Cytosolic phospholipase A$_2$$\alpha$–deficient mice are resistant to experimental autoimmune encephalomyelitis

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Experimental autoimmune encephalomyelitis (EAE), a Th1-mediated inflammatory disease of the central nervous system (CNS), is a model of human multiple sclerosis. Cytosolic phospholipase A$_2$$\alpha$ (cPLA$_2$$\alpha$), which initiates production of prostaglandins, leukotrienes, and platelet-activating factor, is present in EAE lesions. Using myelin oligodendrocyte glycoprotein (MOG) immunization, as well as an adoptive transfer model, we showed that cPLA$_2$$\alpha^{-/-}$ mice are resistant to EAE. Histologic examination of the CNS from MOG–immunized mice revealed extensive inflammatory lesions in the cPLA$_2$$\alpha^{-/-}$ mice, whereas the lesions in cPLA$_2$$\alpha^{-/-}$ mice were reduced greatly or completely absent. MOG–specific T cells generated from WT mice induced less severe EAE in cPLA$_2$$\alpha^{-/-}$ mice compared with cPLA$_2$$\alpha^{-/-}$ mice, which indicates that cPLA$_2$$\alpha$ plays a role in the effector phase of EAE. Additionally, MOG–specific T cells from cPLA$_2$$\alpha^{-/-}$ mice, transferred into WT mice, induced EAE with delayed onset and lower severity compared with EAE that was induced by control cells; this indicates that cPLA$_2$$\alpha$ also plays a role in the induction phase of EAE. MOG–specific T cells from cPLA$_2$$\alpha^{-/-}$ mice were deficient in production of Th1-type cytokines. Consistent with this deficiency, in vivo administration of IL–12 rendered cPLA$_2$$\alpha^{-/-}$ mice susceptible to EAE. Our data indicate that cPLA$_2$$\alpha$ plays an important role in EAE development and facilitates differentiation of T cells toward the Th1 phenotype.
during EAE and MS development; it is likely that a subset of these may be therapeutically useful targets in MS. Lipid mediators of inflammation, including prostaglandins (PGs), leukotrienes (LTs), and platelet-activating factor (PAF), are elevated in the cerebrospinal fluid (CSF) of patients who have MS (17–19). Cytosolic phospholipase A\(_2\) (cPLA\(_2\)) initiates the production of these inflammatory mediators (20, 21); is expressed by endothelial cells, CD4\(^+\) T cells, and macrophages at the site of EAE lesions (22); and is activated by monocyte chemoattractant protein (MCP)-1 and -3, monocyte inflammatory protein–1\(\alpha\), and regulated on activation, normal T cell expressed and secreted (23, 24), which are chemokines that are believed to play a role in EAE and MS (25). These chemokines stimulate the phosphorylation and translocation of cPLA\(_2\), and lead to selective release of arachidonic acid (AA) from phospholipid membranes. The free AA is converted to PG by way of the cyclooxygenase pathway, and LTB\(_4\) and cysteinyl leukotrienes by way of the 5-lipoxygenase (5-LO) pathway. Concurrent with the release of AA, lysophosphatidylcholine is generated, which serves as the precursor for the proinflammatory PAF (26, 27). In addition to being converted into proinflammatory mediators, AA can be converted into lipoxins that may promote resolution of inflammation (28, 29) or peroxisome proliferator-activated receptor \(\gamma\) agonists, including 15-hydroxyeicosatetraenoic acid (30). Agonists of peroxisome proliferator-activated receptor \(\gamma\) were shown to regulate macrophage and T cell functions negatively and to reduce the severity of EAE (31).

The roles of different end-products of cPLA\(_2\) enzymatic activity in EAE development are not well understood, but Kalyvas and David (22), who documented the presence of cPLA\(_2\) at the site of lesions, also demonstrated that arachidonyl trifluoromethylketone (AACOCF3)—which inactivates cPLA\(_2\) by reversible reaction with the active site serine (32)—can reduce EAE severity in myelin oligodendrocyte glycoprotein (MOG)-immunized mice. AACOCF3 was effective at blocking the onset of disease, and, in some cases, preventing relapse. However, AACOCF3 also inhibits calcium-independent phospholipase A\(_2\) (33), and thromboxane synthase, which is downstream of cyclooxygenase (32). AACOCF3 also inhibits fatty acid amidase hydrolase, the enzyme that degrades the natural ligands for the cannabinoid receptors (34, 35), and cannabinoid receptor agonists ameliorate clinical EAE and suppress inflammatory cytokines in a Theiler virus–induced model of EAE (36). Thus, AACOCF3 may be acting on multiple enzymes, and therefore, its effect in EAE may not be due solely to inhibition of cPLA\(_2\).

