Role of Group V Phospholipase A2 in Zymosan-induced Eicosanoid Generation and Vascular Permeability Revealed by Targeted Gene Disruption*

Yoshiyuki Satake†‡¶, Bruno L. Diaz§‖, Barbara Balestrieri‡¶, Bing K. Lam‡‡, Yoshihide Kanaoka§§, Michael J. Grusby‡‡‡, and Jonathan P. Arm§§§§

From the †Department of Medicine, Harvard Medical School, Boston, Massachusetts 02115, ‡Division of Rheumatology, Immunology, and Allergy and ¶Partners Asthma Center, Brigham and Women’s Hospital, Boston, Massachusetts 02115; ††Divisão de Biologia Celular, Coordenação de Pesquisas, Instituto Nacional de Câncer, Rio de Janeiro, Brazil 20231-050, and ‡‡Department of Immunology and Infectious Disease, Harvard School of Public Health, Boston, Massachusetts 02115

Conclusions regarding the contribution of low molecular weight secretory phospholipase A2 (sPLA2) enzymes in eicosanoid generation have relied on data obtained from transfected cells or the use of inhibitors that fail to discriminate between individual members of the large family of mammalian sPLA2 enzymes. To elucidate the role of group V sPLA2, we used targeted gene disruption to generate mice lacking this enzyme. Zymosan-induced generation of leukotriene C4 and prostaglandin E2 was attenuated ~50% in peritoneal macrophages from group V sPLA2-null mice compared with macrophages from wild-type littermates. Furthermore, the early phase of plasma exudation in response to intraperitoneal injection of zymosan and the accompanying in vivo generation of cysteinyl leukotrienes were markedly attenuated in group V sPLA2-null mice compared with wild-type controls. These data provide clear evidence of a role for group V sPLA2 in regulating eicosanoid generation in response to an acute innate stimulus of the immune response both in vitro and in vivo, suggesting a role for this enzyme in innate immunity.

The first step in the biosynthesis of eicosanoids is the release of arachidonic acid from cell membrane phospholipids by phospholipase A2. Several classes of phospholipase A2 have been described in mammals (1, 2). Cytosolic phospholipase A2 (cPLA2)α is an 85-kDa cytosolic enzyme that uses a catalytic serine residue and preferentially cleaves arachidonic acid from cell membrane phospholipids (3). The Ca2+-dependent translocation of cPLA2-α from the cytosol to the nuclear envelope (4), a prominent site of eicosanoid biosynthesis, is dependent on a Ca2+-dependent lipid binding (C-2) domain. Paralogues of cPLA2-α (cPLA2-β and cPLA2-γ) have been described previously (5, 6). cPLA2-β has a M, of 110,000 and shares 30% identity with cPLA2-α, including a functional C-2 domain. cPLA2-γ has a M, of 61,000, shares 29% sequence identity with cPLA2-α, lacks a C-2 domain, and is Ca2+-independent. Mammalian low molecular weight secretory phospholipase A2 (sPLA2) enzymes, which are now 10 in number, are characterized by a conserved motif containing a catalytic histidine residue, by their relatively small size of ~14 kDa, and by their highly disulfide-linked tertiary structures (7–13). They are distinguished from one another by their structures, their biochemical properties, and their tissue distribution. Calcium-independent phospholipase A2 enzymes have been described in myocardiun and in leukocytes (14, 15). They have been implicated in membrane remodeling, regulation of store operated calcium channels, apoptosis, and release of arachidonic acid. The fourth group of phospholipase A2 enzymes comprises the acetyl hydrolases of platelet activating factor (16).

Given the complexity and size of the phospholipase A2 family, targeted gene disruption is a suitable approach to elucidating the role(s) of individual enzymes and proved fruitful in determining the role of cPLA2-α in regulating eicosanoid biosynthesis. Disruption of the gene encoding cPLA2-α led to almost complete abrogation of the rapid generation of prostaglandin (PG) E2, leukotriene (LT) C4, and LTB4 from peritoneal macrophages in response to A23187 and a more delayed generation of PGE2 in response to lipopolysaccharide (LPS) (17, 18). Separate studies demonstrated a marked attenuation of the release of labeled arachidonic acid from cPLA2-α-null peritoneal macrophages in response to zymosan, A23187, phorbol myristate acetate, and okadaic acid (19) and the critical role of this enzyme in immediate and delayed phases of eicosanoid generation by mouse bone marrow culture-derived mast cells (20, 21).

