Group X Secreted Phospholipase A2 Releases ω3 Polyunsaturated Fatty Acids, Suppresses Colitis, and Promotes Sperm Fertility*

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Within the secreted phospholipase A2 (sPLA2) family, group X sPLA2 (sPLA2-X) has the highest capacity to hydrolyze cellular membranes and has long been thought to promote inflammation by releasing arachidonic acid, a precursor of pro-inflammatory eicosanoids. Unexpectedly, we found that transgenic mice globally overexpressing human sPLA2-X (PLA2G10-Tg) displayed striking immunosuppressive and lean phenotypes accompanied by marked elevation of free ω3 polyunsaturated fatty acids (PUFAs) and their metabolites. Studies using Pla2g10-deficient mice revealed that endogenous sPLA2-X, which is highly expressed in the colon epithelium and spermatozoa, mobilized ω3 PUFAs or their metabolites to protect against dextran sulfate-induced colitis and to promote fertilization, respectively. In colitis, sPLA2-X deficiency increased colorectal expression of arachidonic acid (AA; C20:4) released by PLA2 is converted to pro-inflammatory eicosanoids, sPLA2-X have long been implicated in inflammation (1). However, recent studies using sPLA2 transgenic (Tg) or knock-out (KO) mice have revealed more diverse roles of sPLA2s in various events through eicosanoid-dependent or -independent mechanisms in response to given microenvironmental cues (2–8). Individual sPLA2s exhibit distinct tissue or cellular distributions and substrate phospholipid selectivity (in terms of polar head and sn-2 fatty acyl groups), which underlies their non-redundant, tissue-specific functions (9, 10).

Accumulating evidence suggests that sPLA2s, while promoting inflammation, also play anti-inflammatory roles in certain situations (9, 10). sPLA2-IIA protects against sepsis or pneumonia by eliminating bacteria as a “bactericidal” sPLA2 (11), although it also acts as an “inflammatory” sPLA2 that amplifies inflammation by hydrolyzing extracellular mitochondrial membranes (7). sPLA2-V, a “Th2-prone” or “metabolic” sPLA2 that is induced by Th2 cytokines or obesity-associated stress, promotes M2 polarization of macrophages partly by altering the balance between unsaturated and saturated fatty acids or by promoting phagocytic clearance of harmful materials, thereby attenuating infection, arthritis, and obesity (8, 12–15). sPLA2-IIID, a “resolving” sPLA2 that is expressed in lymphatic dendritic cells, attenuates contact dermatitis by mobilizing ω3 polyunsaturated fatty acid (PUFA)-derived pro-resolving lipid mediators such as docosahexaenoic acid (DHA; C22:6)-derived resolvins D1 (RvD1) (5).

Among the phospholipase A2 (PLA2)3 family, which hydrolyzes the sn-2 position of phospholipids to yield fatty acids and lysophospholipids, secreted PLA2 (sPLA2) enzymes comprise the largest subgroup (1). Along with the central dogma that ω6 arachidonic acid (AA; C20:4) released by PLA2 is converted to pro-inflammatory eicosanoids, sPLA2s have long been implicated in inflammation (1). However, recent studies using sPLA2 transgenic (Tg) or knock-out (KO) mice have revealed more diverse roles of sPLA2s in various events through eicosanoid-dependent or -independent mechanisms in response to given microenvironmental cues (2–8). Individual sPLA2s exhibit distinct tissue or cellular distributions and substrate phospholipid selectivity (in terms of polar head and sn-2 fatty acyl groups), which underlies their non-redundant, tissue-specific functions (9, 10).

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3 The abbreviations used are: PLA2, phospholipase A2; AA, arachidonic acid; BM, bone marrow; DSS, dextran sodium sulfate; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; GI, gastrointestinal; 12-HHT, 12(S)-hydroxyheptadecatrienoic acid; IID, inflammatory bowel disease; IEC, intestinal epithelial cell; LPC, lysophosphatidylcholine; LPL, lamina propria lymphocyte; PC, phosphatidylcholine; sPLA2, secreted PLA2; sPLA2-X; group X sPLA2; PG, prostaglandin; PE, phosphoethanolamine; cPLA2α, cytosolic phospholipase A2α; Tg, PLA2G10-Tg.

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ing AA metabolism. Indeed, mice deficient in sPLA2-X (Pla2g10⁻/⁻) are refractory to pulmonary and cardiovascular disorders in association with reduced eicosanoid levels (2, 19–22). In contrast, overexpression of sPLA2-X in cultured macrophages elicits anti-inflammatory responses (23). Furthermore, adoptive transfer of Pla2g10⁻/⁻ bone marrow (BM) cells into LDL receptor-null (Ldlr⁻/⁻) mice exacerbates whereas that of human PLA2G10-Tg (PLA2G10 ω3⁺/⁺) BM cells ameliorates atherosclerosis and associated Th1 immunity (24). These observations suggest that sPLA2-X also has anti-inflammatory roles. Moreover, lipidomics studies of sPLA2-X-treated cells or lipopolysaccharides in vitro have demonstrated the release of ω3 PUFA as in addition to ω6 AA (25, 26). However, the ability of sPLA2-X to release ω3 PUFA and the resulting physiological outcomes have not been investigated in vivo. Here, we show that sPLA2-X releases ω3 PUFA in vivo, thereby suppressing colitis and facilitating fertility in the respective tissues where it is highly expressed.

Experimental Procedures

Mice—Pla2g2d⁻/⁻, Pla2g2e⁻/⁻, Pla2g2f⁻/⁻, Pla2g3⁻/⁻, Pla2g4a⁻/⁻, Pla2g5⁻/⁻, Pla2g6⁻/⁻, Pla2g10⁻/⁻, Pla2g12a⁻/⁻, Ptges⁻/⁻, and PLA2G10 ω3⁺/⁺ mice were described previously (4–6, 8, 22, 27–30). C57BL/6 mice were obtained from SLC Japan (Shizuoka, Japan). All mice were housed in climate-controlled (23 °C) specific-pathogen-free facilities with a 12-h light/dark cycle, with free access to standard diet CE2 (CLEA Japan) and water. All procedures involving animals were approved by the Institutional Animal Care and Use Committees of the Tokyo Metropolitan Institute of Medical Science, in accordance with the Standards Relating to the Care and Management of Experimental Animals in Japan.

Histology and Immunohistochemistry—Formalin-fixed tissues were embedded in paraffin, sectioned, mounted on glass slides, deparaffinized in xylene, and rehydrated in ethanol with increasing concentrations of water. The tissue sections (4 μm thick) were incubated with Target Retrieval Solution (Dako, Glostrup, Denmark) as required, incubated for 10 min with 3% (v/v) H2O2, washed three times with phosphate-buffered saline (PBS) for 5 min each, incubated with 5% (w/v) skim milk in PBS for 30 min, washed three times with PBS for 5 min each, and incubated with rabbit antiserum for mouse sPLA2-X at 1:500 dilution in PBS overnight at 4 °C. The sections were then treated with a CSA system staining kit (Dako) with diaminobenzidine substrate, followed by counterstaining with hematoxylin and eosin.

