNA was digested with BamHI and analyzed by Southern blotting using the genomic 2.5-kb HindIII/NotI (the NotI site is in the iDash 11 multiple donoring sequence) probe from \( \lambda \) (see Fig. 1). PCR analysis of exon 3 was carried out using the following primers: 5'-primer (5'-CTGGTTTCTCCTCCTTCTCAGCTGGTG-3'), 3'-primer (5'-GGAAAACACTGGGACACTGAGGTAGTG-3'). The PCR reaction was performed using 1 \( \mu \)g of genomic DNA, 50 pmol of each primer, and the following cycling conditions: 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s for 35 cycles. The amplified exon 3 DNA fragment was cloned and sequenced as described above. For genotype analysis, the PCR product was digested with BamHI and analyzed on 1% low melt agarose gels.

PLA\textsubscript{2} Activity—PLA\textsubscript{2} activity was measured as described previously (17) using radiolabeled E. coli membrane phospholipid substrate. One unit of PLA\textsubscript{2} activity is defined as the hydrolysis of 56 pmol of phospholipid (representing 1% of total phospholipid) in 30 min at 37 °C. sPLA\textsubscript{2} was prepared from the mouse small intestine using acid extraction as described by Kramer et al. (18). Briefly, mouse small intestine (0.23–0.38 g wet weight) was homogenized in 2 ml of homogenization buffer (30 mM Tris, pH 7.5, 120 mM NaCl, and 2 mM EGTA) for 3 x 20 s on ice. An equal volume of cold 0.36 M H\textsubscript{2}SO\textsubscript{4} was added, mixed by vortexing, and placed on ice for 1 h. The acid extracts were centrifuged for 30 min at 10,000 \( \times g \), the supernatant was recovered, and the protein concentration was determined by Bradford assay (Pierce). A small aliquot of the supernatant (200 \( \mu \)l) was neutralized with 1 M Tris base (35 \( \mu \)l), and the PLA\textsubscript{2} activity was determined. The PLA\textsubscript{2} activity in the media of transfected COS7 cells was also determined.

Lipopolysaccharide (LPS) Treatment of Mice and RNA Isolation—LPS (0111:B4, Sigma) in phosphate-buffered saline (PBS) was used for all injections. Mice were injected intraperitoneally with either 5 or 10 \( \mu \)g of LPS, and 5 h later tissues were removed. LPS up to 500 \( \mu \)g was also injected intravenously into the tail vein, and tissues were collected 18 h after injection. In an attempt to enhance the effect of LPS on sPLA\textsubscript{2} gene expression, mice were first primed with 5 mg of carrageenan (FMC Corp., Rockport ME) (in 0.5 ml of PBS) injected intraperitoneally, and 24 h later injected intravenously with varying concentrations of LPS up to 10 \( \mu \)g as described by Ogata et al. (19). Tissues were removed from these animals 5 h after the LPS injection. Total RNA was prepared from tissue samples using Trizol (Life Technologies, Inc.) reagent as described by the manufacturer. Northern blot analysis of total RNA was performed using 1% agarose formaldehyde gels to separate the RNA, which was then transferred to nitrocellulose. Blots were probed with the mouse sPLA\textsubscript{2} cDNA, stripped, and reprobed with a human glyceraldehyde 3-phosphate dehydrogenase cDNA (Clontech). The 

**RESULTS**

**Mouse sPLA\textsubscript{2} Gene**—As a first step in generating mice with a targeted disruption in the sPLA\textsubscript{2} gene, a CCL2 embryonic stem cell genomic library was screened to obtain an isogenic genomic clone of the mouse sPLA\textsubscript{2} gene (20). The CCL2 line was originally derived from the mouse strain 129/Sv (21). The preliminary screening of the library using a rat sPLA\textsubscript{2} cDNA probe resulted in 30 positive clones, all of them identical to \( \lambda \) (Fig. 1). The sPLA\textsubscript{2} gene was located at one end of this genomic clone, subcloned, sequenced, and was found to be missing exon 5. In order to obtain the complete gene, the library was re-screened with a 3' rat sPLA\textsubscript{2} cDNA probe (see "Materials and Methods"). One positive clone, \( \lambda \) was obtained from this screening, and it contained the complete gene (Fig. 1). The sequence of the mouse sPLA\textsubscript{2} gene is shown in Fig. 2. It is not a large gene, the complete coding sequence including introns and exons spans about 3.5 kb. The organization of the mouse gene is identical to that of the human and rat genes (18, 22–24).