To assess the role of cPLA\(_2\) in EAE, we established the cPLA\(_2\)\(^{-/-}\) mice on the susceptible B6 background, and studied the development of disease in cPLA\(_2\)\(^{-/-}\) and cPLA\(_2\)\(^{+/+}\) littersmates. We found that cPLA\(_2\)\(^{-/-}\) mice are resistant to EAE induction. We demonstrated that this resistance is accompanied by a defect in development of Th1 responses and can be restored by administration of IL-12.

## RESULTS

cPLA\(_2\)\(^{-/-}\) mice do not develop EAE after immunization with MOG

To test if end-products of cPLA\(_2\) enzymatic activity were increased during EAE development, we measured products of the cyclooxygenase and 5-LO pathways in spinal cords of naive mice, as well as mice at the onset, peak, and recovery of the disease. Very low levels of these mediators were found in the spinal cords of naive mice. Conversely, levels of PGE\(_2\) and LTB\(_4\) were increased significantly (\(P < 0.05\)) at the peak and during the recovery phase of EAE (Fig. 1).

To test the potential role of cPLA\(_2\) in EAE development, we immunized cPLA\(_2\)\(^{-/-}\) or cPLA\(_2\)\(^{+/+}\) littersmates with MOG and followed EAE development. In three independent experiments, cPLA\(_2\)\(^{-/-}\) mice were resistant to EAE induction (3 out of 27 mice developed EAE), whereas cPLA\(_2\)\(^{+/+}\) mice developed severe EAE (22 out of 23 mice developed EAE) (Fig. 2). There was no difference in the severity of EAE between cPLA\(_2\)\(^{-/-}\) and cPLA\(_2\)\(^{+/+}\) littersmates (unpublished data); therefore, the cPLA\(_2\)\(^{-/-}\) littersmates were used as controls for cPLA\(_2\)\(^{-/-}\) mice in all further experiments. Microscopic examination of the brain and spinal cord showed remarkable differences between the cPLA\(_2\)\(^{-/-}\) and cPLA\(_2\)\(^{+/+}\) mice (Fig. 3; Table I). The cPLA\(_2\)\(^{-/-}\) mice had numerous multifocal to coalescing inflammatory cell infiltrates in the brain and spinal cord (Fig. 3), and all cPLA\(_2\)\(^{+/+}\) mice had infiltrates in the brain and spinal cord. Infiltrates were present in the leptomeninges, around blood vessels in the leptomeninges and white matter, and in the parenchyma.

![Figure 1](image-url)  
**Figure 1.** Lipid mediators are increased in spinal cords of mice with EAE. Mice were killed and perfused extensively with PBS. Spinal cords from naive mice (N), or mice with onset (O), peak (P), or recovery (R) of EAE were isolated, extracted with methanol, and levels of PGE\(_2\) (A) and LTB\(_4\) (B) were measured using ELISA. Levels of PGE\(_2\) and LTB\(_4\) were increased significantly at peak and recovery of EAE (\(P < 0.05\)).
of the white matter; in the brain they also were localized around the ventricles. Infiltrates in most animals consisted of mononuclear cells, primarily lymphocytes, macrophages, and glial cells. Occasionally, neutrophils and fewer eosinophils also were observed, although in one cPLA<sub>2</sub><sup>α</sup><sup>+/−</sup> mouse they approached 50% of the infiltrating cells. Pallor and vacuolation were associated with the inflammatory cell infiltrates, which were consistent with edema and demyelination, and dilated axons were observed sometimes. Changes were more common in the posterior sections of spinal cord, compared with the anterior sections. Luxol fast blue stains showed demyelination at the sites of inflammatory cell infiltrates. In contrast, brains from two out of six cPLA<sub>2</sub><sup>α</sup><sup>−/−</sup> mice and spinal cords from three out of six cPLA<sub>2</sub><sup>α</sup><sup>−/−</sup> mice had no infiltrates in any section examined. When present, the changes were consistent with edema and demyelination, and were more common in the parenchyma of the brain and spinal cord of cPLA<sub>2</sub><sup>α</sup><sup>−/−</sup> mice compared with cPLA<sub>2</sub><sup>α</sup><sup>+/−</sup> mice. Similar to cPLA<sub>2</sub><sup>α</sup><sup>−/−</sup> mice, the infiltrates in cPLA<sub>2</sub><sup>α</sup><sup>−/−</sup> mice were primarily mononuclear cells.