Quantitative RT-PCR—Total RNA was extracted from tissues or cells using TRIzol reagent (Invitrogen). First-strand cDNA synthesis was performed using a high capacity cDNA reverse transcriptase kit (Applied Biosystems, Foster City, CA). PCR was carried out using Power SYBR Green or TaqMan gene expression assay (Applied Biosystems) on the ABI7700 real time PCR system (Applied Biosystems), as described previously (4–6, 8). The probe/primer sets used are listed in Table 1. Gapdh (4352339E; Applied Biosystems) was used as an internal control.

Preparation of Macrophages—Preparation of resident and thioglycolate-induced mouse peritoneal macrophages was described previously (30). Mouse BM cells were cultured in α-minimal essential medium (Wako, Osaka, Japan) containing 10% (v/v) fetal bovine serum (FBS; Invitrogen), 100 units/ml penicillin, and 100 μg/ml streptomycin supplemented with 100 ng/ml M-CSF (Leukoprol; Kyowa Kirin, Tokyo, Japan) to obtain BM-derived macrophages, as described previously (8). These cells were cultured for 8 h in serum-free medium and then for 24 h in culture medium supplemented with 100 ng/ml lipopolysaccharide (LPS, Escherichia coli O111:B4) (Sigma) plus 10 ng/ml mouse interferon (IFN)–γ (PeproTech, Rocky Hill, NJ).

Flow Cytometry—Mouse tissues were excised, minced in Hanks’ solution (Nissui Pharmaceutical, Tokyo, Japan), with 2% (v/v) heat-inactivated FBS and 0.05% (w/v) sodium azide (Nacalai Tesque, Kyoto, Japan), and incubated with 400 units/ml collagenase type II (Worthington) with shaking for 30 min at 37 °C. After adding 10 mM EDTA, the suspensions were passed through cell strainer 70-μm nylon (BD Biosciences) and then centrifuged at 300 × g for 5 min at 4 °C. Except for analysis of the erythrocyte lineage, splenocytes or thymocytes were treated for 2 min on ice with 10 mM Tris-HCl (pH 7.0) containing 0.84% (w/v) ammonium chloride to lyse red cells, centrifuged, and suspended in Hanks’ solution. For flow cytometry, the cells were subjected to blocking with mouse BlockTM (BD Biosciences), incubated with phycoerythrin (PE)-conjugated anti-mouse CD11c (N418; eBioscience), PE-labeled anti-mouse CD11 b (M1/70; BD Biosciences), fluorescein isothiocyanate (FITC)-labeled anti-mouse CD3e (145–2C11; eBioscience), Alexa Fluor 647-labeled anti-mouse CD45R/B220 (RA3–2B8; BD Biosciences), PE-labeled anti-mouse CD4 (GK1.5; eBioscience), Alexa 647-labeled anti-mouse CD8α (53–6.7; BioLegend, San Diego, CA), PE-labeled anti-mouse CD71 (Ri7217; BioLegend), allophycocyanin (APC)-labeled TER119 (TER-119; BioLegend), or isotype control antibody (BioLegend), and analyzed by flow cytometry with a FACSAria III (BD Biosciences) and FlowJo (Tree Star, Ashland, OR) soft-

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TABLE 1

| Name       | Assay no.          |
|------------|--------------------|
| Pla2g4a    | Mm00479527_m1      |
| Pla2g6     | Mm00447090_m1      |
| Pla2g1b    | Mm00478249_m1      |
| Pla2g2d    | Mm00478250_m1      |
| Pla2g2e    | Mm00478870_m1      |
| Pla2g2f    | Mm00478872_m1      |
| Pla2g5     | Mm00481612_m1      |
| Pla2g10    | Mm00449532_m1      |
| Pla2g3     | Mm01191142_m1      |
| Pla2g12a   | Mm00458226_m1      |
| Mrc1       | Mm00458750_m1      |
| Nos2       | Mm00440502_m1      |
| Il1b       | Mm00434228_m1      |
| Il6        | Mm00446190_m1      |
| Il17a      | Mm00439618_m1      |
| Il22       | Mm00444241_m1      |
| Trf        | Mm00432581_m1      |
| Reg3g      | Mm01181783_g1      |
| Cd4        | Mm0042754_m1       |
| Cd8a       | Mm01182107_g1      |
| Epcam      | Mm00493214_m1      |

Accession numbers for TaqMan probes (Applied Biosystems) are indicated.
Microarray—Total RNA was purified using the RNeasy mini kit (Qiagen, Venlo, Netherlands). Microarray analysis was carried out according to the manufacturer’s protocol (Agilent Technologies, Santa Clara, CA), as described previously (6, 8). In brief, the quality of RNA was assessed with a 2100 Bioanalyzer. cRNA targets were synthesized with a low input QuickAMP labeling kit. Samples were hybridized to the Whole Mouse Genome microarray kit (4x44K), washed, and then scanned using a SureScan Microarray Scanner. Microarray data were analyzed with Feature Extraction software and then imported into GeneSpring GX software. Probes were normalized by quantile normalization among all microarray data. The GEO accession numbers for the microarrays are GSE77336 and GSE77144.

CT Analysis—Mice were anesthetized with Nembutal (0.5 mg/g body weight) (Dainippon Sumitomo Pharmaceutical, Osaka, Japan), and their adiposity was analyzed using the micro-CT system Latheta LCT-100 (Aloka, Tokyo, Japan), as described previously (8).

Measurement of Serum Immunoglobulin (Ig) Levels—Serum titers of IgM, IgG1, IgG2, and IgE were determined by a mouse IgX ELISA quantification kit (Bethyl Laboratories, Montgomery, TX).

Dextran Sodium Sulfate (DSS)-induced Colitis—DSS of average molecular weight 36,000–50,000 (MP Biomedicals, Solon, OH) was orally applied to 8-week-old male mice at a concentration of 1–3% (w/v) in drinking water. Changes in body weight, stool consistency, and occult blood in the stool were scored as follows: 0, normal; 2, loose stools; 4, watery diarrhea. Hemoccult was analyzed by the clinical blood analyzer Vetscan HMII (Abaxis, Union, CA). Circulating blood cells were analyzed by the clinical blood cell analyzer Vetscan HMII (Abaxis, Union, CA).

Preparation of Colorectal Lamina Propria Lymphocytes (LPLs)—LPLs were prepared from C57BL/6 mice treated with 3% DSS for 7 days. Briefly, the colon (1 cm in length) was incubated with PBS before use.