Whereas these data demonstrate the essential function of cPLA2-α in supplying arachidonic acid for leukotriene and prostaglandin biosynthesis, several lines of evidence have suggested that low molecular weight sPLA2 enzymes amplify the actions of cPLA2-α in eicosanoid generation. Transfection of HEK293 cells with the heparin-binding group IA sPLA2, group

* This work was supported by National Institutes of Health Grants HL36110, HL070946, HL7718, and AI40171; an award from The Sandler Family Supporting Foundation (M. J. G.); an American Lung Association Clinical Investigator Award (CI-011-N); Programa de Fixação de Pesquisadores, Conselho Nacional de Desenvolvimento Científico e Tecnológico-PROTIX/CNPq, Brazil; an International Research Grant from the Japan Eye Bank and the Kowa Life Science Foundation; an International Research Grant from the Japan Eye Bank and the Kowa Life Science Foundation; and an International Research Grant from the Japan Eye Bank and the Kowa Life Science Foundation.

† Both authors contributed equally to this work.

‡ To whom correspondence should be addressed: Brigham and Women’s Hospital, Smith Research Building, Room 623B, 1 Jimmy Fund Way, Boston, MA 02115. Tel.: 617-502-1505; Fax: 617-502-1310; E-mail: jarm@rics.bwh.harvard.edu.

§ The abbreviations used are: cPLA2, cytosolic phospholipase A2; HBA, Hanks’ balanced salt solution containing 0.1% bovine serum albumin; LT, leukotriene; sPLA2, secretory phospholipase A2; PG, prostaglandin; LPS, lipopolysaccharide; RT-PCR, reverse transcription-PCR; Pipes, 1,4-piperazinediethanesulfonic acid; PBS, phosphate-buffered saline; RP-HPLC, reverse-phase high performance liquid chromatography; CysLT1R, cysteinyl leukotriene type 1 receptor.
Targeted Gene Disruption of Group V sPLA$_2$

V sPLA$_2$, or group IID sPLA$_2$ amplified the cPLA$_2$-dependent release of arachidonic acid and PGE$_2$ generation in response to A23187 or to LPS and interleukin-1$\beta$ (22, 23). The action of these enzymes was attributed to their ability to bind to phosphatidyl in caveoli, leading to internalization and co-localization with prostaglandin endoperoxide synthase enzymes (22). Similarly, adenoviral transfection of group V sPLA$_2$ or group IIA sPLA$_2$ into mouse mesangial cells amplified H$_2$O$_2$-induced release of arachidonic acid. This was observed only in cPLA$_2$-null cells (24). When P388D$_1$ macrophages were primed with LPS for 1 h and then activated with platelet activating factor, there was biphasic release of labeled arachidonic acid (25). Studies with pharmacological inhibitors and with antisense oligonucleotides suggested that the initial intracellular release of arachidonic acid was dependent upon cPLA$_2$-$\alpha$, whereas the subsequent extracellular release was dependent upon group V sPLA$_2$, which was dependent upon the initial action of cPLA$_2$-$\alpha$ (26, 27). In certain subclones of the P388D$_1$ macrophage, LPS stimulation alone elicited a delayed phase of arachidonic acid release and prostaglandin endoperoxide synthase-2-dependent PGE$_2$ generation. Both cPLA$_2$-$\alpha$ and group V sPLA$_2$ were required; however, in contrast to the primed-immediate response to LPS and platelet activating factor, activation of cPLA$_2$-$\alpha$ led to induction of group V sPLA$_2$ expression that in turn induced prostaglandin endoperoxide synthase-2 (28).

The data implicating low molecular weight sPLA$_2$ enzymes in eicosanoid generation have thus relied on either transfection experiments or pharmacological and antisense inhibition experiments. The latter, although informative with regard to the potential functions of a sPLA$_2$, fail to address the role of the endogenous enzymes. The latter have lacked specificity (25, 27), failed to discriminate between individual low molecular weight phospholipase A$_2$ enzymes (29), or yielded conflicting data (30, 31). To definitively test the role of group V sPLA$_2$ in vivo and in vitro, we have generated mice with targeted disruption of the gene encoding this enzyme.

EXPERIMENTAL PROCEDURES

Materials—Restriction enzymes were from Roche Molecular Biochemicals. Human serum albumin, zymosan A, Evans blue dye, and paraformaldehyde were from Sigma.