To determine if cPLA<sub>2</sub><sup>α</sup><sup>−/−</sup> mice had defective priming of MOG-specific T cells in vivo, we tested antigen-specific proliferation of LN T cells against the immunizing antigen.

Table I. Microscopic changes in CNS of cPLA<sub>2</sub><sup>α</sup><sup>+/−</sup> and cPLA<sub>2</sub><sup>α</sup><sup>−/−</sup> mice immunized with MOG

| Group       | Cervical Incidence (%) | Mean no. of foci | Spinal cord Cervical Incidence (%) | Mean no. of foci | Thoracic Incidence (%) | Mean no. of foci | Lumbar Incidence (%) | Mean no. of foci |
|-------------|------------------------|-----------------|-----------------------------------|-----------------|------------------------|-----------------|----------------------|-----------------|
| cPLA<sub>2</sub><sup>α</sup><sup>+/−</sup> | 6/6 (100)              | 36.0 ± 4.9      | 6/6 (100)                         | 26.0 ± 5.7      | 6/6 (100)              | 33.0 ± 4.3      | 6/6 (100)            | 47.2 ± 23.8     |
| cPLA<sub>2</sub><sup>α</sup><sup>−/−</sup> | 4/6 (67)               | 4.8 ± 2.2       | 1/6 (17)                          | 4.3 ± 4.3       | 3/6 (50)               | 4.0 ± 3.2       | 3/6 (50)             | 6.3 ± 2.9       |

<sup>a</sup>Mice with any inflammatory cell infiltrates were considered positive.

<sup>b</sup>Mean number of foci of ≥20 inflammatory cells ± SEM.

<sup>c</sup>Number of mice affected/number of mice examined (%).

<sup>d</sup>P < 0.0005 compared with cPLA<sub>2</sub><sup>α</sup><sup>+/−</sup>.

<sup>e</sup>P < 0.005 compared with cPLA<sub>2</sub><sup>α</sup><sup>−/−</sup>.
When stimulated with a wide range of concentrations of MOG in vitro, cells that were isolated from the draining LNs of the immunized cPLA\(_2\alpha^{-/-}\) and cPLA\(_2\alpha^{+/+}\) mice proliferated similarly (Fig. 4). The same results were obtained when purified T cells were isolated from the draining LNs and stimulated with MOG in the presence of WT irradiated B6 spleen cells used as APCs (unpublished data).

**cPLA\(_2\alpha\)** plays important roles in the induction and the effector phases of EAE

After establishing that cPLA\(_2\alpha^{-/-}\) mice are resistant to EAE development when immunized with MOG, we sought to determine if cPLA\(_2\alpha\) plays a role in the induction or effector phases of EAE, or both. To examine these possibilities, we developed a robust EAE adoptive transfer model in B6 mice.

We tested multiple procedures of inducing EAE in B6 mice using an adoptive transfer model, and were able to identify conditions under which robust EAE develops in nearly 100% of the recipient mice (Table S1, available at http://www.jem.org/cgi/content/full/jem.20050665). We were able to generate encephalitogenic cells from spleen and draining LNs of immunized donor animals. To induce EAE, spleen or LN cells from the immunized donors needed to be cultured with exogenous IL-12, and the recipient mice needed to be irradiated sublethally (500 R). EAE was induced with as few as 5.8 \(\times\) 10\(^6\) cultured spleen cells. We also isolated CD4\(^+\) cells at the end of the culture period; 3 million of these purified CD4\(^+\) T cells were able to transfer EAE (unpublished data). In all further adoptive transfer experiments, spleens from the immunized mice were used as a source of encephalitogenic T cells and the recipient mice were irradiated sublethally.

To address whether cPLA\(_2\alpha\) plays a role in the effector phase of EAE, we tested the ability of encephalitogenic cells that were generated from immunized WT animals to induce EAE in cPLA\(_2\alpha^{-/-}\) or cPLA\(_2\alpha^{+/+}\) mice. In three independent experiments, cells that were isolated from WT mice induced less severe EAE in cPLA\(_2\alpha^{-/-}\) mice than in cPLA\(_2\alpha^{+/+}\) control mice (Fig. 5 A). The day of onset of EAE was the same in both groups of recipient mice. These results indicate that cPLA\(_2\alpha\) plays a role in the effector phase of EAE.