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Sperm Fertility—Analyses of spermatozoa were carried out as described previously (3). Briefly, female mice (10 weeks old) were injected intraperitoneally with 7.5 IU pregnant mare serum gonadotropin (Asuka Pharmacy, Tokyo, Japan) followed by 48 h later with 7.5 IU human chorionic gonadotropin (Asuka Pharmacy). After 13 h, the oocyte cumulus complexes from the oviduct were placed in 100 μl of HTF medium (ARK Resource, Kumamoto, Japan) in a 60-mm culture dish, and droplets were covered by embryo-tested mineral oil (Nakalai Tesque). Spermatozoa collected from the cauda epididymis from male mice (8 weeks old) were allowed to swim into 50 μl of HTF medium, aspirated, incubated in 200 μl of HTF medium for 60 min at 37 °C to permit capacitation, diluted, and added to the oocyte droplets to achieve a concentration of 200 spermatozoa/μl. After incubation for 6 h at 37 °C, the oocytes were washed and cultured for 24 h. Fertilization was evaluated by the presence of a second polar body and two pronuclei. As required for experiments, lipids (1 μl) were added to the in vitro fertilization assay.

Electrospray Ionization-Mass Spectrometry (ESI-MS)—All procedures were performed as described previously (4, 5). In brief, tissues were soaked in 10 volumes of methanol and homogenized with a Polytron homogenizer. After overnight incubation at −20 °C, H2O was added to the mixture to give a final methanol concentration of 10% (v/v). As internal standards for determination of recovery, 1 ng of d4-labeled EPA, d4-labeled leukotriene B4, d4-labeled prostaglandin (PG) E2, and d4-labeled 15-hydroxyeicosatetraenoic acid (Cayman Chemicals) were added to the samples. The oxygenated lipids in the supernatant were extracted using Sep-Pak C18 cartridges (Waters, Milford, MA), where the samples in 10% methanol were applied to the cartridges, washed with 10 ml of hexane, eluted with 3 ml of methyl formate, dried up under N2 gas, and dissolved in 60% methanol. The analysis of PUFAs and their metabolites was performed using a 4000Q-TRAP quadrupole-linear ion trap hybrid mass spectrometer (AB Sciex, Framingham, MA) with liquid chromatography (LC) (LC-20AP; Shimadzu, Kyoto, Japan) combined with an HTC PAL autosampler (CTC Analytics, Zwingen, Switzerland). The sample was applied to the Develosil C30-UG column (1 × 150 mm inner diameter, 3 μm particles) (Nomura Chemical, Aichi, Japan) coupled for ESI-MS/MS. The samples injected by the autosampler (10 μl) were directly introduced and separated by
A step gradient with mobile phase A (water containing 0.1% acetic acid) and mobile phase B (acetonitrile: methanol/H₂O 4:1; v/v) at a flow rate of 50 l/min and a column temperature of 45 °C.

For detection of phospholipids, tissues were soaked in 10 volumes of 20 mM Tris-HCl (pH 7.4) and then homogenized with a Polytron homogenizer. Phospholipids were extracted and subjected to ESI-MS using a 4000Q-TRAP and LC-20AP with Develosil C30-UG column, as described previously (4). As an internal standard, 1 nmol of LPC(17:0) (Avanti Polar Lipids, Alabaster, AL) was added to each sample. The samples were separated by a step gradient with mobile phase A (acetonitrile/methanol/water/H₂O 1:1:1 (v/v/v) containing 5 mM phosphoric acid and 1 mM ammonium formate) and mobile phase B (2-propanol containing 5 mM phosphoric acid and 1 mM ammonium formate) at a flow rate of 80 l/min at 50 °C.

Identification was conducted using multiple reaction monitoring transition and retention times, and quantification was performed based on peak area of the multiple reaction monitoring transition and the calibration curve obtained with an authentic standard for each lipid (Avanti Polar Lipids and Cayman Chemicals).

Statistical Analysis—Data are expressed as mean ± S.E. or S.D. Statistical significance between groups was evaluated by two-tailed Student’s t test or one-way analysis of variance at a significance level of p < 0.05.

Results

Immunosuppressive Phenotypes in PLA2G10⁰⁻/⁻ Mice—Although our analysis of PLA2G10⁰⁻/⁻ mice was underway (4), we noticed that PLA2G10⁰⁻/⁻ mice had fewer circulating lymphocytes than did wild-type (WT) mice (Fig. 1A), contrary to our prediction that sPLA₂-X overexpression would increase immune cells through its proposed pro-inflammatory action.

Consistent with the lymphopenia, the weight of the spleen relative to that of the heart was significantly lower (Fig. 1, B and C); the number of splenocytes was 50% lower (Fig. 1D), and the splenic white pulps appeared smaller (Fig. 1E) in PLA2G10⁰⁻/⁻ mice than in WT mice. Although the proportions of splenic CD4⁺ or CD8⁺ T cells and CD11b⁺ monocytes/macrophages were unchanged, the proportion of CD45R⁺ B cells was lower in PLA2G10⁰⁻/⁻ mice than in WT mice (Fig. 1, F and G). As the absolute number of splenocytes was reduced in PLA2G10⁰⁻/⁻ mice, the total counts of splenic T cells and monocytes/macrophages were also lower.
phages were proportionally lower in PLA2G10tg/H11001 mice than in WT mice. Furthermore, the median fluorescence intensity of CD45R on B cells was greater in PLA2G10tg/H11001 mice than in WT mice (Fig. 1G), indicative of altered B cell differentiation. Despite the lower proportion of B cells, serum levels of IgG1 and IgE, but not IgM and IgG2a, were higher in PLA2G10tg/H11001 mice compared to WT mice.

TABLE 2
Microarray gene profiling of the thymus of PLA2G10tg/H11001 mice versus WT mice
Total RNAs were isolated from the thymus of PLA2G10tg/H11001 and littermate WT mice at 6 months. Equal amounts of total RNA (pooled from four mice for each genotype) were subjected to two-color gene expression microarray analysis. Data were processed using the Feature Extraction software from Agilent. Representative genes that showed decreased expression in transgenic (Tg) mice relative to WT mice are listed.

| Gene name | Accession no. | Tg/WT | Description |
|-----------|---------------|-------|-------------|
| Klf2      | NM_008452     | 0.403 | Kruppel-like factor 2 |
| Cd8a      | AK088128      | 0.481 | CD8 antigen, α-chain |
| Ccr9      | NM_009913     | 0.526 | Chemokine (C-C motif) receptor 9 |
| Dynl1     | NM_019682     | 0.532 | Dynein light chain LC8-type 1 |
| Ets2      | NM_011809     | 0.544 | E2F avian leukemia oncogene 2, 3’ domain |
| Wdr78     | NM_146254     | 0.584 | WD repeat domain 78 |
| Rorc      | NM_011281     | 0.623 | RAR-related orphan receptor γ |
| Traf4     | NM_009423     | 0.623 | TNF receptor-associated factor 4 |
| Rip14     | NM_016758     | 0.642 | Regulator of G-protein signaling 14 |
| Kpna2     | NM_010655     | 0.643 | Karyopherin (importin) α2 |
| Phlda1    | NM_009344     | 0.657 | Pleckstrin homology-like domain family A member 1 |
| Soat1     | NM_009230     | 0.663 | Sterol O-acyltransferase 1 |
| Cpn1      | NM_170588     | 0.666 | Copine (Cpn1), transcript variant 1 |
| Nup35     | NM_027091     | 0.671 | Nucleoporin 35 |
| Tdrd5     | XM_129603     | 0.673 | Tudor domain containing 5 |
| Plf23     | NM_030064     | 0.674 | PHD finger protein 23 |
| Bys1      | NM_016859     | 0.679 | Bystin-like |
| Jinj3     | NM_001017426  | 0.686 | Jinjoni domain containing 3 |
| Klf3      | NM_008453     | 0.690 | Kruppel-like factor 3 (basic) |
| Srrm      | NM_009272     | 0.693 | Spermidine synthase |
| Sla2      | BC052655      | 0.693 | Src-like adaptor 2 |
| Oli6c2a   | BC095967      | 0.705 | Oligonucleotide/oligosaccharide-binding fold containing 2A |
| Hsp110    | NM_013559     | 0.706 | Heat shock protein 110 |
| Zicch7    | NM_177027     | 0.709 | Zinc finger, CCHC domain containing 7 |
| Ppp1r10   | NM_175934     | 0.711 | Protein phosphatase 1, regulatory subunit 10 |
| Hsp4a     | NM_008300     | 0.729 | Heat shock protein 4 |
| Lxn       | NM_016753     | 0.734 | Latexin (Lxn) |
| Dhrs3     | NM_011303     | 0.736 | Dehydrogenase/reductase (SDR family) member 3 |