Isolation and Activation of Mouse Peritoneal Macrophages—The peritoneal cavities of mice were flushed with 5 ml of ice-cold PCG buffer (25 mm Pipes, 110 mm NaCl, 5 mm KCl, 1 mm CaCl$_2$, 1 g/liter glucose, pH 7.4). After washing, 3.3 $\times$ 10$^6$ cells were plated on glass coverslips in 24-well tissue culture plates in 500 ml of PCG buffer, 10% fetal bovine serum, 2 ml/l-glutamine, 100 ml/penicillin, and 100 mg/ml streptomycin and incubated overnight at 37 °C with 5% CO$_2$. Nonadherent cells were removed by washing three times with PCG buffer. Cells were fixed with 2% paraformaldehyde (Sigma) in phosphate-buffered saline (PBS) for 15 min at room temperature, washed once in Hanks’ balanced salt solution without Mg$^{2+}$ or Ca$^{2+}$ (HBSS–) containing 0.1% bovine serum albumin (HBA), permeabilized with 0.025% saponin (Sigma) in PBS for 10 min at room temperature, and washed twice with HBA. Macrophages were then blocked in HBA containing 5% normal donkey serum (Jackson ImmunoResearch, West Grove, PA) for 1 h at room temperature. Cells were incubated with 5 ml/5 ml rabbit anti-mouse group V sPLA$_2$ (32) in blocking buffer for 2 h at room temperature, washed extensively with HBA, and incubated for 1 h at room temperature with fluorescein isothiocyanate-conjugated donkey anti-rabbit IgG (heavy and light chains; Jackson ImmunoResearch), diluted 1:400. Cells were washed five times with HBA, mounted in Vectashield™ mounting medium (Vector Laboratories, Burlingame, CA), and imaged with a Nikon Eclipse TE2000U microscope coupled with a Spot-RT digital camera.

Isolation and Activation of Mouse Peritoneal Macrophages—The peritoneal cavities of mice were flushed with 5 ml of ice-cold Dulbecco’s modified Eagle’s medium (Life Sciences, Rockville, MD) containing 10% fetal bovine serum, 2 ml/l-glutamine, 100 ml/ml penicillin, 100 mg/ml streptomycin, and 10 ml/ml heparin. After two washes in HBSS—, 3.75 $\times$ 10$^5$ cells in 500 ml of Dulbecco’s modified Eagle’s medium, 10% fetal bovine serum, 2 ml/l-glutamine, 100 ml/ml penicillin, and 100 mg/ml streptomycin were placed in individual wells of a 48-well tissue culture plate and incubated for 3 h at 37 °C with 5% CO$_2$. Nonadherent cells were removed by washing with HBSS—. Nonadherent cells from each mouse were pooled and counted to determine the fraction of adherent cells, which did not differ between group V sPLA$_2$-null and wild-type mice. RNA was extracted from 100 ml of Dulbecco’s modified Eagle’s medium containing 0.1% human serum albumin and added to each well, and adherent cells were stimulated in a dose- and time-dependent manner with zymosan A (0–100 particles/cell; 0–6 h) (35). Supernatants were collected for measurements of cysteiny1 leukotrienes and PGE$_2$, by enzyme-linked immunosassays (Amersham Biosciences). In some experiments, supernatants were preincubated for 1 h with 50 ml/ml monoclonal antibody for cysteinyl leukotriene content by reverse phase high performance liquid chromatography (RP-HPLC).

Zymosan-induced Peritonitis—Each mouse received an injection of 0.5% Evans blue dye in PBS into the tail vein (100 mg dye/kg body weight) immediately before injection of 1 ml of zymosan A (1 mg/ml in sterile PBS, Sigma) into the peritoneal cavity (26). At selected time points (0, 5, 10, and 30 min), the mice were euthanized by CO$_2$ inhalation, and the peritoneal cavity was lavaged with 4 ml of ice-cold PBS. The peritoneal lavage fluid was centrifuged at 500 $\times$ g for 5 min, and the optical density of the supernatant was measured at 610 nm to assess extravasation of Evans blue with a [3H]dUTP random prime-labeled (Stratagene) 308-bp portion of DNA from the 3′-untranslated region of the group V sPLA$_2$ gene that lies outside the targeting construct (Fig. 1A).

Northern Blotting—Mouse hearts were homogenized in Tri Reagent (Molecular Research Center, Cincinnati, OH), and RNA was extracted according to the manufacturer’s protocol. Ten mg of RNA from each heart were resolved in separate lanes of 1.2% agarose formaldehyde gels, blotted to Immobilon M (Millipore), and probed with a [32P]dCTP-labeled cDNA spanning the open reading frame of group V sPLA$_2$ as described previously (34).

