Published in final edited form as:
J Biol Chem. 1998 November 20; 273(47): 31215–31221.

Decreased Neonatal Dietary Fat Absorption and T Cell Cytotoxicity in Pancreatic Lipase-related Protein 2-Deficient Mice*

Mark E. Lowe[‡,§,¶], **Mark H. Kaplan**[‖,**], **Laurie Jackson-Grusby**[‡‡], **Dymphna D'Agostino**[‡], and **Michael J. Grusby**[‖,§§,¶¶]

[‡]Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri 63110

[§]Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, Missouri 63110

[‖]Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, Massachusetts 02115

[§§]Department of Medicine, Harvard Medical School, Boston, Massachusetts 02115

[‡‡]The Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02142

Abstract

The pancreas secretes several different lipases. The most abundant is pancreatic triglyceride lipase (PTL). The pancreas also synthesizes two homologues of PTL, the pancreatic lipase-related proteins 1 and 2 (PLRP1 and PLRP2). Cytotoxic T-lymphocytes also express PLRP2 under certain conditions. We sought to determine the role of PLRP2 in fat absorption and in T-cell cytotoxicity by creating a PLRP2-deficient mouse. Adult PLRP2-deficient mice had normal fat absorption. In contrast, suckling PLRP2-deficient mice had fat malabsorption evidenced by increased fecal weight, increased fecal fats, and the presence of undigested and partially digested dietary triglycerides in the feces. As a result, the PLRP2-deficient pups had a decreased rate of weight gain. To assess T cell cytotoxicity, we immunized PLRP2-deficient mice with a mastocytoma cell line, P815, and determined the ability of splenocytes from the immunized mice to kill P815 cells in a ^{51}Cr release assay. PLRP2-deficient cells had deficient killing activity in this assay, and PLRP2-deficient splenocytes released fewer fatty acid from the target cells than did control cells. Our results provide the first evidence of a physiological function for PLRP2. PLRP2 participates in T cell cytotoxicity, and PLRP2 performs a crucial role in the digestion of dietary fats in suckling animals.

During the last 10 years, data from cDNA and genomic cloning have provided evidence for a lipase gene family. The first speculation about a lipase gene family began after the cloning of cDNAs for hepatic lipase, lipoprotein lipase, and pancreatic triglyceride lipase (PTL)[1] showed similarities among the predicted amino acid sequences of these lipases (1).

---

*This work was supported by National Institutes of Health Grants CA-56462 and DK 53100 and by the Mathers Foundation.

[¶]To whom correspondence should be addressed: Dept. of Pediatrics, Washington University School of Medicine, Box 8116, St. Louis, MO 63110. Tel.: 314-454-2576; Fax: 314-454-4218; Lowe@Kids.wustl.edu.
[**]Special Fellow of the Leukemia Society of America.
[¶¶]Scholar of the Leukemia Society of America.

[1]The abbreviations used are: PTL, pancreatic triglyceride lipase; PLRP1, pancreatic lipase related protein 1; PLRP2, pancreatic lipase related protein 2; PBS, phosphate-buffered saline; ANOVA, analysis of variance; CTL, cytotoxic T lympocytes.

Subsequently, the cloning of other cDNAs encoding lipases from various species and the realization that many lipases and other hydrolases share a common protein folding pattern expanded the family greatly (2). Two of the recently described lipases have higher sequence homology to PTL than any other members of the gene family. Because of their similarities to PTL, Giller *et al.*(3) named these two lipases pancreatic lipaserelated proteins 1 and 2 (PLRP1 and PLRP2) (3).

One of these related proteins, PLRP2, was also independently cloned for reasons other than its similarity to PTL. Mouse PLRP2 was cloned as an interleukin-4-inducible gene in CD8+ T cells (4). Rat PLRP2 was cloned as GP3, a zymogen granule membrane-associated protein (5). These studies suggested potential physiological functions for PLRP2 in T cell-mediated cytotoxicity, in zymogen granule fusion with the acinar cell plasma membrane, and in the digestion of dietary fats.

Additional investigations of PLRP2 have supported some of these functions. Each of these postulated functions requires that PLRP2 have lipase activity. Studies of recombinant PLRP2 have demonstrated a high level of triglyceride lipase activity similar to PTL (6). Unlike PTL, PLRP2 also hydrolyzes other lipid substrates and possesses phospholipase and galactolipase activities (6, 7). PLRP2 is also secreted by the pancreas and by isolated pancreatic acinar cells in culture (8, 9). This secretion places PLRP2 in the duodenum, where dietary fat digestion occurs. Secretion of PLRP2 by T cells has not been reported. Finally, the pancreas expresses mRNA encoding PLRP2 at birth but does not express mRNA encoding PTL until near the suckling-weanling transition (10). This temporal pattern of expression for mRNA encoding PLRP2 and PTL led to the speculation that PLRP2 mediates dietary fat digestion in suckling animals.

We sought to define the function of PLRP2 in dietary fat metabolism and in T cell cytotoxicity. To accomplish this aim, we created a line of PLRP2-deficient mice by homologous recombination. We characterized T cell function in adult animals and dietary fat absorption in animals at various ages.

MATERIALS AND METHODS

Targeting Construct and Generation of Mice with Null Allele for PLRP2

The targeting construct was made by cloning a *Sma*I/*Cla*I fragment of the mouse PLRP2 gene into a vector containing a thymidine kinase cassette (Fig. 1) (11). The gene fragment contained exons I–V out of the 12 total PLRP2 exons (12). A LacZ/neomycin cassette was cloned into a unique *Bam*HI site in exon IV (13). The LacZ fusion was created so that cells expressing the gene encoding PLRP2 could be detected *in situ*.

D3 embryonic stem cells were transfected with the targeting construct. We screened for targeted stem cells by Southern blot of genomic DNA isolated from neomycin-resistant clones and digested with *Eco*RI. The blot was hybridized to a 5′-probe, which detected a 5-kilobase pair wild-type band and a 7-kilobase pair fragment from the targeted allele. The targeting efficiency was 3.6% (7/190). Embryonic stem cells from a targeted clone were injected into BALB/c mouse blastocysts to generate chimeric animals whose offspring from BALB/c matings were subsequently screened for the targeted allele by a polymerase chain reaction with three primers (lipase upstream, 5′-GTAACTGTATTTGCGTTGA-3′; lipase downstream, 5′-ACCGACAACACTTGCACCAA-3′; and LacZ, 5′-ATTCAGGCTGCGCAACTGTT-3′) (Fig. 1). These primers amplified a 113-base pair wild-type fragment and a 190-base pair fragment from the targeted allele (Fig. 1). Mice with the targeted allele were backcrossed six generations to BALB/c mice before intercrossing.