FIGURE 2. Immunosuppressive and lean phenotypes in PLA2G10tg/H11001 mice. A, expression of M1 or M2 macrophage markers in resident peritoneal macrophages from WT and X-Tg mice (n = 6). B, counts of thioglycollate-induced macrophages in the peritoneal cavity of WT and X-Tg mice (n = 4). C, expression of the M2 macrophage marker Arg1 in BM-derived macrophages from WT or X-Tg mice with or without stimulation for 24 h with LPS + IFN-γ (n = 4). D, body weights of WT and X-Tg mice at indicated ages (n = 5). E, CT scanning of visceral (red) and subcutaneous (yellow) fat (upper panel) and quantification of total, visceral, and subcutaneous fat (lower panel) in 1-year-old WT and X-Tg mice (n = 5). F, hematoxylin-eosin staining of the skin of 1-year-old WT and X-Tg mice. Arrow indicates subcutaneous fat. Mean ± S.E. (A–C) or mean ± S.D. (D and E), *, p < 0.05, and **, p < 0.01.

Phages were proportionally lower in PL"A2G10tg/H11001 mice than in WT mice. Furthermore, the median fluorescence intensity of CD45R on B cells was greater in PL"A2G10tg/H11001 mice than in WT mice (Fig. 1G), indicative of altered B cell differentiation. Despite the lower proportion of B cells, serum levels of IgG1 and IgE, but not IgM and IgG2a, were higher in PL"A2G10tg/H11001 mice compared to WT mice.
than in WT mice (Fig. 1H), suggesting preferential skewing toward a Th2 response, which on the one hand promotes allergies and on the other hand suppresses Th1/Th17-based diseases such as arthritis, atherosclerosis, obesity, and colitis (32).

In the thymus, PLA2G10tg/H11001 mice had fewer CD4CD8 double-positive and more CD4CD8 double-negative cells than did WT mice (Fig. 1I), indicating perturbed thymocyte transition from the double-negative to the double-positive stage in the thymic cortex of PLA2G10tg/H11001 mice. In support of this, microarray gene profiling of the thymus revealed lower expression of genes crucial for differentiation, proliferation, survival, and migration of thymocytes (e.g. Cd8a, Ccr9, Rorc, Klf2, and Ets2) (33–37) in PLA2G10tg/H11001 mice than in WT mice (Table 2).

Resident peritoneal macrophages in PLA2G10tg/H11001 mice showed greater expression of the M2 macrophage markers Arg1 and Cd206 than did WT mice, although expression of the M1 macrophage marker Cd68 was comparable in both genotypes (Fig. 2A). The count of thioglycolate-induced macrophages in the peritoneal cavity was lower in PLA2G10tg/H11001 mice than in WT mice (Fig. 2B), suggesting a reduced ability of monocytes to migrate to sites of inflammation or to differentiate into pro-inflammatory M1-like macrophages. M-CSF-driven BM-derived macrophages from PLA2G10tg/H11001 mice showed greater expression of the M2 marker Arg1 than did WT mice, even when they were cultured with M1 polarizers (LPS + IFN-γ) (Fig. 2C).

In agreement with the view that M2 macrophages and Th2 immunity counteract metabolic diseases (8, 32), PLA2G10tg/H11001 mice had lower body weight (Fig. 2D) and adiposity (Fig. 2E) than did WT mice throughout their life span. The subcutaneous fat layer, which was obviously present in WT mice, was scarcely seen in PLA2G10tg/H11001 mice (Fig. 2F). Thus, Tg overexpression of sPLA2-X facilitates M2 polarization of macrophages, which may account, at least partly, for the anti-inflammatory and lean phenotypes.

We next assessed whether the anti-inflammatory phenotypes observed in PLA2G10tg/H11001 mice might be ascribed to the capacity of sPLA2-X to alter lipid profiles in vivo. ESI-MS revealed that the splenic levels of AA, EPA, and DHA were significantly greater in PLA2G10tg/H11001 mice than in WT mice (Fig. 3A). The levels of AA metabolites tended to be slightly higher in PLA2G10tg/H11001 mice than in WT mice, but none of them reached statistical significance. Notably, the levels of ω3 PUFA metabolites, such as hydroxyeicosapentaenoic acids and hydroxydocosahexaenoic acids (including protectin D1 (PD1)), were significantly increased in PLA2G10tg/H11001 mice relative to WT mice (Fig. 3A). The increase of ω3 PUFAs and their metabolites in PLA2G10tg/H11001 mice was not limited to the spleen, because the skin levels of DHA and its metabolite PD1 were also higher in PLA2G10tg/H11001 mice than in WT mice (Fig. 3B), although AA and its metabolite PGE2 were also increased in the transgenic skin (4). In the colon, significant increases of EPA, rather than AA, metabolites were evident (Fig. 3C).
together, these results suggest a previously unappreciated capacity of sPLA2-X to mobilize ω3 PUFAs and their metabolites in vivo. Given the well established anti-inflammatory role of ω3 PUFAs and their metabolites (38, 39), the lipid profiles altered thus far could explain, at least in part, the immunosuppressive phenotypes in PLA2G10+/− mice.

Exacerbation of Colitis in Pla2g10−/− Mice—Given these observations, we next searched for a particular pathophysiological condition under which endogenous sPLA2-X would play an anti-inflammatory role. To this end, we focused on inflammation in the gastrointestinal (GI) tract, where endogenous sPLA2-X is abundantly expressed (40, 41). Inflammatory bowel disease (IBD) is a chronic, relapsing, and remitting condition of unknown origin that exhibits various features of immunological disorders, including impaired mucosal barrier function, pronounced innate and acquired immunity, and dysregulated production of cytokines, chemokines, and lipid mediators (42–44). Both ω6 AA metabolites, such as PGE2 and 12(S)-hydroxyeicosatetraenoic acid (12-HHT) (31, 45, 46), and ω3 PUFAs or their metabolites, such as resolvins D and E (47–49), are protective against IBD. However, the PLA2 subtypes that lie upstream of the production of these lipid mediators in this disease are currently unknown.