Reverse Transcription-PCR (RT-PCR)—Expression of transcripts for group V sPLA$_2$ was also analyzed by RT-PCR. One mg of RNA was reverse transcribed using Moloney murine leukemia virus reverse transcriptase and the Advantage RT for PCR kit (Clontech). Five ml of the resulting reaction mixture were used in a PCR reaction with Taq Gold polymerase (PerkinElmer Life Sciences), using the following primers that span the open reading frame: forward primer, 5′-ACACTGGCTTGTGTCGTCGC-3′ in exon II; and reverse primer, 5′-GCATAGAGAAGTGGG-3′ in exon V. PCR conditions were as follows: a denaturing step at 95 °C for 5 min; followed by 35 cycles of 95 °C for 45 s, 65 °C for 45 s, and 72 °C for 90 s; followed by a final extension step at 72 °C for 10 min. Primers for S15 ribosomal protein (Ambion, Austin, TX) were used as a positive control. Products were resolved on 2% agarose gels and visualized with ethidium bromide.

Immunofluorescence Analysis of Group V sPLA$_2$—Expression—The peritoneal cavities of mice were flushed with 5 ml of ice-cold PCG buffer (25 mm Pipes, 110 mm NaCl, 5 mm KCl, 1 mm CaCl$_2$, 1 g/liter glucose, pH 7.4). After washing, 3.3 $\times$ 10$^5$ cells were plated on glass coverslips in 24-well tissue culture plates in 500 ml of PCG buffer, 10% fetal bovine serum, 2 ml/l-glutamine, 100 ml/ml penicillin, and 100 mg/ml streptomycin and incubated overnight at 37 °C with 5% CO$_2$. Nonadherent cells were removed by washing three times with PCG buffer. Cells were fixed with 2% paraformaldehyde (Sigma) in phosphate-buffered saline (PBS) for 15 min at room temperature, washed once in Hanks’ balanced salt solution without Mg$^{2+}$ or Ca$^{2+}$ (HBSS–) containing 0.1% bovine serum albumin (HBA), permeabilized with 0.025% saponin (Sigma) in HBSS– for 10 min at room temperature, and washed twice with HBA. Macrophages were then blocked in HBA containing 5% normal donkey serum (Jackson ImmunoResearch, West Grove, PA) for 1 h at room temperature. Cells were incubated with 5 ml/gml rabbit anti-mouse group V sPLA$_2$ (32) in blocking buffer for 2 h at room temperature, washed extensively with HBA, and incubated for 1 h at room temperature with fluorescein isothiocyanate-conjugated donkey anti-rabbit IgG (heavy and light chains; Jackson ImmunoResearch), diluted 1:400. Cells were washed five times with HBA, mounted in Vectashield™ mounting medium (Vector Laboratories, Burlingame, CA), and imaged with a Nikon Eclipse TE2000U microscope coupled with a Spot-RT digital camera.
dye. In some experiments, 4 volumes of ethanol and 60 mg of PGB, (as internal standard for RP-HPLC) were added to the lava fluid, mixed well, incubated on ice for 30 min, and centrifuged at 10,000 x g for 10 min at 4 °C. The supernatants were then dried by vacuum centrifugation, resuspended in 50 mM HEPES (pH 7.6) and methanol (1:2, v/v), and analyzed for cysteinyl leukotriene content by RP-HPLC.

RP-HPLC Analysis of Cysteinyl Leukotrienes—Cysteinyl leukotrienes were measured by RP-HPLC as described previously (36, 37). Briefly, samples were applied to a 5-μm, 4.6 x 250-mm C18 Ultrasphere RP column (Beckman) equilibrated with a solvent of methanol/acetone/water/acetic acid (10:15:100:0.2, v/v), pH 6.0 (solvent A). After injection of the sample, the column was eluted at a flow rate of 1 ml/min with a programmed concave gradient to 55% of solvent A and 45% methanol over 2.5 min. After 5 min, methanol was increased linearly to 75% over 15 min and maintained at this level for an additional 15 min. The UV absorbance at 280 nm was recorded. The retention times for PGB2, LTC4, LTD4, and LTE4 were 21.4, 22.2, 23.7, and 25.1 min, respectively.

Quantitative analyses of cysteinyl leukotrienes were calculated from the ratio of the peak area of each leukotriene to the peak area of the PGB, internal standard after correction for the differences in molar extinction coefficients.