RNA Analysis

We isolated total RNA from the pancreas of animals at birth, 10 days, 21 days, and 4 months of age as described previously (10). The integrity of the RNA was assessed by denaturing agarose gel electrophoresis (14). The mRNA encoding PTL, PLRP1, and PLRP2 was quantitated by an RNase protection assay utilizing 300-base pair probes complementary to each mRNA species. The PTL and PLRP1 probes went from the middle of exon 3 to the middle of exon 5, and the PLRP2 probe extended from the middle of exon 2 to the middle of exon 4 and was 5′ of the LacZ-neomycin cassette. Standard curves were prepared by hybridizing the probe to known amounts of *in vitro* transcribed mRNA encoding each individual lipase. Values were normalized to levels of cyclophyllin. The cyclophyllin standard cDNA was obtained from Ambion, Inc. mRNA transcription was done with reagents from Ambion, Inc. (Austin TX) according to the manufacturer's directions. The Ambion RPA II kit was used for the RNase protection assay. The products were separated by acrylamide gel electrophoresis according to the instructions with the kit and were transferred to Ambion's Bright-Star Plus membrane in a semidry electroblot apparatus (Bio-Rad). Bands were detected with the BrightStar BioDetect (Ambion) kit according to the instructions. The signal from the standard curves and the unknowns was quantitated using SigmaGel software (SSPS, Inc., Chicago, IL), and the amount of mRNA was determined from the standard curve.

Immunohistochemistry and β-Galactosidase Staining

For Immunoperoxidase staining, tissues were fixed in 10% formalin and embedded in paraffin, and thin sections were made. Paraffin was removed with xylene and isopropyl alcohol washes followed by three 5-min rinses in PBS-T (137 mM NaCl, 2.7 mM KCl, 4.3 mM $Na_2HPO_4 \cdot H_2O$, 1.4 mM KH_2PO_4, 0.2% Tween 20). Endogenous peroxidase activity was quenched with 0.3% hydrogen peroxide in H_2O. After washing three times for 5 min in PBS-T, the slides were incubated in blocking serum, 1.5% preimmune goat serum in PBS-T for 30 min. They were then incubated with the primary antibody diluted 1:2000 in PBS-T overnight at 4 °C. The primary antibody was a rabbit polyclonal antibody prepared against a unique peptide from mouse PLRP2 (^{422}TVQRGKDGKEFNFC^{435}) coupled to keyhole lympet hemocyanin. The slides were washed with PBS-T three times for 5 min each wash and processed following the directions in the Vectastain *Elite* ABC Kit (Vector Laboratories, Burlingame CA). The slides were viewed under a standard light microscope.

β-Galactosidase staining was done on fresh frozen sections embedded in O.C.T. (Sakura Finetek, Inc., Torrance CA). The sections were airdried and postfixed in 0.5% glutaraldehyde in PBS (PBS-T without the Tween 20) containing 2 mM $MgCl_2$. The slides were then processed as described (15).

Animal Diets

Diets were from PicoLab™. The standard chow was 5053 and contained 11.9% of energy as fat, 23.6% as protein, and 64.5% as carbohydrate. The medium fat diet was 5058, and the high fat diet was a special test diet. The former contained 21.6% of energy as fat, 21.9% as protein, and 56.5% as carbohydrate. The latter had 59.7% of energy as fat, 15% as protein, and 25.3% as carbohydrate. The fat was derived from lard and corn oil in the same proportions for all diets. *Ad libitum* access to food and water was allowed. The mice were adapted to the diet for at least 2 weeks before samples were collected. Nursing mothers were fed standard 5053 chow.

Fecal Fat Analysis

Adult mice were placed in a cage with a metabolic screen. They were given water but no food during the 4-h collection period. The stool was dried to a constant weight, and fats were extracted as described (16). To obtain stool from suckling animals, 10-day-old animals were sacrificed, and the entire colon was removed. The feces was removed by extrusion and processed as for adult animals. The percentage of recovery was 90 ± 2.0% as determined by the recovery of radiolabeled triolein.

Analysis of Lipid Classes

Extracted fecal fats from 100 mg of dried feces were dissolved in 1 ml of chloroform, and 10 μl of each sample was spotted onto a silica G TLC plate along with a standard mixture containing 10 μg each of monoolein, 1,2-diolein, 1,3-diolein, and triolein. The plate was developed by a two-stage, one-dimensional TLC and developed in cupric acetate/phosphoric acid as described (17, 18).

T Cell Cytotoxicity Assays

Three wild-type and PLRP2-deficient mice were injected intraperitoneally with 3×10^7 P815 cells. After 10 days, the mice were sacrificed, and the spleens were removed. Pooled single cell suspensions were diluted for use in the cytotoxicity assay or for *in vitro* stimulation. Spleen cells were cultured at 5×10^6 cells/ml and stimulated with 3×10^4 mitomycin C-treated P815 cells/ml. After 24 h, 20 units/ml interleukin-2 was added to each culture. The cells were cultured for 6 days before use as effectors in the secondary cytotoxicity assay.

To test cytotoxicity, 2×10^7 P815 cells were labeled with 200 μCi of sodium chromate-51 (NEN Life Science Products) in 10 ml at 37 °C for 1 h or with ^{3}H-labeled oleic acid for 18 h. Labeled target cells were washed extensively and plated in a round bottom 96-well microtiter plate at a concentration of 2×10^4/well. Effector cells were added to the wells in triplicate at the indicated effector:target ratios in a total volume of 200 μl. Plates were spun down, and supernatants were collected after 3 h for fatty acid release or 4 h for chromium release. Samples were counted in a β-counter. The percentage of cytotoxicity was calculated as (experimental release – spontaneous release)/(maximum release – spontaneous release). Spontaneous release was less than 10% of the maximum in lipid release assays and less than 20% of the maximum in chromium release assays.

Statistical Analysis

The data were analyzed by Student's *t* test and by two-way ANOVA followed by the Tukey test for pairwise multiple comparison procedures. The SigmaStat statistical package was used for all calculations.

RESULTS

PLRP2-deficient Mice

To investigate the role of PLRP2 in dietary fat digestion and T cell-mediated cytotoxicity, we generated PLRP2-deficient mice by targeted homologous recombination. Adult and suckling PLRP2-deficient mice were grossly indistinguishable from wild-type and heterozygote littermates. Adult mice had normal numbers and populations of cells in peripheral lymphoid organs. The frequency of PLRP2-deficient mice in heterozygote by heterozygote matings was diminished. The ratio of wild-type:heterozygous:homozygous deficient pups was 1.3:1.9:0.8 in over 300 live births.