Among the sPLA2s, Pla2g10 (X) was expressed most abundantly in C57BL/6j colon, followed in order by Pla2g5 (V), Pla2g2f (IIF), Pla2g3 (III), and Pla2g12a (XIIA), whereas Pla2g1b (IB), Pla2g2d (IID), and Pla2g2e (IE) were detected only at trace levels (Fig. 4A). Pla2g4a and, to a lesser extent Pla2g6 (which encode group IVA cytosolic PLA2 (cPLA2α) and group VIA Ca2++-independent PLA2 (iPLA2β), respectively), were also expressed at substantial levels in the colon. Immunohistochemistry of the colon showed that sPLA2-X protein was localized in IECs and goblet cells, although its staining was absent in Pla2g10−/− mice (Fig. 4B). Consistently, Pla2g10 mRNA was enriched in Epcam-positive IECs isolated from WT colon (Fig. 4C). In DSS-induced ulcerative colitis, a well-known model of IBD (50), the colorectal expression of Pla2g10 as well as Pla2g2f, Pla2g3, Pla2g12a, and Pla2g6 was decreased in mice treated for 7 days with 3% DSS (Fig. 4A), probably due to the collapse of the mucosal epithelium or in unknown ways. The expression of Pla2g4a and Pla2g5 was constant regardless of DSS challenge, suggesting that they are distributed mainly in cells other than IECs.

To assess the roles of sPLA2s in IBD, we applied the DSS-induced colitis model to mice lacking individual sPLA2s expressed in the colon. Notably, Pla2g10−/− mice exhibited more severe colitis than did WT mice. After a lag period of several days after exposure to 1% DSS, Pla2g10−/− mice displayed more severe body weight loss (Fig. 5A), fecal bleeding plus diarrhea (summarized as the clinical score) (Fig. 5B), and colon shortening (Fig. 5C) than did WT mice. Histologically, more advanced epithelial loss, crypt damage, ulceration, and submucosal infiltration of immune cells were evident in the colon of DSS-treated Pla2g10−/− mice than was the case for WT mice (Fig. 5D). In comparison, mice lacking other sPLA2s, including Pla2g2d−/−, Pla2g2e−/−, Pla2g2f−/−, Pla2g3−/−, and Pla2g5−/− mice, showed no obvious phenotypes in this model (Fig. 5E).

Quantitative RT-PCR of the colon revealed that the expression levels of genes related to pro-inflammatory and Th17-related cytokines (Il1b, Il6, Il17a, Il22, and Tnf) were increased more robustly in Pla2g10−/− mice than in Pla2g10+/− mice after DSS challenge (Fig. 5F). Expression of Reg3g, which encodes an IL-22-inducible anti-bacterial protein (42, 43), as well as that of CD4+ and CD8+ T cell markers (Cd4 and Cd8α; the latter in particular) also tended to be higher in DSS-treated Pla2g10−/− than in Pla2g10+/− mice (Fig. 5F). Expression of both M1 and M2 macrophage markers (Nos2 and Arg1, respectively) was also greater in DSS-treated Pla2g10−/− mice than in Pla2g10+/− mice, suggesting that the absence of sPLA2-X affected recruitment, rather than polarization, of macrophages in this setting. These results were further supported by microarray gene profiling, where colorectal expression of various cytokines, chemokines, macrophage markers, and other inflammatory genes was elevated in DSS-treated Pla2g10−/− mice relative to Pla2g10+/− mice (Table 3). Even in the control group, expression of the pro-inflammatory and anti-bacterial genes S100a8 and S100a9 was higher in Pla2g10−/− mice than in Pla2g10+/− mice, suggesting that some colorectal abnormal-
Group X sPLA₂ Releases ω3 Lipids in Vivo

**Figure A**

Pla2g10

- None +/+ (n = 8)
- None −/− (n = 5)
- DSS +/+ (n = 23)
- DSS −/− (n = 17)

**Figure B**

Pla2g10

- None +/+ (n = 8)
- None −/− (n = 5)
- DSS +/+ (n = 23)
- DSS −/− (n = 17)

**Figure C**

(+/+) −/−

**Figure D**

Pla2g10 +/+  Pla2g10 −/−

**Figure E**

Pla2g2d  Pla2g2e  Pla2g2f  Pla2g5  Pla2g3

- Body weight (%)
- Clinical score
- Hematocrit (%)

**Figure F**

II1b  II6  II17a  II22  Tnf  Reg3g  Cd4  Cd8  Nos2  Arg1

- II1b/Gapdh (x10⁹)
- II6/Gapdh (x10⁹)
- II17a/Gapdh (x10⁹)
- II22/Gapdh (x10⁹)
- Tnf/Gapdh (x10⁹)
- Reg3g/Gapdh (x10⁹)
- Cd4/Gapdh (x10⁹)
- Cd8/Gapdh (x10⁹)
- Nos2/Gapdh (x10⁹)
- Arg1/Gapdh (x10⁹)
cytes is disturbed by Pla2g10 deficiency, particularly under the conditions of colitis.

To evaluate the relative contribution of sPLA2-X in the hematopoietic and non-hematopoietic compartments to DSS-induced colitis, BM cells from Pla2g10+/+ or Pla2g10−/− mice were adoptively transferred into lethally irradiated Pla2g10++/+ or Pla2g10−/− mice, which were then subjected to the colitis model (Fig. 7A). When the donor BM cells from Pla2g10++/+ or Pla2g10−/− mice were transferred into recipient Pla2g10++/+ mice (WT → WT or KO → WT), there were no differences in body weight (Fig. 7B) or clinical score (Fig. 7C) between the groups. In contrast, weight loss (Fig. 7B) and an increased clinical score (Fig. 7C) were obvious in Pla2g10−/− mice that received Pla2g10++/+ BM cells (WT → KO) in comparison with WT → WT or KO → WT chimeras. When Pla2g10−/− mice were used as both donors and recipients (KO → KO), the weight loss (Fig. 7B) and increased clinical score (Fig. 7C) were similar to those in WT → KO chimeras. These results suggest that sPLA2-X in non-hematopoietic cells, most likely IECs, is mainly responsible for the protection from colitis. Of note, DSS-induced splenomegaly (Fig. 7D) and the decrease in hematocrit (Fig. 7E) were significantly more severe in KO → KO chimeras than in WT → KO chimeras, implying an additional contribution of hematopoietic sPLA2-X to these processes in the absence of non-hematopoietic sPLA2-X.

sPLA2-X Mobilizes ω3 PUFAs in Colitis—To gain insights into the mechanism underlying the anti-inflammatory action of sPLA2-X in colitis, lipids extracted from colon tissues of Pla2g10+/+ and Pla2g10−/− mice with or without DSS treatment were subjected to ESI-MS. We found that the colon levels of EPA, docosapentaenoic acid (DPA; C22:5), and DHA were increased in WT mice following DSS treatment, whereas these changes were not evident in Pla2g10−/− mice (Fig. 8A). AA also showed a similar trend but did not reach statistical significance (Fig. 8A). Pla2g10 deficiency did not alter the basal levels of these PUFAs. Strikingly, the colorectal levels of AA metabolites were not affected by Pla2g10 deficiency (Fig. 8B), whereas those of EPA or DHA metabolites, such as resolvins and 18-HEPE, were substantially lower in DSS-treated Pla2g10−/− mice than in Pla2g10+/+ mice (Fig. 8C). These results, together with the results of Pla2g10−/− mice (see above) and the reported role of ω3 PUFA metabolites in the protection against colitis (47–49), raise the possibility that the mobilization of ω3 PUFAAs or their metabolites may underlie the anti-inflammatory role of sPLA2-X in colitis.