**sPLA\textsubscript{2}-deficient Inbred Mouse Strains**

**Fig. 1. Genomic organization of the mouse sPLA\textsubscript{2} gene.** Restriction map and localization of the sPLA\textsubscript{2} gene in the \( \lambda \) and \( \lambda \) sPLA\textsubscript{2} genomic clones are illustrated. The exons are denoted by numbers below the corresponding box. The additional BamHI site in exon 3 of \( \lambda \) is shown, and the HindIII genomic fragment from the 3'-end of \( \lambda \) used as the probe for Southern blotting is underlined.

stem cell genomic library was screened to obtain an isogenic genomic clone of the mouse sPLA\textsubscript{2} gene (20). The CCL2 line was originally derived from the mouse strain 129/Sv (21). The preliminary screening of the library using a rat sPLA\textsubscript{2} cDNA probe resulted in 30 positive clones, all of them identical to \( \lambda \) (Fig. 1). The sPLA\textsubscript{2} gene was located at one end of this genomic clone, subcloned, sequenced, and was found to be missing exon 5. In order to obtain the complete gene, the library was re-screened with a 3' rat sPLA\textsubscript{2} cDNA probe (see "Materials and Methods"). One positive clone, \( \lambda \) was obtained from this screening, and it contained the complete gene (Fig. 1). The sequence of the mouse sPLA\textsubscript{2} gene is shown in Fig. 2. It is not a large gene, the complete coding sequence including introns and exons spans about 3.5 kb. The organization of the mouse gene is identical to that of the human and rat genes (18, 22–24).

It consists of five exons, each representing a specific domain of the enzyme; exon 1 is a 5'-untranslated sequence, exon 2 codes for the signal peptide sequence, exon 3 codes for the calcium binding domain, and exons 4 and 5 code for catalytic activity domains. The mouse and rat sPLA\textsubscript{2} gene sequences including introns are nearly 80% identical.

However, between the two mouse sPLA\textsubscript{2} \( \lambda \) genomic clones there appeared to be a BamHI polymorphism. When \( \lambda \) was digested with BamHI, two DNA fragments hybridized with the sPLA\textsubscript{2} cDNA probe, a 5.7-kb 5'-fragment and a 600-bp 3'-fragment representing the end of this clone. Based on the sequence from the sPLA\textsubscript{2} gene in \( \lambda \), this BamHI site occurs in exon 3. This appears to be a conserved sequence since this BamHI site is also present in exon 3 of the rat and human genes. A similar BamHI digest of \( \lambda \) or of embryonic stem cell genomic DNA resulted in a single 8.2-kb fragment. An explanation for this discrepancy is that \( \lambda \) originated from the stem cell, whereas \( \lambda \) was from contaminating STO feeder cell genomic DNA. The STO feeder cells are mouse fibroblasts derived from the inbred Swiss strain SIM (25). Subsequent sequencing of the sPLA\textsubscript{2} gene in \( \lambda \) revealed that the BamHI site in exon 3 was lost because of an insertion of an extra T residue (GGATCC → GGATTCC). This insertion not only
Fig. 2. DNA sequence of the mouse sPLA2 gene. The sequence shown is from α6, with the mutation in exon 3 removed, in order to show the correct translation of the gene. Highlighted within the sequence are the putative TATA box, which is overlined; the five exons and the deduced amino acid sequence, which are underlined; the BamHI site in exon 3, which contains the T insertion mutation is in boldface and has an asterisk below it; and a putative polyadenylation site, which is in boldface. The TATA box, exon 1, and the polyadenylation site are based on the structure of the rat sPLA2 gene (23, 24).
sPLA₂ enzymewould betotally inactivesince both the calcium sequences that make up the active site (see Fig. 5). This and terminate out of frame in exon 4, losing both the exon 4 and would disrupt the BamHI recognition sequence, but it would also cause a frameshift in the expressed mRNA. The resulting sPLA₂ enzyme would have a disrupted calcium binding domain and terminate out of frame in exon 4, losing both the exon 4 and 5 sequences that make up the active site (see Fig. 5). This sPLA₂ enzyme would be totally inactive since both the calcium and catalytic activity domains are absolutely required for activity. This result would indicate that the full-length sPLA₂ gene in A6 isolated from the ES stem cell genomic library codes for an inactive gene product. There are at least four possible explanations for this result. First, the mutation was a sequencing error (this is unlikely since this region was sequenced with a number of different primers on both strands, and in addition this sequence is not cleaved by BamHI); second, the mutation was generated during cloning: third, it could be an artifact of the ES cell line; and fourth, the mutation originated from the 129/Sv mouse strain from which the ES cells were originally derived. If this last possibility was correct, then there already existed a mouse strain that had a natural disruption of the sPLA₂ gene.