Analysis of PLRP2 Expression

Expression of PLRP2 was determined by immunohistochemistry and by β-galactosidase histochemistry. A polyclonal rabbit antibody against a carboxyl-terminal peptide unique to PLRP2 was generated. This antibody recognized purified rat PLRP2 but not purified rat PTL or PLRP1 on a protein immunoblot.[2] Sections of pancreas from wild-type and PLRP2-deficient mice were immunoperoxidase-stained using the anti-PLRP2 antibody as the primary reagent. No staining was detected in the acinar cells of the PLRP2-deficient pancreas (Fig. 2*A*), whereas acinar cells in the wildtype pancreas stained with the antibody (Fig. 2*B*). The staining pattern was heterogeneous with some acinar cells staining darkly and others not staining at all. The absence of PLRP2 immunoreactivity confirms that the mice containing the targeted allele did not synthesize PLRP2 protein.

β-Galactosidase activity could also be detected in the acinar cells of the PLRP2-deficient animals (Fig. 2*C*). Staining was not present in the islets. The acinar cell-specific expression of β-galactosidase was consistent with proper targeting of the PLRP2 gene. Like the staining pattern of the antibody, β-galactosidase activity varies over regions of the pancreas. Not all acinar cells stain to the same extent, demonstrating that individual acinar cells express PLRP2 at different levels.

The temporal pattern of expression for mRNA encoding PTL, PLRP1, and PLRP2 was quantitated by an RNase protection assay. Total RNA was prepared from wild-type and PLRP2-deficient pancreas taken from mice at different ages. Fig. 3 shows the temporal pattern of expression for the mRNAs encoding the three lipase species. The expression pattern of mRNA encoding PLRP1 and PLRP2 in wild-type mice mirrored the pattern previously reported in the rat (Fig. 3, *A* and *B*). Both were present at birth, and expression persisted throughout adulthood. mRNA encoding PTL was not detected at birth or 10 days of age but was present in both wild-type and PLRP2-deficient mice at 21 days of age and 4 months of age (adults). A faint signal with the PLRP2 probe was found in the PLRP2-deficient animals. The signal was about 5% of the signal in wild-type mice and, presumably, represented a chimeric mRNA between the 5′-end of the PLRP2 mRNA and the β-galactosidase mRNA because the targeted deletion was downstream from the region complementary to the probe. Representative gels are shown in Fig. 3*C*. No expression of PTL was detected in newborn and 10-day-old mice even with overexposure of the gel (Fig. 3*C*, *far right part*). Importantly, these results demonstrate that the temporal pattern of expression for the genes encoding PTL, PLRP1, and PLRP2 are the same in the mouse as in the rat and that expression of PTL and PLRP1 was not altered in the PLRP2-deficient animals.

Absorption of Dietary Fats

We next addressed the role of PLRP2 in dietary fat digestion. The secretion of PLRP2, a lipase with high triglyceride lipase activity, by the pancreas suggests that it may play a role in dietary fat digestion. Because earlier studies showed that newborn and suckling animals express PLRP2 but not PTL, we examined both suckling and adult animals. We determined if PLRP2 contributes significantly to fat digestion by measuring fecal fat. The feces from the PLRP2-deficient suckling mice were loose, yellow, and of larger volume than the formed brown feces of wild-type and heterozygous pups. This observation was supported by the increased dry weight of the colonic contents from the PLRP2-deficient pups (Table I). These findings suggested that the PLRP2 pups had steatorrhea.

[2]M. Lowe, unpublished observations

We confirmed that the pups had fatty stools by measuring the fecal fat content in the stools (Fig. 4). Fats comprised 55% of the fecal dry weight in the PLRP2-deficient pups compared with 10.6% in heterozygous and 7.5% in wild-type pups. There were no differences in fecal fat between wild-type and PLRP2-deficient mice by 23 days of age. Challenging the adult mice with higher fat diets did not cause the PLRP2-deficient mice to malabsorb fat (Fig. 4). These findings demonstrated that PLRP2 makes a major contribution to the digestion of dietary fats in suckling animals but not in adult animals. The observation that the defect disappears as the pups age can be explained by the induction of pancreatic triglyceride lipase, which compensates for the lack of PLRP2 activity in the older pups.

The malabsorbed lipid classes were identified by thin layer chromatography. The extracted lipids were separated in a two-solvent, one-dimensional system and quantitatively stained with cupric acetate/phosphoric acid. Fatty acids were the predominant species in the feces from wild-type and heterozygous suckling mice (Fig. 5, *lanes 1* and *2*) and in adult wild-type and PLRP2-deficient mice (Fig. 5, *lanes 5* and *6*). In contrast, diglycerides and triglycerides comprised a much greater proportion of the lipids in the feces of PLRP2-deficient animals (Fig. 5, *lanes 3* and *4*). These results confirm that suckling animals require PLRP2 for efficient dietary fat digestion and show that diglycerides and triglycerides are physiological substrates for PLRP2.

Growth of PLRP2-deficient Mice

If the PLRP2-deficient mice malabsorb fat, their rate of weight gain might be affected. To test this possibility, we weighed all of the pups in multiple litters of heterozygous intercrosses from day 4 to weaning at day 23 (Fig. 6). The curves for the wild-type and heterozygote pups overlap and were not significantly different by two-way ANOVA analysis. The rate of weight gain for the PLRP2-deficient pups differed from the rates for the wild-type and heterozygote pups (0.33 g/day *versus* 0.43 and 0.44 g/day). Two-way ANOVA revealed a significant difference between the PLRP2-deficient pups and the wild-type pups ($F(1, 19) = 78.9$, $p < 0.0001$) and the heterozygote pups ($F(1, 19) = 87.9$, $p < 0.0001$). The decreased weight gain in PLRP2-deficient pups confirmed the importance of PLRP2 in dietary fat digestion by suckling animals.