Statistical Analyses—Data are presented as arithmetic means and S.E. Statistical differences between groups were calculated using Student’s t test.

RESULTS AND DISCUSSION

Generation of Mice Lacking Group V sPLA2—To generate mice lacking group V sPLA2, we used targeted gene disruption. Southern blotting of wild-type genomic DNA for group V sPLA2 yielded bands of ~5 and ~3.7 kb with NcoI (Fig. 1B) and EcoRI (data not shown) digestion, respectively. Southern blotting of DNA from animals with disruption of the group V sPLA2 gene yielded bands 2.6 and 0.8 kb smaller than wild-type DNA after digestion with NcoI (Fig. 1B) and EcoRI (data not shown), respectively. Quantities of cysteinyl leukotrienes were calculated from the ratio of the peak area of each leukotriene to the peak area of the PGB, internal standard after correction for the differences in molar extinction coefficients.

Zymosan-induced Eicosanoid Generation by Mouse Peritoneal Macrophages—Stimulation of group V sPLA2-null and wild-type macrophages with unopsonized zymosan particles elicited maximum cysteinyl leukotriene and PGE2 generation at 3 h (data not shown). Cysteinyl leukotriene generation plateaued at 30–100 zymosan particles/cell, whereas PGE2 generation plateaued at a maximal response at 3–10 particles/cell (Fig. 3, A and B). Both cysteinyl leukotriene generation and PGE2 generation were attenuated at all doses of zymosan in group V sPLA2-null peritoneal macrophages compared with wild-type control cells, with no change in the position of the dose-response curve.

Immunofluorescence analysis (Fig. 2) confirmed the lack of cPLA2 with cPLA2-α-null mice (19). Combined with our data, this suggests an absolute requirement for cPLA2-α in eicosanoid generation by zymosan-stimulated peritoneal macrophages and an amplifying role for group V sPLA2. This is consistent with the conclusions obtained in other cells. In HEK293 cells, the function of transfected group V sPLA2 in supplying arachidonic acid for eicosanoid generation was abolished by pharmacological inhibition of cPLA2-α (22). In primary cultures of mouse mesangial cells, adenoviral transfection with group V sPLA2 augmented arachidonic acid release in response to H2O2 by cells obtained from cPLA2-α-sufficient mice but not from cPLA2-α-null mice (24).

The precise mechanism by which group V sPLA2 amplifies the essential function of cPLA2-α is unknown and is the subject of ongoing experiments.

The participation of group V sPLA2 and its possible cross-talk with cPLA2-α in the release of arachidonic acid has been demonstrated in the mouse P388D1 macrophage cell line. When these cells were labeled with [3H]arachidonic acid for 5 h, primed with LPS for 1 h, and then activated with platelet-
activating factor, there was a biphasic release of labeled arachidonate (25). In the first phase, which was complete within 2 min, arachidonic acid was released and retained within the cell, whereas in the second phase, extracellular release of labeled arachidonic acid occurred over the succeeding 10 min. Studies with pharmacological inhibitors and with antisense oligonucleotides demonstrated that the initial intracellular release of arachidonic acid was dependent upon cPLA₂α, whereas the subsequent extracellular release was dependent upon group V sPLA₂ (26). The initial action of cPLA₂α appeared necessary for the cellular function of group V sPLA₂ (27). In certain subclones of the P388D₁ macrophage, LPS
stimulation alone elicited a delayed phase of arachidonic acid release and prostaglandin endoperoxide synthase-2-dependent PGE₂ generation (28). Both cPLA₂/H9251 and group V sPLA₂ were required, but, in contrast to the primed-immediate response to LPS and PAF, activation of cPLA₂/H9251 led to induction of group V sPLA₂ expression, which in turn induced prostaglandin endoperoxide synthase-2. A third pathway of arachidonic acid release in P388D1 macrophages was described in response to zymosan (44). Zymosan alone elicited the Ca²⁺/H₁₁₀₀₁-dependent activation of cPLA₂/H₉₂₅₁, which elicited an immediate and sustained release of arachidonic acid that was inhibited by methyl arachidonyl fluorophosphonate, an inhibitor of cPLA₂/H₉₂₅₁, and not by LY311717, an inhibitor of group V sPLA₂ (44). Priming with LPS augmented the response to zymosan, providing an increment in release of arachidonic acid that was inhibited by LY311717. Exogenous group V sPLA₂ also augmented the response to zymosan. By comparison, our findings indicate a role for group V sPLA₂ in peritoneal macrophages stimulated with zymosan alone in the absence of LPS priming. The differences between our data and those obtained with the P388D₁ cell line may be due to the transformed nature of P388D₁ cells or may relate to the observation that release of arachidonic acid in response to zymosan was only observed in the MAB clone of this cell line (44) and/or to the observation that P388D₁ cells have low levels of esterified arachidonic acid compared with primary cultures of mouse macrophages (45).