However, because a complete absence of clinical EAE never was observed in cPLA\(_2\alpha^{-/-}\) mice that received encephalitogenic cells from WT animals, we tested the possibility that cPLA\(_2\alpha\) also plays a role in the induction of the encephalitogenic immune responses. We immunized cPLA\(_2\alpha^{-/-}\) or cPLA\(_2\alpha^{+/+}\) mice with MOG, and used the cells that were derived from
these mice to induce EAE in WT recipients. Cells that were isolated from cPLA$_2$$\alpha^{-/-}$ and cPLA$_2$$\alpha^{+/+}$ mice were able to induce EAE in the WT recipients. However, cells that originated from cPLA$_2$$\alpha^{-/-}$ mice induced less severe EAE with delayed onset (Fig. 5 B). These results suggest that, in addition to playing a role in the effector phase, cPLA$_2$$\alpha$ plays a role in the induction phase of the disease.

Development of Th1-type responses is impaired in cPLA$_2$$\alpha^{-/-}$ mice

To assess why cells from the immunized cPLA$_2$$\alpha^{-/-}$ mice did not induce EAE that was as severe as that observed with cells from cPLA$_2$$\alpha^{+/+}$ controls, we tested antigen-specific proliferation of these effector cells. In the presence of various amounts of MOG, spleen cells that were isolated from cPLA$_2$$\alpha^{-/-}$ and cPLA$_2$$\alpha^{+/+}$ control animals proliferated similarly (Fig. 6 A). The proliferation of both types of cells was reduced in the presence of IL-12, but cells from cPLA$_2$$\alpha^{-/-}$ and cPLA$_2$$\alpha^{+/+}$ animals proliferated similarly (Fig. 6 B). However, when we measured the amounts of

Figure 6.

Proliferation and cytokine production by splenic cells from immunized cPLA$_2$$\alpha^{-/-}$ and cPLA$_2$$\alpha^{+/+}$ mice stimulated with MOG. The mice were immunized at two sites s.c. with MOG/CFA, and spleens were collected 10 d later. T cell stimulation was set up in the presence of various concentrations of MOG and in the (A) absence or (B) presence of 30 ng/ml IL-12. Cells were cultured for 62–72 h and pulsed with [3H]thymidine during the last 14–18 h of culture. Proliferation data are shown as a mean cpm ± SD of six wells. Supernatants were collected after 48 h of culture and the amounts of (C) IFN-$$\gamma$$ and (D) TNF were determined in the pools of supernatants of six wells. Data shown are representative of two independent experiments.

Figure 7.

Absence of IL-12 during the culture period impaired the ability of T cells from cPLA$_2$$\alpha^{-/-}$ mice to induce EAE. Encephalitogenic cells were prepared by immunizing cPLA$_2$$\alpha^{-/-}$ or cPLA$_2$$\alpha^{+/+}$ mice and culturing their spleen cells in the presence of MOG without addition of exogenous IL-12. In two independent experiments, (A) 10$^7$ or (B) 4.5 × 10$^7$ cells were injected i.p. into sublethally irradiated (500 R) WT mice. EAE clinical scores were assessed as described in Fig 2. (A) Data are shown as a mean clinical score ± SE of 10 mice/group. The incidence of EAE in mice that were injected with cPLA$_2$$\alpha^{-/-}$ cells was 50%; it was 10% in mice that were injected with cPLA$_2$$\alpha^{+/+}$ cells. The statistical significance of the difference between the curves was determined using ANOVA, $P < 0.0001$. (B) Data are shown as a mean clinical score ± SE of 10 cPLA$_2$$\alpha^{-/-}$ mice and 9 cPLA$_2$$\alpha^{+/+}$ mice. The incidence of EAE in mice that were injected with cPLA$_2$$\alpha^{-/-}$ cells was 100%; it was 50% in mice that were injected with cPLA$_2$$\alpha^{+/+}$ cells. The statistical significance of the difference between the curves was determined using analysis of variance, $P < 0.0001$. 
cells in these cultures to produce (A) IFN-γ or (B) TNF was measured after stimulation with MOG (20 μg/ml) or anti-CD3 (1 μg/ml).

Figure 8. The defect in production of Th1-type cytokines in cells from cPLA₂α−/− is acquired during in vivo priming. CD4+ cells and non-CD4+ cells (APCs) were isolated from the draining LNs of cPLA₂α−/− or cPLA₂α+/− mice that were immunized previously with MOG/CFA. CD4+ cells and APCs from the two types of mice were mixed and the ability of cells in these cultures to produce (A) IFN-γ or (B) TNF was measured after stimulation with MOG (20 μg/ml) or anti-CD3 (1 μg/ml).