Cytotoxic Activity in PLRP2-deficient Mice

Since murine PLRP2 was originally identified as an interleukin-4-inducible gene in CD8+ T cells, we also examined the PLRP2-deficient mice for alterations in cytotoxic activity (4). To determine whether PLRP2 plays an important role in T cell-mediated cytotoxicity, we immunized wild-type and PLRP2-deficient mice with the P815 mastocytoma cell line. P815 was derived from DBA/2 mice and is syngeneic at the major histocompatibility complex locus ($H\text{-}2^d$) but has other histocompatibility differences from the BALB/c background of the mice. Ten days following injection of tumor cells, mice were sacrificed and spleen cells were tested as effectors in a ^{51}Cr release assay. Control splenocytes displayed a dose-dependent cytotoxicity against ^{51}Cr-labeled P815 targets (Fig. 7*A*). However, PLRP2-deficient splenocytes killed targets at a significantly lower level.

In parallel, splenocytes were expanded *in vitro* by stimulation with mitomycin C-treated P815 cells. After 6 days in culture, cells were used as effectors in a cytotoxic assay with ^{51}Cr-labeled P815 targets. Cytotoxicity as measured by ^{51}Cr release was similar to the results of the primary assay. CTL from wild-type mice killed targets in a dose-dependent manner, but killing was less efficient by PLRP2-deficient cells (Fig. 7*B*). This deficiency in killing activity was not due to differences in the percentages of CD8+ cells in the cultures (data not shown). Furthermore, levels of granzyme A production by wild-type and PLRP2-

deficient mice were indistinguishable. A similar defect in killing was seen in assays using RENCA cells, another syngeneic tumor target (data not shown).

CTL-mediated lysis has been shown to result in the liberation of free fatty acids from target cells through an undefined mechanism (19). To determine whether free fatty acid release was affected by PLRP2 deficiency, we used [^{3}H]oleic acid-labeled P815 cells as targets in the CTL assay described above. Fig. 7*C* demonstrates that free fatty acid release from syngeneic targets is decreased when PLRP2-deficient CTLs are used as effectors compared with wild-type cells. This correlates with the decreased cytotoxicity seen in the ^{51}Cr release assays.

DISCUSSION

Lipases are ubiquitous enzymes present in many tissues and organisms. One recently described lipase, PLRP2, is expressed in pancreas and cytotoxic T cells (4, 5, 10). Although the function of PLRP2 in these tissues is not known, one hypothesis is that pancreatic PLRP2 participates in dietary fat digestion and that cytotoxic T cell PLRP2 mediates cell lysis by hydrolyzing membrane lipids. To address the physiological function of PLRP2, we created and characterized mice deficient in PLRP2. Our results show that PLRP2 contributes to cytotoxic T cell cytotoxicity and to fat digestion in the suckling animal.

PLRP2 in Cytotoxic T Cells

PLRP2-deficient mice had a clear decrease in cytotoxicity as measured by both ^{51}Cr and ^{3}H-fatty acid release when compared with normal cytotoxic T cells. The decrease was greater than the decrease in cytotoxicity observed in granzyme B-deficient mice, but the defect was not as great as when pathways dependent on perforin or Fas were compromised (20-22). Each of these pathways has been implicated in cytotoxic T cell killing activity. These findings suggested that PLRP2 may play an auxiliary role in some types of cytotoxic T cell-mediated lysis. The molecular details of PLRP2-mediated cytotoxicity remain unclear, and further studies are necessary to elucidate these mechanisms.

Expression in Pancreas

Our evaluation of PLRP2 expression by immunohistochemistry and β-galactosidase histochemistry revealed patchy expression of PLRP2 by acinar cells, suggesting that acinar cells contain varying amounts of PLRP2. The notion of heterogeneous stores of pancreatic exocrine enzymes is not new (23, 24). Earlier studies demonstrated that enzyme contents differ among individual zymogen granules and that enzyme secretion is nonparallel. The heterogeneity of exocrine proteins in acinar cells provides a potential explanation for the nonparallel, adaptive changes in relative amounts of digestive enzymes in response to diet.

PLRP2 in Dietary Fat Digestion

Although the role of pancreatic lipases in dietary fat absorption is widely accepted, the participation of PLRP2 in fat digestion has not been established. The PLRP2-deficient mice provided the opportunity to directly test the function of PLRP2 in dietary fat digestion. Our results demonstrate that adult mice do not require PLRP2 for efficient dietary fat digestion. Although PLRP2 did not make a significant contribution to overall fat absorption in the adult, we did not eliminate the possibility that PLRP2 hydrolyzes a minor species of dietary fat. Also, the potential participation of PLRP2 in zymogen granule release was not tested in these studies. If PLRP2 has an important function in the adult animals, additional investigations are required to define that role.

In contrast, PLRP2 was essential for efficient fat digestion in suckling mice. We found that the PLRP2-deficient suckling mice had 10–15 times more fat in their feces than wild-type or heterozygote animals, suggesting a marked decrease in fat absorption. The fecal fat from PLRP2-deficient mice contained undigested and partially digested acylglycerides, confirming the importance of PLRP2 in dietary triglyceride digestion. Additionally, the weight gain of the suckling animals was decreased compared with wild type and heterozygotes, which is consistent with significant loss of calories through fat malabsorption. Clearly, PLRP2 contributes significantly to dietary fat digestion in suckling animals.

Our findings provide an explanation for the efficient fat digestion in suckling animals. Newborns have a relative PTL deficiency (25). Yet, newborn infants do not have significant steatorrhea despite consuming 3–5-fold more fat per kg, body weight, than adults. Previous models of fat absorption in newborns centered on the ready absorption of undigested monoacylglycerols, which made the complete digestion of dietary fats unnecessary for efficient absorption, or on the presence of bile salt-stimulated lipase in breast milk, which could compensate for the lack of PTL (26-28).

Against these models are *in vitro* experiments demonstrating that a combination of gastric lipase, bile salt-stimulated lipase, and PTL are required for efficient digestion of breast milk triglycerides (28-30). Digestion was incomplete unless all three lipases were present. In addition, there is ample evidence that the low intraluminal concentrations of bile salts found in premature and full-term infants not only decrease bile saltstimulated lipase activity but favor the absorption of fatty acids over monoacylglycerols, making the complete digestion of triglycerides beneficial for the newborn (31-33). Given these data, the low levels of PTL in newborns should hamper the digestion of dietary fats, but most newborns efficiently absorb their large dietary fat intakes, suggesting that other lipases may be active. PLRP2 is clearly one of those lipases.