Zymosan-induced Peritonitis—Disruption of the genes encoding LTC₄ synthase (43) and of CysLT₁R (36), revealed the contribution of the cysteinyl leukotrienes, acting through CysLT₁R, to plasma exudation in zymosan-induced peritonitis. We therefore assessed cysteinyl leukotriene generation (by RP-HPLC) and plasma exudation (by leak of Evans blue dye) in group V sPLA₂-null and wild-type mice in response to intraperitoneal injection of a suspension of zymosan A. There was a significant ~50% attenuation of the leak of Evans blue dye 15 and 30 min after injection of zymosan in mice lacking group V sPLA₂ compared with that observed in wild-type littermates (Fig. 4). No difference in plasma exudation was apparent at 60 and 120 min. We measured cysteinyl leukotrienes in the peritoneal lavage fluid 30 min after intraperitoneal injection of zymosan, when there was significant attenuation of plasma exudation. We therefore assessed cysteinyl leukotriene generation (by RP-HPLC) and plasma exudation (by leak of Evans blue dye) in group V sPLA₂-null and wild-type mice in response to intraperitoneal injection of a suspension of zymosan A. There was a significant ~50% attenuation of the leak of Evans blue dye 15 and 30 min after injection of zymosan in mice lacking group V sPLA₂ compared with that observed in wild-type littermates (Fig. 4). No difference in plasma exudation was apparent at 60 and 120 min. We measured cysteinyl leukotrienes in the peritoneal lavage fluid 30 min after intraperitoneal injection of zymosan, when there was significant attenuation of plasma exudation. We therefore assessed cysteinyl leukotriene generation (by RP-HPLC) and plasma exudation (by leak of Evans blue dye) in group V sPLA₂-null and wild-type mice in response to intraperitoneal injection of a suspension of zymosan A. There was a significant ~50% attenuation of the leak of Evans blue dye 15 and 30 min after injection of zymosan in mice lacking group V sPLA₂ compared with that observed in wild-type littermates (Fig. 4). No difference in plasma exudation was apparent at 60 and 120 min. We measured cysteinyl leukotrienes in the peritoneal lavage fluid 30 min after intraperitoneal injection of zymosan, when there was significant attenuation of plasma exudation. We therefore assessed cysteinyl leukotriene generation (by RP-HPLC) and plasma exudation (by leak of Evans blue dye) in group V sPLA₂-null and wild-type mice in response to intraperitoneal injection of a suspension of zymosan A. There was a significant ~50% attenuation of the leak of Evans blue dye 15 and 30 min after injection of zymosan in mice lacking group V sPLA₂ compared with that observed in wild-type littermates (Fig. 4). No difference in plasma exudation was apparent at 60 and 120 min. We measured cysteinyl leukotrienes in the peritoneal lavage fluid 30 min after intraperitoneal injection of zymosan, when there was significant attenuation of plasma exudation.
extravasation. LTE_4 was the predominant cysteinyl leukotriene present in the lavage fluid, as reported previously (43), and its generation was attenuated in group V sPLA_2-null animals compared with wild-type controls (Fig. 5).

The attenuation of enhanced vascular permeability in response to zymosan A in group V sPLA_2-null mice at early time points but not at later time points is somewhat distinct from the findings in mice lacking LTC_4 synthase or CysLT_1R (36, 43). In the former, attenuated vascular permeability was observed up to 1 h after intraperitoneal injection of zymosan, and in the latter, it extended to 4 h. Nevertheless, the greatest attenuation of plasma exudation in both strains of mice was observed at early time points and was less marked at 2 and 4 h, consistent with our findings in group V sPLA_2-null mice and the means ± S.E. for each group of mice (B). *p < 0.05.

In summary, this is the first report of mice with targeted disruption of group V sPLA_2. Our studies reveal the participation of the enzyme in zymosan-induced eosinoid generation both in vitro and in vivo and suggest a role for the enzyme in acute innate immune responses.