Disruption of the sPLA₂ Gene in Inbred Mouse Strains In order to determine if this sPLA₂ gene mutation originated from the 129/Sv mouse strain or was due to a cloning or cell line artifact, genomic DNA was prepared from these mice, digested with BamHI, and analyzed by Southern blotting (Fig. 3A). The presence of the 8.2-kb BamHI fragment would be indicative of this mutation, whereas hybridization to 5.7- and 2.5-kb fragments would suggest a wild-type sPLA₂ gene structure. In addition to the 129/Sv mouse strain, genomic DNA from, C57BL/6, BALB/c, and CD-1 Swiss Webster mice was also analyzed (Fig. 3). The Southern blotting indicates that both the 129/Sv and the C57BL/6 mouse strains are homozygous for this sPLA₂ mutation. The BALB/c mouse strain has a wild-type genotype and the CD-1 mouse chosen for this experiment was heterozygous for the mutation. The heterozygosity of the sPLA₂ gene in CD-1 mice is due to the fact that this is an outbred mouse strain.

To confirm the Southern blotting result and prove that the loss of the BamHI site in exon 3 was due to the T insertion, exon 3 was amplified from each of these mouse strains, cloned, and sequenced. An example of this analysis is shown in Fig. 3B, in which exon 3 clones from these mouse strains were digested with BamHI. The C57BL/6 exon 3 clones were not cleaved by BamHI, whereas all of the BALB/c exon 3 clones contained the BamHI site and gave the appropriate cleavage products. The CD-1 exon 3 clones, as expected, consisted of both types. Analysis of the exon 3 DNA sequences revealed that 129/Sv, C57BL/6 and the mutant allele in the CD-1 all had the T insertion mutation.

We have also analyzed the sPLA₂ gene structure for a number of additional mouse strains, and the results are presented in Table I. Most of the mouse strains tested had a homozygous wild-type sPLA₂ BamHI digestion pattern. The heterozygosity of the B6/D2F1 mouse would indicate that the DBA/2 mouse strain has the wild-type sPLA₂ gene, since C57BL/6 is homozygous for the sPLA₂ mutation. The congenic strain B10.RIII has the mutated sPLA₂ BamHI digestion pattern, which would mean that the C57BL/10 mouse strain also has the mutated sPLA₂ gene. This is very likely since C57BL/6 and C57BL/10 are substrains of C57BL. Based on the known genealogy (26) of inbred mouse strains, it is possible that 129/Sv and C57BL could have originated from a common ancestor that passed on these characteristics. The origin of the other mouse strains listed in Table I are much further removed from these two strains. Mutant and Wild-type sPLA₂ Gene Expression—The expression of sPLA₂ mRNA and enzyme activity was next analyzed to confirm that the sPLA₂ gene mutation results in an inactive enzyme. Mulherkar et al. (27, 28) have cloned the BALB/c mouse sPLA₂ cDNA from the small intestine where it is present at very high levels and localized in the Paneth cell (27-31). sPLA₂ mRNA expression and enzyme activity were determined in the small intestine of control and LPS-injected, BALB/c mice (sPLA₂ wild-type) and C57BL/6 mice (sPLA₂ mutant). Northern blot analysis of total RNA isolated from these animals and hybridized with a mouse sPLA₂ cDNA probe is shown in Fig. 4A. High levels of sPLA₂ mRNA can be detected in both control and LPS-treated BALB/c mice. In control and LPS-treated C57BL/6 mice, sPLA₂ mRNA was undetectable.