When LPLs isolated from DSS-treated WT mice were incubated with PUFAAs ex vivo, production of Th17 cytokines, IL-17A and IL-22, was partially suppressed by ω3 PUFAAs (EPA, DPA and DHA) as well as of AAAs, although their metabolites (resolvins and 18-HEPE) were ineffective in this assay (Fig.

Table 3: Microarray gene profiling of the colon of Pla2g10−/− mice versus Pla2g10+/+ mice in DSS-induced colitis

| Gene name | Accession no. | DSS(−) KO/WT | DSS(+) KO/WT |
|-----------|---------------|--------------|--------------|
| Cytokines and their receptors | | | |
| Il1b | NM_003361 | 1.74 | 2.92 |
| Il6 | NM_003361 | 0.70 | 2.85 |
| Il17a | NM_010552 | 0.45 | 2.94 |
| Il2 | NM_003366 | 1.07 | 7.12 |
| Il7r | NM_003372 | 1.15 | 2.05 |
| Chemokines and their receptors | | | |
| Ccl4 | NM_013652 | 1.24 | 4.07 |
| Ccl7 | NM_013654 | 0.60 | 2.25 |
| Ccr6 | NM_009835 | 1.35 | 2.44 |
| Cxcl13 | NM_018866 | 1.21 | 5.49 |
| Cxcl2 | NM_009140 | 0.93 | 4.60 |
| Macrophages | | | |
| Emr1 | NM_001330 | 0.76 | 2.24 |
| Nos2 | NM_019997 | 0.76 | 2.72 |
| Cd68 | NM_009835 | 0.59 | 2.04 |
| Arg1 | NM_007482 | 1.00 | 3.36 |
| Chi3l3 | NM_009892 | 1.07 | 3.58 |
| Inflammation-related | | | |
| S100a9 | NM_00281852 | 3.60 | 5.18 |
| S100a8 | NM_0013650 | 3.35 | 5.86 |
| Ptg2 | NM_011198 | 1.14 | 2.64 |
| Mmp3 | NM_010809 | 0.66 | 2.98 |
| Mmp9 | NM_013599 | 0.52 | 3.02 |
| Mmp10 | NM_019471 | 0.72 | 3.65 |
| Epithelial cells | | | |
| Krt78 | NM_012487 | 0.76 | 0.43 |
| Krt1 | NM_008473 | 1.33 | 0.15 |
| Defb23 | NM_00137933 | 1.03 | 0.08 |
| Defb45 | NM_00137752 | 0.99 | 0.25 |

Figure 5. Exacerbation of DSS-induced colitis in Pla2g10−/− mice. A and B, daily monitoring of body weight loss (A) and clinical score (B) in Pla2g10+/+ and Pla2g10−/− mice (8-week-old, male) that were untreated or orally administered 1% DSS. C and D, gross appearance (C) and histology (D) of the colon in Pla2g10−/− mice after treatment with DSS for 7 days. Bar, 50 μm. E, DSS-induced colitis in knock-out mice for various sPLA2s, Pla2g2f−/−, Pla2g2e−/−, Pla2g10−/−, Pla2g2f−/−, Pla2g2e−/−, Pla2g2f−/−, Pla2g2f−/−, or Pla2g2f−/− mice and their corresponding control mice were administered 1% DSS orally and evaluated for body weight on day 7 (top), clinical score at the indicated times (middle), and hematocrit on day 7 (bottom) (n = 4–6). F, quantitative RT-PCR of inflammation-associated genes in the colon of Pla2g10−/− and Pla2g10−/− mice after treatment with or without DSS for 7 days (n = 5 (without DSS) or 6 (with DSS)). Mean ± S.E., *, p < 0.05, and **, p < 0.01.
Because these PUFAs can act on the fatty acid receptor GPR120 or GPR40 (51), we tested the effect of GSK137647, a GPR120-selective agonist, on cytokine production by LPLs. The release of IL-17A and IL-22 was suppressed by GSK137647 as efficiently as DHA (Fig. 8E), indicating that PUFAs may act, at least in part, on GPR120 on LPLs, thereby partially dampening the Th17 cytokine production. Moreover, daily intrarectal injection of /H9275 PUFAs (a mixture of EPA and DHA) into Pla2g10/H11002/Pla2g10/H11002 mice prevented DSS-induced body weight loss (Fig. 8F). Overall, these results further support the notion that sPLA2-X prevents colitis by releasing /H9275 PUFAs. Nonetheless, because the colorectal level of AA tended to be lower in DSS-treated Pla2g10/H11002/Pla2g10/H11002 mice than in WT mice (Fig. 8A), the /H9275 PUFAs were present at 30 times lower than the AA metabolites (Fig. 8, B and C), and AA suppressed IL-17A release by LPLs (Fig. 8D), it is possible that AA itself released by sPLA2-X might also contribute to the protection from colitis and that the background level of PGs might mask a pool of PGs potentially formed following mobilization of AA by sPLA2-X in a subset of cells.

**Protective Role of the cPLA2α-PGE2 Axis against Colitis**—The above observations prompted us to ask which PLA2 subtype(s) is linked to AA metabolism in colitis. We therefore applied the DSS-induced colitis model to mice null for Pla2g4a and Pla2g6, which are expressed in the colon (Fig. 4A). Severe weight gain, fecal bleeding, and diarrhea were seen in Pla2g4a/H11002/Pla2g4a/H11002 mice, but not WT mice, soon after oral application of DSS (Fig. 9, A and B). On day 7, colorectal damage with epithelial loss and massive immune cell infiltration (Fig. 9C), splenomegaly (Fig. 9D), and decrease of hematocrit (Fig. 9E) were far more prominent in Pla2g4a/H11002/Pla2g4a/H11002 mice than in WT mice. In contrast, exacerbation of these parameters was not evident in Pla2g6/H11002/Pla2g6/H11002 mice (Fig. 9, A and B). The overall phenotypes in Pla2g4a/H11002 mice were similar to those in Ptger4/H11002 mice, which lack the PGE2 receptor.