References

1. Kirchgessner TG, Chuat J-C, Heinzman C, Etienne J, Guilhot S, Svenson K, Ameis D, Pilon C, D'Auriol L, Andalibi A, Schotz MC, Galibert F, Lusis AJ. Nucleic Acids Res. 1989; 86:9647–9651.
2. Cygler M, Schrag JD, Sussman JL, Harel M, Silman I, Gentry MK, Doctor BP. Protein Sci. 1993; 2:366–382. [PubMed: 8453375]
3. Giller T, Buchwald P, Blum-Kaelin D, Hunziker W. J Biol Chem. 1992; 267:16509–16516. [PubMed: 1379598]
4. Grusby MJ, Nabavi N, Wong H, Dick RF, Bluestone JA, Schotz MC, Glimcher LH. Cell. 1990; 60:451–459. [PubMed: 2302735]
5. Wishart MJ, Andrews PC, Nichols R, Blevins GT Jr, Logsdon CD, Williams JA. J Biol Chem. 1993; 268:10303–10311. [PubMed: 8486693]
6. Jennens ML, Lowe ME. J Lipid Res. 1995; 36:2374–2381. [PubMed: 8656075]
7. Andersson L, Carriere F, Lowe ME, Nilsson A, Verger R. Biochim Biophys Acta. 1996; 1302:236–240. [PubMed: 8765145]
8. Lowe ME. Methods Enzymol. 1997; 284:285–297. [PubMed: 9379941]
9. Wagner ACC, Wishart MJ, Mulders SM, Blevins PM, Andrews PC, Lowe AW, Williams JA. J Biol Chem. 1994; 269:9099–9104. [PubMed: 8132647]
10. Payne RM, Sims HF, Jennens ML, Lowe ME. Am J Physiol. 1994; 266:G914–G921. [PubMed: 8203536]
11. Grusby MJ, Johnson RS, Papaioannou VE, Glimcher LH. Science. 1991; 253:1417–1420. [PubMed: 1910207]
12. Kaplan MH, Boyer SN, Grusby MJ. Genomics. 1996; 35:606–609. [PubMed: 8812501]
13. Gossler A, Joyner AL, Rossant J, Skarnes WC. Science. 1989; 244:463–465. [PubMed: 2497519]

14. Davis, LG.; Dibner, MD.; Battey, JF. Basic Methods in Molecular Biology. Elsevier; New York: 1986. p. 143-146.
15. Hogan, B.; Beddington, R.; Costantini, F.; Lacy, E. Manipulating the Mouse Embryo: A Laboratory Manual. 2. Cold Spring Harbor Laboratory; Cold Spring Harbor, NY: 1994. p. 373-375.
16. Schwarz M, Lund E, Setchell K, Kayden H, Zerwekh J, Bjorkhem I, Herz J, Russell D. J Biol Chem. 1996; 271:18024–18031. [PubMed: 8663430]
17. Bitman J, Wood DL. J Liq Chromatogr. 1981; 4:1023–1034.
18. Bitman J, Wood DL, Ruth JM. J Liq Chromatogr. 1981; 4:1007–1021.
19. Richeri GV, Kleinfeld AM. J Immunol. 1991; 147:2809–2815. [PubMed: 1918994]
20. Kagi D, Vignaux F, Ledermann B, Burki K, Depraetere V, Nagata S, Hengartner H, Golstein P. Science. 1994; 265:528–530. [PubMed: 7518614]
21. Kagi D, Ledermann B, Burki K, Seiler P, Odermatt B, Olsen KJ, Podack ER, Zinkernagel RM, Hengartner H. Nature. 1994; 369:31–37. [PubMed: 8164737]
22. Heusel JW, Wesselschmidt RL, Shresta S, Russell JH, Ley TJ. Cell. 1994; 76:977–987. [PubMed: 8137431]
23. Adelson JW, Miller PE. Am J Physiol. 1989; 256:G817–G825. [PubMed: 2655473]
24. Miller PE, Adelson JW. Am J Physiol. 1987; 252:G768–G775. [PubMed: 3296783]
25. Fredikzon A, Olivecrona T. Pediatr Res. 1978; 43:247–257.
26. Cohen M, Morgan RGH, Hofmann AF. Gastroenterology. 1971; 60:1–15. [PubMed: 5544089]
27. Carey MC, Hernell O. Semin Gastrointest Dis. 1992; 3:189–208.
28. Bernback S, Blackberg L, Hernell O. J Clin Invest. 1990; 85:1221–1226. [PubMed: 2318975]
29. Chen Q, Sternby B, Nilsson A. Biochim Biophys Acta. 1989; 1004:372–385. [PubMed: 2503032]
30. Hernell O, Blackberg L, Chen Q, Sternby B, Nilsson A. J Pediatr Gastroenterol Nutr. 1993; 16:426–431. [PubMed: 8315552]
31. Hofmann, AF.; Mekhijan, HS. The Bile Acids. Nair, PP.; Kritchevsky, D., editors. Plenum Publishing Corp.; New York: 1971. p. 103-152.
32. Lindstrom MB, Sternby B, Borgstrom B. Biochim Biophys Acta. 1988; 959:178–184. [PubMed: 3349096]
33. Morgan RGH, Borgstrom B. Q J Exp Physiol. 1969; 54:228–243. [PubMed: 5193736]

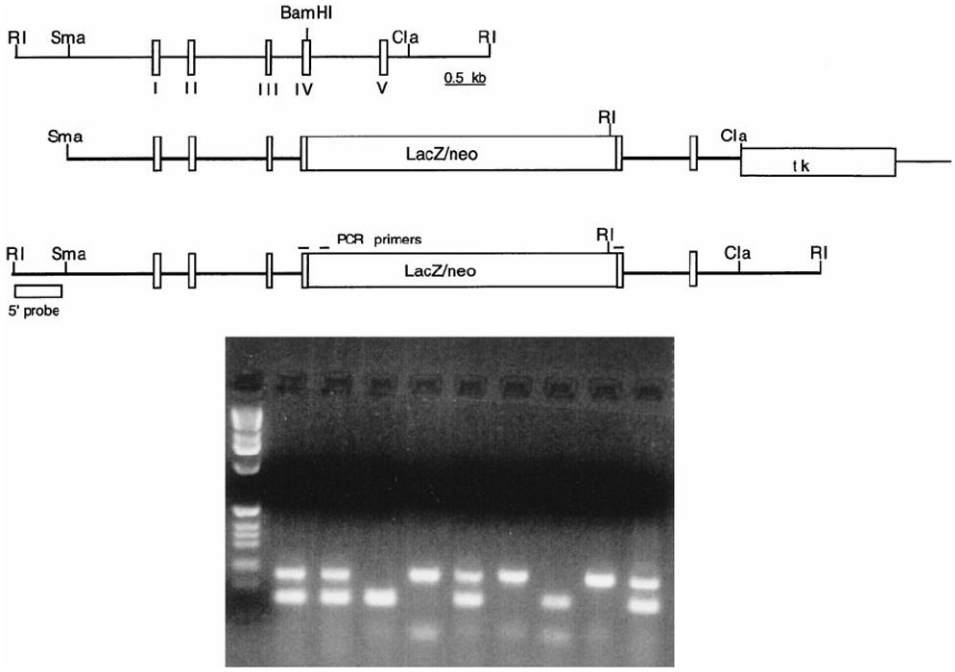

Fig. 1. Targeting construct for creating PLRP2-deficient mice and polymerase chain reaction (*PCR*) screening of tail DNA from matings of mice heterozygous for the PLRP2 allele