![Fig. 3. Genomic analysis of the sPLA₂ gene. A, Southern blot analysis of BamHI-digested mouse genomic DNA from the mice strains shown and the ES cell line D3 (129/Sv-derived) was hybridized with the the 3'-end HindII probe of A3 (Fig. 1). The two C57BL/6 lanes represent DNA isolated from two separate animals. The BALB/c genomic DNA was from Clontech. All other genomic DNAs were prepared. B, analysis of PCR clones of exon 3 from the mice indicated. Exon 3 of the sPLA₂ gene was amplified from mouse genomic DNA as described under "Materials and Methods" and cloned into the pCR II vector. Four clones from each amplification were digested with EcoRI, which releases the inserted DNA and with BamHI. Lane 6 from the amplification of CD-1 genomic DNA contains two exon 3 fragments, one that is digested by BamHI and the other that is not.](image-url)
for the majority of the acid-stable PLA2 activity in the BALB/c weight secretory phospholipases A2, which are also acid-stable. Likely due to one or a combination of the other low molecular weight mRNA expression in the rat (33). One likely explanation is that the level of PLA2 expression is already so high in the BALB/c small intestine that it would be difficult to see any amount of induction in this tissue. In other tissues like the aorta and in the small intestine of mice with the PLA2 mutation, sPLA2 mRNA can be induced by LPS (see below).

In order to prove that the BALB/c sPLA2 mRNA codes for an active sPLA2 enzyme and the mutant sPLA2 gene codes for an inactive form, both mutant and BALB/c sPLA2 cDNAs were cloned by RT-PCR. We had found that it was possible to induce expression of the sPLA2 mRNA in mice carrying the mutated sPLA2 gene if extreme conditions are used for the induction. When mice are first pretreated with carragenan and then injected 24 h later with LPS there is a tremendous enhancement of LPS-induced TNF production and mouse mortality by endotoxin shock (19). Under these conditions, sPLA2 mRNA can be detected in the small intestine by Northern blot analysis and in the aorta by RT-PCR (not shown) of mice with the mutated sPLA2 gene. It should be noted that BALB/c mice do not survive this treatment; they die shortly after the carragenan injection. We have used small intestine total RNA prepared from a CD-1 mouse that was homozygous for the sPLA2 mutation (–/–/CD-1) (verified by BamHI genomic Southern blotting) and treated in the above fashion and from BALB/c for RT-PCR to amplify and clone both the mutant and wild-type mouse sPLA2 cDNAs. The PCR products from these amplifications are shown in Fig. 4B. Both the BALB/c and rat osteoblast (control) RNA produced the correct size 595-bp PLA2 cDNA product (Fig. 4B), which could also be digested by BamHI (not shown). The sPLA2 cDNA products from amplification of total RNA isolated from the –/–/CD-1 mouse (or from any of the mutant sPLA2 mice, 129/Sv, C57BL/6) gave multiple sPLA2 cDNA products (Fig. 4B, lane 2) that could not be digested by BamHI (not shown). Both the BALB/c sPLA2 cDNA and the mutant sPLA2 cDNA RT-PCR products were cloned and sequenced and are shown in Fig. 5. The two more strongly amplified products from the –/– mouse were the full-length sPLA2 cDNA with the T insertion (InsPLA2) and a sPLA2 cDNA with exon 3 removed (X3sPLA2). A third sPLA2 cDNA with exon 4 spliced out was also found (X4sPLA2). These sPLA2 cDNAs would most likely code for inactive enzymes since they all have disrupted calcium binding and catalytic activity domains. Both the mutant and wild-type sPLA2 cDNAs were cloned into the eukaryotic expression vector pSG5 and transiently transfected into COS7 cells. 48 h after transfection, PLA2 activity in the media of the transfected cells was determined. Cells transfected with any of the mutant sPLA2 cDNAs did not release any detectable PLA2 activity into the media, confirming that mice strains carrying the mutant sPLA2 gene do not express an active sPLA2 enzyme. In contrast, the media of cells transfected with the BALB/c sPLA2 cDNA contained greater than 3,000 units/ml of PLA2 activity, confirming that the BALB/c sPLA2 gene codes for an active enzyme.