**Figure 6. Altered extramedullary erythropoiesis in DSS-treated Pla2g10/H11001—**

A–C, representative appearance of the spleen (A), spleen weight (B), and hematocrit (C) in Pla2g10/H11001 and Pla2g10/H11002 mice after treatment for 7 days with or without 1% DSS. D–G, flow cytometry of the erythrocyte lineage in the blood (D and E) and spleen (F and G) of Pla2g10/H11001 and Pla2g10/H11002 mice with or without administration of DSS for 7 days. Representative FACS profiles (D and F) and quantified results (n = 3) are shown. Mean ± S.E., *, p < 0.05, and **, p < 0.01.
EP4 (31), or Ptges−/− mice, which lack microsomal PGE2 synthase-1 (mPGES-1) (Fig. 9, A–E) (52), even though the overall symptoms, as revealed by the delay in body weight loss, were milder in Ptges−/− mice than in Pla2g4a−/− mice.

Lipidomics studies of the colon revealed that PGE2 was present at a markedly lower level in DSS-treated Pla2g4a−/− mice than in WT mice (Fig. 9F). 12-HHT was also ~50% lower, although the changes in other prostanoids were relatively small, in DSS-treated Pla2g4a−/− mice compared with WT mice. These results indicate that cPLA2α is preferentially coupled with PGE2 and to a lesser extent 12-HHT in DSS-induced colitis. In contrast, the levels of EPA and DHA metabolites were unaffected by Pla2g4a deficiency. The level of PGE2 was markedly decreased in Ptges−/− mice (to a level similar to that in Pla2g4a−/− mice, but not to zero), with reciprocal increases of other prostanoids, relative to WT mice (Fig. 9F). These results suggest the following. (i) The severe exacerbation of DSS-induced colitis in Pla2g4a−/− mice is due to the marked reduction of colon-protective prostanoids such as PGE2 and 12-HHT (31, 45, 46). (ii) The milder outcome in Ptges−/− mice than in Pla2g4a−/− mice is probably because the former harbors the reduction of PGE2 only, which may be counterbalanced by the increases in 12-HHT and/or other prostanoids through a shunting effect (53). (iii) The cPLA2α-mPGES-1 axis accounts mostly, if not entirely, for a pool of PGE2 responsible for this disease model. (iv) There is an alternative route for the basal, cPLA2α-independent production of prostanoids such as PGF2α and 6-keto-PGF1α (a stable end product of PGI2) in the colon. Overall, cPLA2α and sPLA2-X exert a protective effect against colitis by mobilizing distinct sets of lipid metabolites, i.e. ω6 AA and ω3 PUFA metabolites, respectively.

Reduced Release of DHA and DPA in Pla2g10−/− Spermatozoa—In addition to the GI tract, sPLA2-X is abundantly expressed in the testis, being stored in and released from the acrosomes of spermatozoa during capacitation and the acrosome reaction, and Pla2g10−/− spermatozoa display reduced fertility, with no alteration in motility (40, 54). However, the phospholipid metabolism underlying the action of sPLA2-X in this context remains to be determined.

To address this issue, we performed lipidomics analysis of Pla2g10+/+ and Pla2g10−/− spermatozoa before and after capacitation. Consistent with the view that sPLA2-X is dispensable for sperm maturation (40), the PC compositions of spermatozoa before capacitation were identical between the genotypes (Fig. 10A). Notably, after capacitation, the levels of PC species with DHA or DPA, but not those with AA and other fatty acids, were significantly lower in Pla2g10−/− cells than in Pla2g10+/+ cells (Fig. 10A). In accordance with this, the release of DHA and DPA but not AA and linoleic acid (Fig. 10B), as well as LPC with C18:0 (and to a lesser extent with C18:1 and C16:0) (Fig. 10C), was greater in Pla2g10+/+ than in Pla2g10−/− spermatozoa. Release of EPA was very low, because EPA-bearing PC was a minor phospholipid component in mouse sperm (Fig. 10, A and B) (3). These results suggest that sPLA2-X secreted from activated spermatozoa preferentially cleaves DHA- or DPA-containing PC in the sperm membrane to release DHA, DPA, and LPC.
We then evaluated the effects of these lipid products on fertilization. The fertilization ability of \( \text{Pla2g10}^{+/+} \) sperm with WT oocytes was lower than that of \( \text{Pla2g10}^{-/-} \) sperm, as reported previously (40, 54), whereas addition of DPA and to a lesser extent LPC restored the fertilization ability of \( \text{Pla2g10}^{+/+} \) sperm (Fig. 10D). Thus, the lipid products released from the sperm membrane by sPLA2-X, particularly DPA, facilitate optimal fertilization.

**Discussion**

The roles of sPLA2s, including sPLA2-X, in promoting or attenuating inflammation or other pathophysiological events may be dictated by the cells from which they are secreted, the target membranes on which they act, or when and how their phospholipid-hydrolytic products are associated with the particular biological processes in cell-, tissue-, or disease-specific contexts. Given the current proposal that sPLA2-X is a pro-inflammatory enzyme (2, 16–22), our present observation that \( \text{Pla2g10}^{+/+} \) mice exhibited a global immunosuppressive phenotype was initially unexpected but appeared to be compatible with studies reporting that sPLA2-X overexpression in cultured macrophages elicited anti-inflammatory responses (23) and that atherosclerosis worsened in \( \text{Ldlr}^{-/-} \) mice that had been received \( \text{Pla2g10}^{+/+} \) BM cells by adoptive transfer (24). In this study, by employing \( \text{Pla2g10} \) gene-manipulated mice in combination with lipidomics, we have revealed the anti-inflammatory, rather than pro-inflammatory, features of sPLA2-X in vivo.

Endogenous sPLA2-X is constitutively expressed at a high level in the GI tract and testis (40, 41, 54), and this study using \( \text{Pla2g10}^{-/-} \) mice has shown that sPLA2-X mobilizes \( \text{HEPE} \) in addition to, or even in favor of, \( \text{6 AA} \) in the processes of colitis and fertilization, respectively. Even in \( \text{Pla2g10}^{+/+} \) mice, which globally overexpress human sPLA2-X at a super-physiological level, there are modest trends toward selective increases of \( \text{HEPE} \) metabolites over \( \text{6 AA} \) metabolites in multiple if not all tissues. These observations suggest that sPLA2-X has the intrinsic ability to mobilize \( \text{HEPE} \)-derived metabolites in vivo. We have

**FIGURE 8.** ESI-MS profiling of PUFA metabolites in DSS-induced colitis. A–C, ESI-MS analysis of PUFAs (A), AA metabolites (B), and EPA/DHA metabolites (C) in the colon of \( \text{Pla2g10}^{+/+} \) and \( \text{Pla2g10}^{-/-} \) mice with or without administration of 1% DSS for 7 days (n = 6–7; *, p < 0.05, and **, p < 0.01). D and E, effects of PUFAs and their metabolites (D) or GSK137647 (GSK; a GPR120 agonist) (E) on Th17 cytokine production by LPLs isolated from DSS-treated WT mice (n = 4; *, p < 0.05, and **, p < 0.01 versus treatment with lipids or GSK137647). Results are expressed as percentages, with values in the absence of lipids or GSK137647 as 100% (dashed line). Results are expressed as percentages, with the value of DSS-untreated mice as 100% (dashed line). Values are mean ± S.E.
recently shown that sPLA₂-IID, which is highly expressed in dendritic cells in lymphoid tissues, resolves contact hypersensitivity by mobilizing DHA-derived pro-resolving lipid mediators (5). Our results thus reveal a novel role of sPLA₂-X as another /H92753 PUFA-mobilizing sPLA₂, thereby regulating tissue-specific homeostasis.