Acknowledgments—We are grateful to Luis A. Sanchez-Perez and Huiya Gilbert for excellent technical assistance.

REFERENCES
1. Six, A. D., and Dennis, E. A. (2000) Biochim. Biophys. Acta 1488, 1–19
2. Diaz, B. L., and Arm, J. P. (2003) Prostaglandins Leukotrienes Essent. Fatty Acids 69, 87–97
3. Clark, J. D., Lin, L. L., Kriz, R. W., Ramesha, C. S., Sultzman, L. A., Lin, A. Y., Milona, N., and Knopf, J. L. (1999) Cell 96, 1045–1051
4. McNish, R. W., and Peters-Golden, M. (1993) Biochem. Biophys. Res. Commun. 196, 147–153
5. Underwood, K. W., Sung, C., Kriz, R. W., Chang, X. J., Knopf, J. L., and Lin, L.-L. (1998) J. Biol. Chem. 273, 21926–21932
6. Pickard, R. T., Strifler, B. A., Kramer, R. M., and Sharp, J. D. (1999) J. Biol. Chem. 274, 8823–8831
7. Chen, J., Engle, S. J., Seilhamer, J. J., and Tischfeld, J. A. (1994) J. Biol. Chem. 269, 23859–23867
8. Capillard, L., Kounanov, K., Mattéi, M.-G., Lazdunski, M., and Lambeau, G. (1997) J. Biol. Chem. 272, 15745–15752
9. Valentin, E., Koduri, R. S., Scimeca, J. C., Carle, G., Gelb, M. H., Lazdunski, M., and Lambeau, G. (1999) J. Biol. Chem. 274, 19152–19160
10. Valentin, E., Ghomashchi, F., Gelb, M. H., Lazdunski, M., and Lambeau, G. (1999) J. Biol. Chem. 274, 31195–31202
11. Gelb, M. H., Valentin, E., Ghomashchi, F., Lambeau, G., and Lazdunski, M. (2000) J. Biol. Chem. 275, 39823–39826
12. Ho, I. C., Arm, J. P., Bingham, C. O., III, Choi, A., Austen, K. F., and Glimcher, J. L. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 13117–13122
13. Ackermann, E. J., and Dennis, E. A. (1994) J. Biol. Chem. 269, 9227–9233
14. Tjoelker, L. W., and Stafforini, D. M. (2000) Biochem. Biophys. Acta 1488, 102–123
15. Grases, R. (1995) J. Lipid Mediat. Cell Signal. 12, 131–137
16. Ackermann, E. J., and Dennis, E. A. (1994) J. Biol. Chem. 269, 9227–9233
17. Uozumi, N., Kume, K., Nagase, T., Nakatani, N., Ishii, S., Tashiro, H., Komagata, Y., Mak, K., Ikuta, K., Ouchi, Y., Miyazaki, J., and Shimizu, T. (1997) Nature 389, 619–622
18. Bonventre, J. V., Huang, Z., Taheri, M. R., O’Leary, E., Li, E., Moskowitz, M. A., and Sapirstein, A. (1997) Nature 389, 622–625
19. Gijon, M. A., Spencer, D. M., Siddiq, A. R., Bonventre, J. V., and Leslie, C. C. (2000) J. Biol. Chem. 275, 20146–20156
20. Fujishima, H., Sanchez Mejia, R., Bingham, C. O., III, Lam, B. K., Sapirstein, A., Bonventre, J. V., Austen, K. F., and Arm, J. P. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 13117–13122
21. Nakatani, N., Ureumi, N., Kame, K., Murakami, M., Kudo, I., and Shimizu, T. (2000) Biochem. J. 352, 311–317
22. Murakami, M., Shimbara, S., Kambe, T., Kawata, H., Winstead, M. V., Tischfield, J. A., and Kudo, I. (1998) J. Biol. Chem. 273, 14411–14423
23. Murakami, M., Koduri, R. S., Enomoto, A., Shimbara, S., Seki, M., Yoshihara, K., Singer, A., Valentin, E., Ghomashchi, F., Lambeau, G., Gelb, M. H., and Kudo, I. (2001) J. Biol. Chem. 276, 10083–10096
24. Han, W. K., Sapirstein, A., Hung, C. C., Alessandrini, A., and Bonventre, J. V. (2003) J. Biol. Chem. 278, 24153–24163
25. Balsinde, J., Barbour, S. E., Bianco, I. D., and Dennis, E. A. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 11060–11064