The *top half* of the *figure* shows a schematic map of the PLRP2 wild-type allele (*top*), targeting construct (*middle*), and the targeted allele (*bottom*). Exons are numbered with roman numerals. The positions of the polymerase chain reaction primers and fragments used for Southern analysis are indicated. *tk*, thymidine kinase. The *bottom half* of the *figure* shows a representative polymerase chain reaction screening of tail DNA. Samples were separated by agarose gel electrophoresis and stained with ethidium bromide. Markers are in the *first lane*. The next *nine lanes* show the results from screening. The upper, 190-base pair band is the targeted allele, and the lower, 113-base pair band is the wild-type allele.

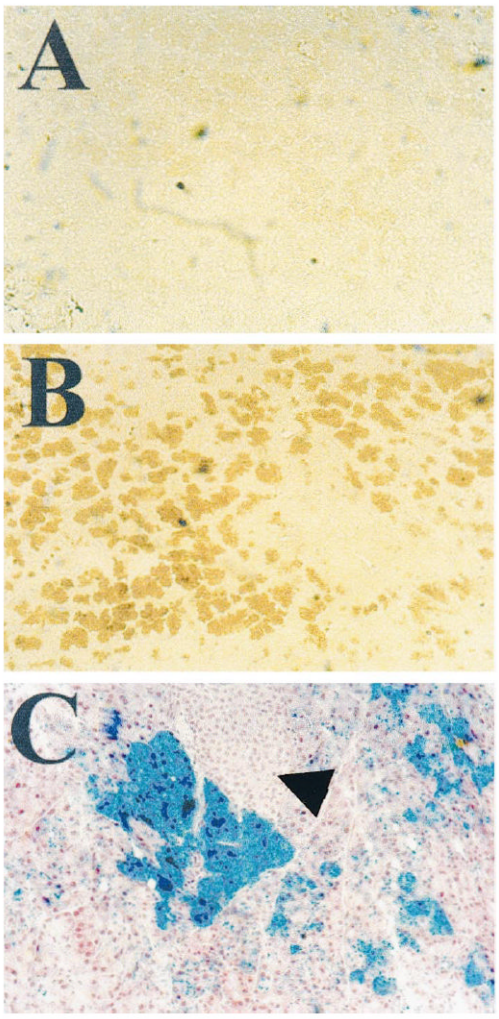

Fig. 2. PLRP2 expression in the pancreas determined by immunohistochemistry and β-galactosidase histochemistry

Sections of pancreas from wild-type and PLRP2-deficient adult mice were stained as described under "Materials and Methods." *A*, PLRP2-deficient pancreas stained with antibody against mouse PLRP2. *B*, wildtype pancreas stained with antibody against mouse PLRP2. *C*, PLRP2-deficient pancreas stained for β-galactosidase activity. The *arrowhead* marks an islet.

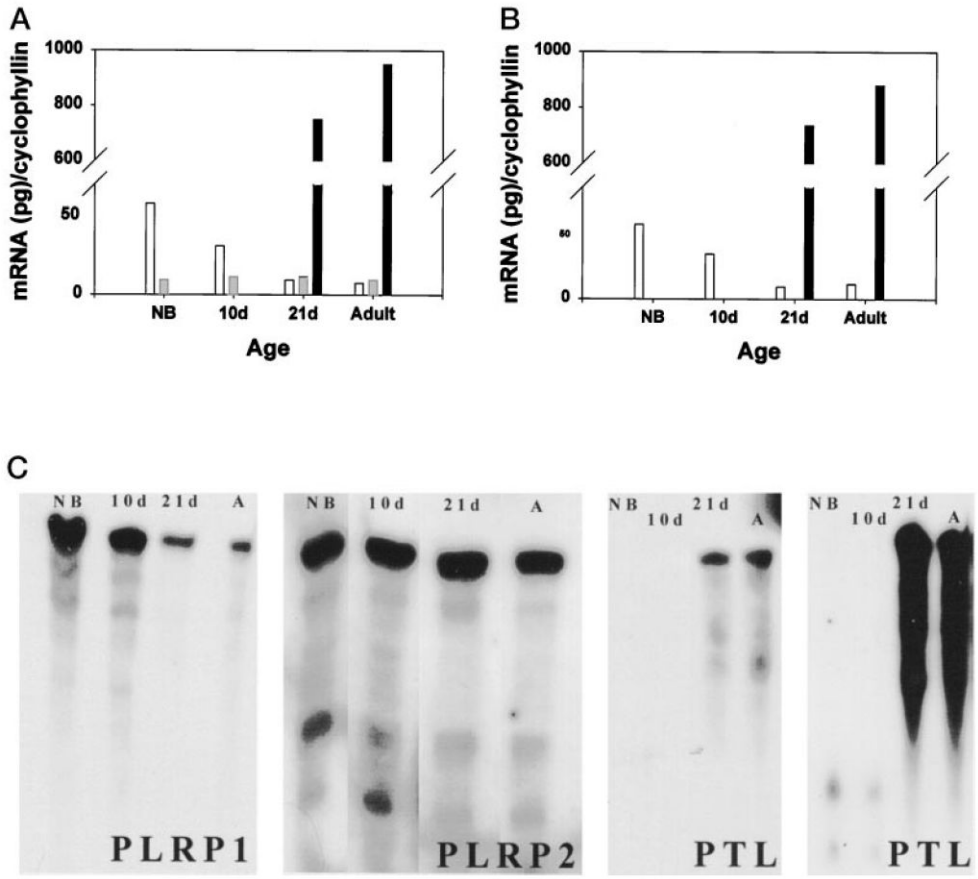

Fig. 3. Temporal expression of mRNA encoding PLRP1, PLRP2, and PTL

The levels of mRNA encoding PLRP1 (*white bars*), PLRP2 (*gray bars*), and PTL (*black bars*) are shown for wild-type pancreas (*A*) and for PLRP2-deficient pancreas (*B*). Duplicate assays on 1 and 2 μg of total RNA were performed for each mRNA species and each genotype. The results were averaged and normalized for 1 μg of total RNA and to arbitrary cyclophyllin units. *C*, representative RNase protection gels are shown. The mRNA species is given *below* each gel, and the age of the animal is shown *above* each *lane* in the respective *gels*. The exposure times for each of the gels is not equivalent. The *third* and *fourth gels* show two different exposure times, 1 min (gel 3) and 15 min (gel 4), for the same PTL samples.