/H92753 PUFAs such as EPA and DHA resolve various types of inflammation, obesity, and atherosclerosis by acting on fatty acid-sensing receptors (e.g. PPARs and GPR120; see below) (51, 55), by being metabolized to pro-resolving lipid mediators (e.g. resolvins and protectins) (38, 39), by attenuating endoplasmic reticulum stress (56), or by increasing membrane fluidity, thus eventually altering membrane signaling or trafficking (57). It is likely that the anti-inflammatory actions of sPLA₂-X occur through any of these mechanisms. Indeed, changes in the tissue levels of /H92753 PUFAs and their metabolites are correlated with the levels of sPLA₂-X expression. /H92753 PUFA metabolites promote M2 macrophage polarization (58, 59), prevent T cell activation or differentiation (60, 61), and alter antibody production by B cells (62), a view that is relevant to the phenotypes observed in PLA2G10tg/−/− mice. Our results are also in accord with the aggravating role of sPLA₂-X in asthma (2), where M2 macrophages are associated with the Th2-skewed airway inflammation (13). Therefore, we speculate that the reported roles of sPLA₂-X in protection against atherosclerosis and obesity (24, 63) may also involve, at least in part, the mobilization of /H92753 PUFAs by this enzyme in a local tissue or even at a distal site (e.g. the GI tract), thus affecting the disease indirectly.

Our results do not rule out the contribution of sPLA₂-X to AA metabolism, because this enzyme can release AA in various cultured cells (at 10–100 ng/ml or more) (16–18), and because several in vivo studies have shown that Pla2g10 ablation results

![Figure 9](image-url)
in reduction of eicosanoids (2, 19–22). However, many of the previous in vivo studies did not measure ω3 PUFA metabolites or discriminate whether sPLA2-X directly mobilizes eicosanoids or whether the observed changes in eicosanoids reflected changes in cPLA2α expression or activation in the ongoing process of a given pathology. In fact, in the context of asthma, sPLA2-X secreted from the airway epithelium acts on infiltrating eosinophils in a paracrine manner to produce LPC, which in turn increases Ca2+ influx leading to cPLA2α-dependent leukotriene generation (64), although it may directly mobilize AA metabolites from lung epithelial cells in an autocrine manner (20). In this study, we have shown that cPLA2α and sPLA2-X are functionally segregated in the large intestine, driving non-overlapping lipid pathways (∆6 AA metabolism and ∆3 PUFA metabolism, respectively), which eventually culminates in a common outcome, i.e. protection against colitis.

To the best of our knowledge, this is the first demonstration of the PLA2 enzymes that are responsible for the release of distinct PUFA metabolites in IBD. Moreover, the sPLA2-X-driven ω3 PUFAs are capable of suppressing Th17 cytokine production by intestinal LPLs through GPR120, providing the first evidence for the functional linkage from a particular sPLA2 to a fatty acid receptor. Although our study failed to show the ameliorating effect of resolvins and 18-HEPE on Th17 cytokine production by these cells, it is possible that these pro-resolving mediators could affect other steps of colitis, for instance by acting directly on epithelial cells to protect from injuries and on neutrophils to suppress their migration and to promote their clearance. In fact, resolvins block colitis when administered exogenously (47–49), and an endogenous EPA-derived epoxide attenuates allergic colitis (65).

Presumably, the mobilization of ω6 AA versus ω3 PUFA metabolites, or even other fatty acids and lysophospholipids, by sPLA2-X or other sPLA2s would rely not only on their intrinsic enzymatic properties but also on tissue- or disease-specific contexts such as the lipid composition of target membranes or the spatiotemporal availability of downstream enzymes, which may explain why the same enzyme often exerts pro- or anti-inflammatory effects with different lipid mediator profiles in distinct settings. Indeed, sPLA2-IID mobilizes DHA-derived RvD1 in draining lymph nodes to suppress contact dermatitis (5) and AA-derived PGD2 in the lung to counteract anti-viral immunity (66). sPLA2-V in adipose tissue releases oleic acid from lipoproteins in the process of obesity (8). Moreover, mobilization of a particular class of lysophospholipids, rather than fatty acids, is important for the function of sPLA2-IIF in the epidermis (67). Thus, caution should be exercised when interpreting the results of studies in which the actions of sPLA2 are assigned only to AA metabolism.

FIGURE 10. sPLA2-X mobilizes DPA, DHA, and LPC from the sperm membrane. A, ESI-MS of PC molecular species in spermatozoa from 8-week-old Pla2g10+/− and Pla2g10−/− mice before and after capacitation (n = 4–6). B and C, ESI-MS of PUFAs (B) and LPC species (C) released from Pla2g10+/− and Pla2g10−/− spermatozoa after capacitation (n = 4). LA, linoleic acid. D, effects of the indicated lipids (1 μM) on in vitro fertilization ability of Pla2g10+/− and Pla2g10−/− spermatozoa with WT oocytes (n = 3). Mean ± S.E., *, p < 0.05, and **, p < 0.01.
Apart from their roles in inflammation, multiple sPLA₂s are expressed in male genital organs (68), among which two particular isoforms, sPLA₂-III and -X, participate in sperm maturation and activation, respectively (3, 54). Several lines of evidence suggest that DHA insufficiency causes asthenozoospermia with hypomotility and infertility (69, 70). sPLA₂-III is secreted from the epididymal epithelium and acts on immature spermatozoa passing through the epididymal duct to promote sperm membrane remodeling (3). As such, mature spermatozoa gain a higher proportion of DPA/DHA-containing PC species, which are crucial for sperm motility and thereby fertility. After ejaculation into the female duct, mature sperm undergo capacitation to allow hypermotility and acrosome reaction for fertilization, where the acrosome-derived sPLA₂-X plays a promoting role (54). DPA, an intermediate in the biosynthesis from EPA to DHA, has recently attracted attention as a precursor of novel 13-series resolvins with potent pro-resolving activity (71). Beyond this function, DPA is highly valuable as a precursor of novel 13-series resolvins with potent pro-resolving activity (71). Beyond this function, DPA is highly valuable.

Author Contributions—M. M. conceived and coordinated the study and wrote the paper. R. M. and Y. T. designed, performed, and analyzed the experiments shown in Figs. 4–9. K. Y., H. S., A. U., and K. I. designed, performed, and analyzed the experiments shown in Figs. 1–3 and 10. Y. N. designed, performed, and analyzed microarray experiments. T. K. and T. Y. provided technical advice. All authors reviewed the results and approved the final version of the manuscript.

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