**Discussion**

In this study we have shown that the murine sPLA2 gene is naturally disrupted in a number of inbred strains. This discovery came about following initial experiments to disrupt sPLA2 by targeted mutagenesis in order to derive a sPLA2 mutant model. It has been recognized that to increase the efficiency of gene targeting in ES cells, the targeting vector must be derived from DNA that is isogenic to the cell line (20). While screening a genomic DNA bacteriophage library prepared from the ES cell C112, a BamHI restriction polymorphism was discovered between an isogenic sPLA2 clone and a clone that originated from the contaminating STO feeder cells. This polymorphism was due to the insertion of a thymidine residue in exon 3 of the ES cell gene. The resulting transcribed mRNA would contain a
frameshift mutation and code for an inactive enzyme, since both the calcium binding and catalytic activity domains encoded by exons 3, 4, and 5 were absent. The CC1.2 ES cell line, having been derived from the mouse strain 129/Sv, suggested that a sPLA2 mutant already existed in mouse populations, while STO cells, having been derived from an inbred Swiss line (25), indicated that the gene was not mutant in all strains. A survey of inbred mouse stains demonstrated that the sPLA2 gene is disrupted in 129/Sv and C57BL/6 but not in BALB/c. It is likely that this mutation would have been overlooked had the polymorphism between CC1.2 and STO not been observed. Therefore, it may be advisable that before attempting to generate targeted mutations in mice, one should first check the expression of the particular gene in a panel of inbred strains.

Based on the above observations, there exists the possibility that “knockout” mice with no overt phenotype, may have had the gene of interest already disrupted.

The loss of the sPLA2 gene appears not to have a detrimental effect on the viability of these mice strains. C57BL/6 is one of the mostly widely used mouse strains and accounts for more than 14% of studies in which an inbred strain is used (34). Thus, the sPLA2 gene does not appear to play a critical role in the development of the animal, nor does it appear to have an effect on fertilization as has been suggested (35, 36).

The high levels of sPLA2 present in inflammatory synovial fluids of rheumatoid arthritis patients was suggestive of a proinflammatory role for sPLA2 in this disease (1–3). The levels of circulating sPLA2 also correlated very well with disease severity in both adult and juvenile rheumatoid arthritis (4, 5). However, the function of sPLA2 in rheumatoid arthritis is unknown. It has been suggested that its involvement in rheumatoid arthritis may be to potentiate an already established inflammatory process (1). The mouse has been used extensively as a model for arthritis. Knowing the sPLA2 genotype of several of the inbred mouse strains, we attempted to see if there was any correlation between mouse strain, the functionality of the sPLA2 gene, and the arthritic model. In antigen-induced arthritic models, both sPLA2-deficient and sPLA2 wild-type mouse strains are susceptible to arthritis. The sPLA2-deficient strains C57BL/6 and B10.RIII have been used in methylated FIG. 5. DNA sequence of wild-type and mutant sPLA2 cDNAs. The three sPLA2 cDNAs isolated from the CD-1 sPLA2-deficient mouse are identified as follows: 1msPLA2 contains the complete sPLA2 coding region, but it has the T insertion (asterisk) at position 166 from the ATG; X3sPLA2 has exon 3 deleted; X4sPLA2 has exon 4 deleted. The BALB/c sPLA2 cDNA, which codes for an active enzyme, is identified as MsPLA2. The ATG initiation codon and the termination codons for each sequence are shown in boldface. The position of the exon sequences within the cDNA sequence are underlined. Two polymorphisms at positions 101 and 231 between the BALB/c and CD-1 sPLA2 cDNA sequences were found and are underlined.
sPLA2 to the Paneth cell of the BALB/c small intestine and this type of analysis to other sPLA2 proinflammatory activities arthritic models. A similar problem arises when trying to apply complicated by a number of factors, such as the genetic variability of each inbred strain and the complex nature of these arthritic models. A similar problem arises when trying to apply this type of analysis to other sPLA2 proinflammatory activities such as its involvement in eicosanoid production and in septic shock. The best approach to address these issues and obtain a better understanding of the role of sPLA2 in these diseases would be to generate sPLA2 wild-type and sPLA2-deficient mice with the same genetic background. Such mouse strains could then be used to reevaluate the role of sPLA2 in these disease models.