Figure 9. Administration of IL-12 renders cPLA₂α−/− mice susceptible to EAE. cPLA₂α−/− or cPLA₂α+/− mice were immunized with MOG/CFA and injected with pertussis toxin on day 0. Then cPLA₂α−/− mice received PBS or IL-12 (0.1 mg/dose) i.p. on days 1, 3, 5, 7, and 9. EAE clinical scores were assessed as described in Fig 1. Data are shown as a mean clinical score ± SE of 10 cPLA₂α−/− mice that were treated with PBS, 8 cPLA₂α+/− mice that were treated with IL-12, and 10 untreated cPLA₂α+/− mice. The incidence of EAE was 20% for PBS-treated cPLA₂α−/− mice, 75% for IL-12-treated cPLA₂α−/− mice, and 100% for cPLA₂α+/− mice. The statistical significance of the difference between the curves for PBS-treated cPLA₂α−/− mice versus IL-12-treated cPLA₂α−/− mice was determined using ANOVA, P < 0.0001. There was no statistically significant difference between the curves for IL-12-treated cPLA₂α−/− mice versus cPLA₂α+/− mice as determined using analysis of variance, P = 0.416.

cPLA₂α−/− mice induced mild disease with a delayed onset (Fig. 7 B). These results indicate that when IL-12 is added to the cultures of MOG-specific T cells, it contributes to the generation of encephalitogenic cells and may mask endogenous defect in their development.

We next tested if T cells that were isolated from cPLA₂α−/− mice have an intrinsic defect in producing Th1-type cytokines or if this defect was acquired during the priming in vivo. We separated CD4+ and non-CD4+ cells (designated APCs thereafter) from the draining LNs of MOG-immunized cPLA₂α−/− and cPLA₂α+/− mice. When CD4+ cells and APC cells were from cPLA₂α−/− mice, stimulation by MOG produced eight- and fivefold lower levels of IFN-γ and TNF, respectively compared with the levels that were produced in co-cultures of the cells from cPLA₂α+/− mice (Fig. 8). Much of this difference was maintained when CD4+ cells from cPLA₂α−/− and cPLA₂α+/− mice were stimulated with APCs from the cPLA₂α+/− mice. However, the source of APCs did have an effect on IFN-γ levels when CD4+ cells from cPLA₂α−/− mice were stimulated with MOG and APCs from cPLA₂α+/− mice. The levels of IFN-γ produced were almost twice as high as the levels that were produced in cultures in which APCs were from cPLA₂α−/− mice. Changing the source of APCs had no effect on the levels of TNF that were produced in these cultures. To test if the defect in Th1 cytokine production was limited to MOG-specific T cells or was attributed to all T cells from cPLA₂α−/− mice, the cultures were stimulated with anti-CD3 mAb’s. In these cultures, T
cells from cPLA2α−/− and cPLA2α+/− mice produced similar levels of IFN-γ, regardless of the source of the APCs. Amounts of TNF produced by cPLA2α−/− CD4 cells were lower (~30%) than amounts of TNF that were produced by CD4+ cells from cPLA2α+/− mice. This indicated that an intrinsic defect in production of TNF may exist in cells from cPLA2α−/− mice. However, this defect is much less pronounced than the fivefold defect in TNF that was observed for MOG-specific T cells from cPLA2α−/− mice.

Injection of IL-12 during disease induction period renders cPLA2α−/− mice susceptible to EAE

The data obtained in the above-described studies indicated that Th1 differentiation is impaired in cPLA2α−/− animals. Therefore, we tested whether injection of recombinant mouse IL-12, a potent inducer of Th1 differentiation, during the immunization phase would render cPLA2α−/− mice susceptible to EAE. When cPLA2α−/− mice were immunized with MOG/CFA and injected with 0.1 μg recombinant murine IL-12 on the day of immunization and every other day after that for a total of five injections, most of the cPLA2α−/− mice developed EAE (Fig. 9).

DISCUSSION

In the present study, we demonstrate that cPLA2α is essential for the development of EAE. cPLA2α plays a role in the induction of the encephalitogenic immune responses and in the effector phase of EAE. cPLA2α plays an important role in enhancing the production of Th1-type cytokines. The defect in Th1-type cytokine production and the reduced encephalitogenic potential of cPLA2α−/− cells can be reversed, in part, by providing exogenous IL-12. Similarly, in vivo administration of IL-12 during the induction phase of the disease can render cPLA2α−/− mice susceptible to EAE.