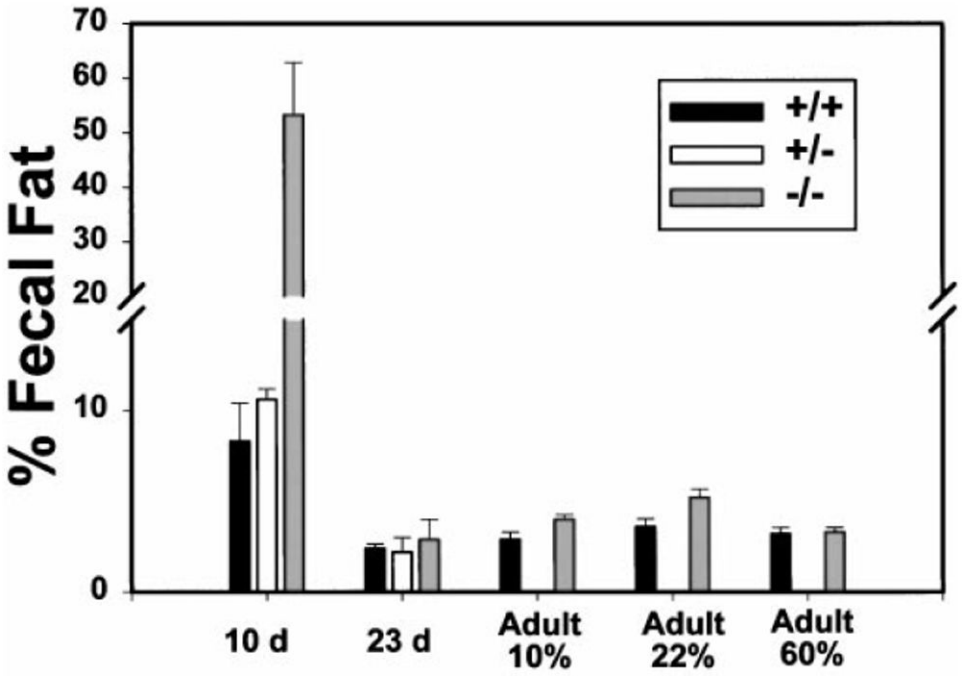

Fig. 4. Fecal fat in PLRP2-deficient mice at various ages

Feces were collected, and fats were extracted as described under "Materials and Methods." The results are expressed as the percentage of fat comprising the total stool dry weight. The ages of the animals are given on the *x* axis. The percentage of calories as fat is given for the adults. *Black bars*, wild type; *white bars*, heterozygotes; *gray bars*, PLRP2-deficient. The *error bars* are one S.D. Heterozygote adults were not studied. The fecal fat is significantly different for the 10-day animals compared with the corresponding genotype in the other groups ($p < 0.05$). In the 10-day group, the value for the PLRP2-deficient mice is significantly different from the wild-type and heterozygote animals; $p < 0.0001$ for both.

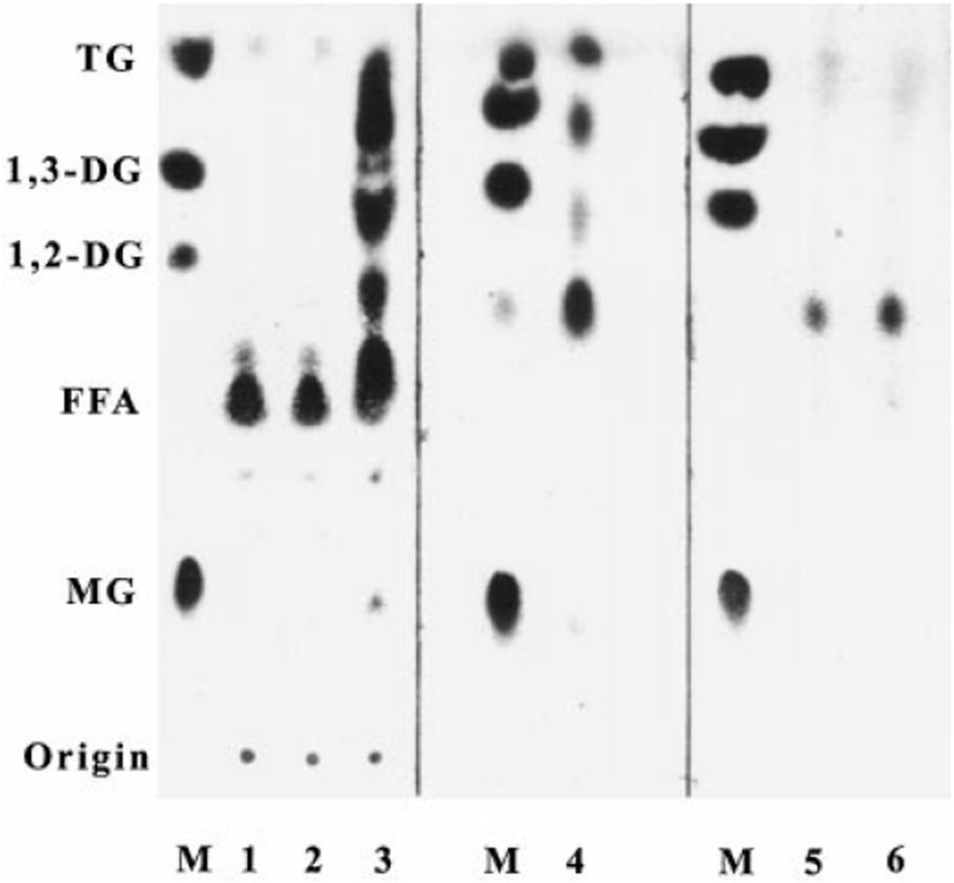

Fig. 5. Separation of lipid classes by thin layer chromatography

Feces were collected and fats extracted as described under "Materials and Methods." Thin layer chromatography was done in a onedimensional two-solvent system as described under "Materials and Methods." The fats extracted from 100 mg of dried feces were dissolved in 1 ml of chloroform, and the indicated volume was spotted on a TLC plate. *M*, standards; *lanes 1–4* are fecal fats from 10-day pups. *Lane 1*, wild type; *lane 2*, heterozygote; *lane 3*, PLRP2-deficient, 5.0 μl; *lane 4*, PLRP2-deficient 0.5 μl; *lane 5*, adult wild type; *lane 6*, adult PLRP2-deficient. The adults samples were collected while the animals were eating regular chow. *TG*, triglyceride; *1,3-DG*, diglyceride; *FFA*, free fatty acid; *MG*, monoglyceride.