Given the potential role of sPLA2 in inflammation, our results suggest that caution must be exercised in generating genetic models for inflammation based on the application of targeted mutagenesis in the mouse. The targeted disruption of a gene is always carried out in ES cells derived from the 129/sv mouse strain, while the mutation is bred into either the 129/sv or C57BL/6 background, both of which bear the natural sPLA2 gene disruption. Such mice will have mutations in two proinflammatory genes, which would greatly complicate the interpretation of results. Therefore, it is perhaps advisable that gene targeting experiments of proinflammatory genes be conducted in a genetic background where the sPLA2 gene is intact.

A number of other functions have also been attributed to sPLA2. Mulherkar et al. (27-31) have localized the murine sPLA2 to the Paneth cell of the BALB/c small intestine and have proposed that sPLA2 acts as an enhancing factor for epidermal growth factor binding to cells, thus playing a role in cellular proliferation within the small intestine (27-31). Another group has recently purified sPLA2 from the BALB/c small intestine and has shown it to have significant bactericidal properties (32). They suggest that the anti-microbial activity of sPLA2 protects the small intestine from microbial infection. Consequently, sPLA2-deficient mice might be expected to be more susceptible to small intestine infections and have problems in regulating the high cell turnover that occurs in this tissue. However, sPLA2-deficient mouse strains appear not to have any gross observable differences in the physiology of the small intestine (34). This would suggest that either the mice survive without these sPLA2 activities or that perhaps the minimal PLA2 activity still present in the intestine of the mutant mice is sufficient to maintain homeostasis. There are at least three other low molecular weight sPLA2s identified in humans and rodents. These are the pancreatic PLA2 and two recently cloned PLA2s, one of which appears to be localized in the heart (42) and the other in the testes (43). Whether or not these other secretory PLA2s compensate for the loss of sPLA2 activity in these mice remains to be determined.

Nonsense or frameshift mutations have been shown to cause reduced steady-state levels of mRNA (for review, see Refs. 44 and 45). The insertion of a single T residue into exon 3 of the mouse sPLA2 gene demonstrates this quite effectively. In mice carrying this mutation, no sPLA2 mRNA was detectable by Northern blot analysis in the small intestine, whereas in wild-type animals, it is present at very high levels. However, the

\[ E = \frac{1}{2}mv^2 \]

\[ s = ut + \frac{1}{2}at^2 \]

\[ a = \frac{v}{t} \]

\[ F = ma \]

\[ V = \frac{S}{T} \]

\[ I = \frac{V}{R} \]

\[ P = IV \]

\[ Q = \int P \, dt \]

\[ d = \frac{1}{2}a(t^2 - t_0^2) \]

\[ T = \frac{1}{2} \pi \sqrt{\frac{2m}{k}} \]

\[ W = \int F \, dx \]

\[ H = K + U \]

\[ F = \sum \frac{k}{r^2} \]

\[ E = \frac{1}{2}k \frac{1}{2} \]

\[ m = \frac{V^3}{4 \pi^2} \]

\[ E = m \frac{V^2}{2} \]

\[ F = \frac{d}{dx} \frac{1}{2}mv^2 \]

\[ P = \frac{d}{dt} \frac{1}{2}mv^2 \]

\[ P = \frac{d}{dt} \frac{1}{2}mv^2 \]

\[ E = \frac{1}{2}m(v^2 - u^2) \]

\[ E = \frac{1}{2}m \frac{v^2 - u^2}{2} \]

\[ F = \frac{d}{dx} \frac{1}{2}mv^2 \]

\[ E = \frac{1}{2}mv^2 \]

\[ P = \frac{d}{dt} \frac{1}{2}mv^2 \]

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