26. Balboa, M. A., Balsinde, J., Winstead, M. V., Tischfield, J. A., and Dennis, E. A. (1996) J. Biol. Chem. 271, 32381–32384
27. Balsinde, J., and Dennis, E. A. (1996) J. Biol. Chem. 271, 6758–6765
28. Shinohara, H., Balboa, M. A., Johnson, C. A., Balsinde, J., and Dennis, E. A. (1999) J. Biol. Chem. 274, 12263–12268
29. Tischfield, J. A. (1997) J. Biol. Chem. 272, 17247–17250
30. Reddy, S. T., and Herschman, H. R. (1997) J. Biol. Chem. 272, 3231–3237
31. Dennis, E. A. (1994) J. Biol. Chem. 269, 13057–13061
32. Arm, J. P., Nwankwo, C., and Austen, K. F. (1997) J. Immunol. 158, 2342–2349
33. Penrose, J. F., Spector, J., Lambeau, G., Friend, D. S., Zweifach, A., and Leslie, C. C. (2001) J. Biol. Chem. 276, 30150–30160
34. Kanaoka, Y., Maekawa, A., Penrose, J. F., Austen, K. F., and Lam, B. K. (2001) J. Biol. Chem. 276, 22608–22613
35. Qiu, Z. H., Gijon, M. A., de Carvalho, M. S., and Leslie, C. C. (1998) J. Biol. Chem. 273, 8203–8209
36. Rouzer, C. A., Scott, W. A., Cohn, Z. A., Blackburn, P., and Manning, J. M. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 4028–4032
37. Sanchez Meija, R. O., Lam, B. K., and Arm, J. P. (2000) Am. J. Respir. Cell Mol. Biol. 22, 557–565
38. Degouvee, N., Ghomashchi, F., Stefanski, E., Singer, A., Smart, B. P., Borregaard, N., Reithmeier, R., Lindsay, T. F., Lichtenberger, C., Reinsch, W., Lambeau, G., Arm, J., Tischfield, J., Gelb, M. H., and Rubin, B. B. (2002) J. Biol. Chem. 277, 5061–5073
39. Qiu, Z. H., de Carvalho, M. S., Spencer, D. M., and Leslie, C. C. (1998) J. Biol. Chem. 273, 8203–8211
40. Rouzer, C. A., Scott, W. A., Cohn, Z. A., Blackburn, P., and Manning, J. M. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 4028–4032
41. Rouzer, C. A., Scott, W. A., Keppe, J., and Cohn, Z. A. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 4279–4322
42. Deherty, N. S., Pouhelle, P., Borgeat, P., Beaver, T. H., Westrich, G. L., and Schrader, N. L. (1985) Prostaglandins 30, 769–789
43. Kanaoka, Y., Maekawa, A., Penrose, J. F., Austen, K. F., and Lam, B. K. (2001) J. Biol. Chem. 276, 22608–22613
44. Balsinde, J., Balboa, M. A., and Dennis, E. A. (2000) J. Biol. Chem. 275, 22544–22549
45. Balsinde, J., and Dennis, E. A. (1996) Eur. J. Biochem. 235, 480–485
46. Han, S. K., Kim, K. P., Koduri, R., Bittova, L., Munoz, N. M., Leff, A. R., Wilton, D. C., Gelb, M. H., and Cho, W. (1999) J. Biol. Chem. 274, 11881–11888
47. Munoz, N. M., Kim, Y. J., Meliton, A. Y., Kim, K. P., Han, S. K., Boetticher, E., O’Leary, E., Myou, S., Zhu, X., Bouventre, J. V., Leff, A. R., and Cho, W. (2003) J. Biol. Chem. 278, 38813–38820
48. Penrose, J. F., Spector, J., Lam, B. K., Friend, D. S., Xu, K., Jack, R. M., and Austen, K. F. (1995) Am. J. Respir. Cell Mol. Biol. 132, 283–289
49. Woods, J. W., Evans, J. F., Ether, D., Scott, S., Vickers, P. J., Hearn, L., Hiebein, J. A., Charleson, S., and Singer, I. I. (1993) J. Exp. Med. 178, 1935–1946
50. Evans, J. H., Spencer, D. M., Zweifach, A., and Leslie, C. C. (2001) J. Biol. Chem. 276, 30150–30160
51. Glover, S., Bayburt, T., Jonas, M., Chi, E., and Gelb, M. H. (1995) J. Biol. Chem. 270, 15359–15367