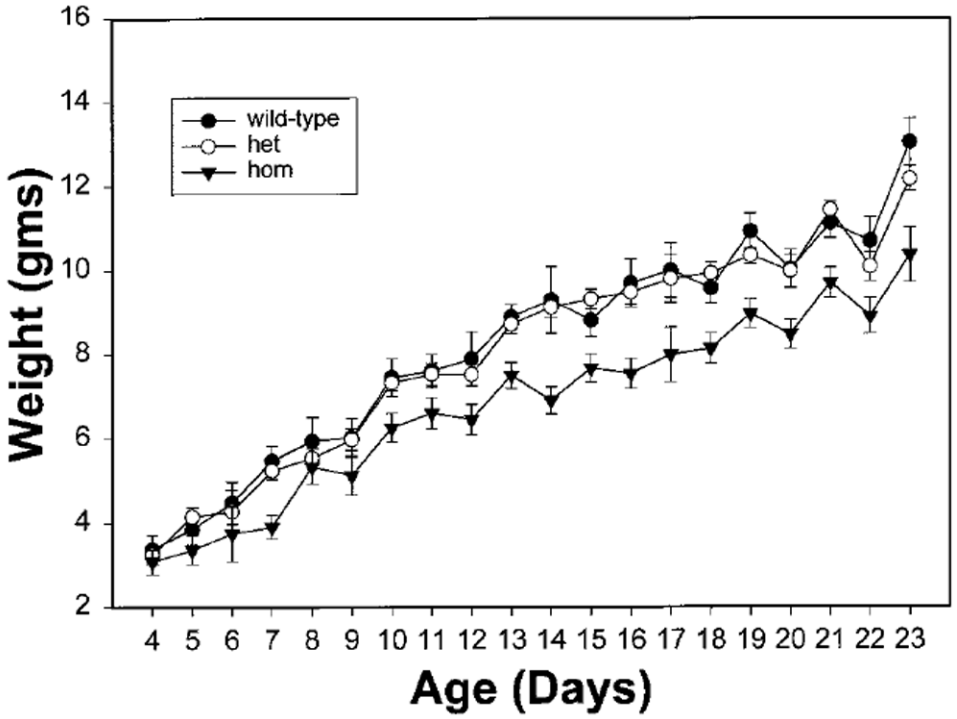

Fig. 6. Weight gain of PLRP2-deficient mice compared with wild-type and heterozygote mice

All pups from six different heterozygote breeding pairs were weighed starting at 4 days of life. Only litters of four or more pups were included. After weaning, tail DNA was prepared, and the genotype of the animals was determined by polymerase chain reaction as described under "Materials and Methods." *Filled circles*, wild type; *open circles*, heterozygotes (*het*); *filled inverted triangles*, PLRP2-deficient (*hom*). The data was analyzed by two-way ANOVA.

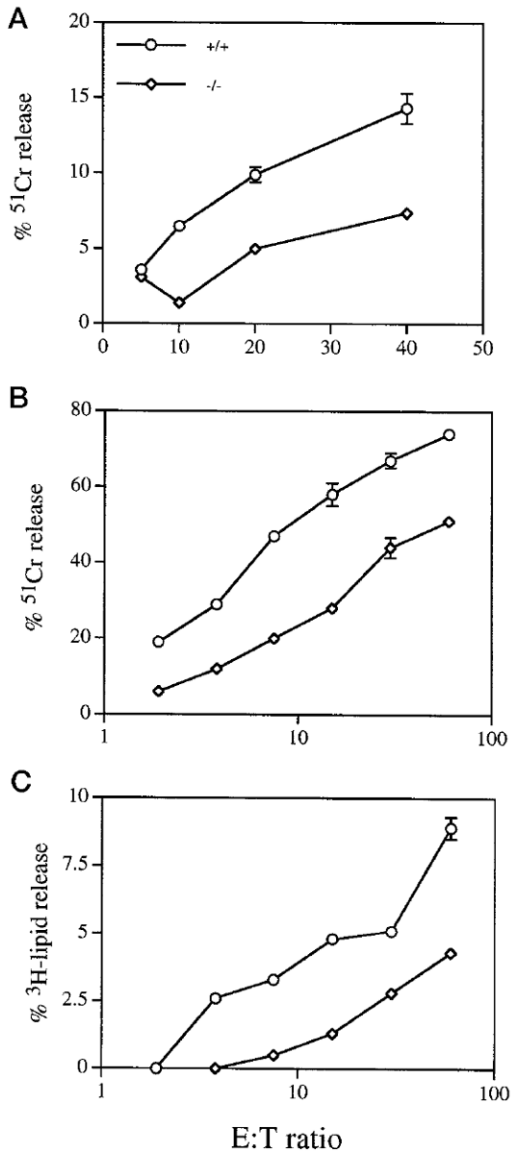

Fig. 7. Cytotoxicity in PLRP2-deficient mice

A, wild-type (*circles*) and PLRP2-deficient (*diamonds*) mice were immunized with P815 tumors. Ten days after immunization, splenocytes were removed and incubated at indicated dilutions with ^{51}Cr-labeled target cells for 4 h. Supernatants were collected and counted in a β-counter. *Symbols* represent the mean ± S.E. of triplicate determinations and are representative of several independent experiments. *B*, splenocytes from mince immunized as in *A* were stimulated *in vitro* with mitomycin C-treated P815 cells for 6 days. Cultures were then used as effector cells as in *A*. *C*, *in vitro* cultured splenocytes were used as effectors in a cytotoxic assay as described in *A* with P815 cells that had been labeled with [^{3}H]oleic acid. Supernatants were collected after 3 h for scintillation counting.

Table I

Stool weight of 10-day suckling mice

Genotype	No. of mice	Stool
		mg/mouse
+/+	44	5.5 ± 0.2
+/−	49	5.5 ± 0.3
−/−	25	13.8±0.8[a]

[a] $p < 0.05$ compared with +/+ and +/− by Student's *t* test.