During the course of EAE development, we detected significant increases in the levels of PGE2 and LTB4, which were indicative of PLA2 activation during the peak and recovery of the disease. This is consistent with the published observation that the levels of cPLAα are increased during EAE development (22), and with the observation that these mediators are increased in CSF of patients who have MS (17).

When immunized with MOG, cPLA2α−/− mice were almost completely resistant to EAE induction. Our finding is consistent with the previously published report that treatment of MOG-immunized mice with AACOCF3, which inhibits cPLA2α, inhibits development of EAE in a dose-dependent manner (22). The observed clinical resistance to EAE development was confirmed by microscopic examination of the brains and spinal cords, which revealed a great reduction in the number of inflammatory foci in the CNS tissues from the immunized cPLA2α−/− mice, compared with cPLA2α+/− mice. However, several immunized cPLA2α−/− mice had reduced, but detectable, inflammation in CNS in the absence of clinical signs of EAE. Such clinically silent inflammatory lesions have been described in MOG-induced EAE in mice and rats (37, 38), and clinically silent MRI lesions are common in patients who have MS (39). It has been suggested that the size and location of the lesions, as well as their ability to recruit macrophage/microglia may be related to the clinical silence of the lesions (37–39). The presence of these inflammatory lesions in ~50% of cPLA2α−/− mice demonstrates that MOG-specific T cells that are capable of entering the CNS were generated in cPLA2α−/− mice. There are at least two possible explanations for the lack of clinical signs in these mice. First, it is possible that in cPLA2α−/− mice, the number of encephalitogenic cells or their function is not adequate for a full development of EAE. In this case, the induction phase of EAE would be deficient in cPLA2α−/− mice. Second, it is possible that encephalitogenic T cells, once in the CNS, are not able to mediate full EAE development. In this latter case, resistance to EAE induction in cPLA2α−/− mice would be a result of a defective effector phase of EAE. It is well-established that encephalitogenic T cells represent only a small fraction of the cells in the inflammatory lesions, whereas most of the cells are recruited to the site in an antigen-independent manner (25, 40).

To determine whether cPLA2α plays a role in the induction or the effector phase of EAE, we used the adoptive transfer model of the disease. Transfer of encephalitogenic cells that were generated from immunized WT donors into cPLA2α−/− recipients resulted in EAE with the same time of onset, but lesser severity, compared with transfer of the same cells into cPLA2α+/− recipients. This indicates that cPLA2α plays a role in the effector phase of EAE, and is consistent with the previous report that cPLA2α is expressed by endothelial cells and macrophages as well as CD4+ T cells in the EAE lesions (22). Multiple downstream products of cPLA2α enzymatic activity may play a role in enhancing and sustaining inflammatory responses in CNS during the effector phase of EAE. For example, LTαβ is a potent chemoattractant of myeloid cells as well as CD4 and CD8 effector cells (41–44). In addition, LTαβ enhance phagocytic capacity and the generation of other proinflammatory mediators by leukocytes (45). In addition to generating AA, cPLA2α also generates the lysophospholipid precursor of PAF—which may play an important role during the effector phase of EAE—by acting as a strong chemoattractant and by increasing the permeability of blood–brain barrier, and thereby, facilitating entry of proinflammatory cells into CNS (24, 46). Recent work by Kihara et al. (47) showed that PAF receptor–deficient mice have reduced incidence and severity of EAE.

Because active immunization of cPLA2α−/− mice resulted in almost no clinical EAE, whereas the adoptive transfer of encephalitogenic cells into cPLA2α−/− mice resulted in mild but significant EAE, we considered the possibility that cPLA2α also plays a role in the induction of encephalitogenic immune responses. We compared EAE development in WT recipient mice that were injected with encephalitogenic cells that were generated from MOG-immunized cPLA2α−/− or cPLA2α+/− mice. EAE that was induced by cPLA2α−/− cells had a delayed onset and lower severity compared with EAE that was induced by control cells; this
showed that cPLA2α also plays a role in the induction of encepha
tigeneic immune response.

The reduced encephalitogenic potential of the cPLA2α−/−
cells coincided with reduced production of IFN-γ and
TNF by these cells in vitro without an increase in Th2-type
cytokines. At the same time, there was no reduction in
MOG-stimulated proliferation of T cells from immunized
cPLA2α−/− mice, which indicated that priming and expa
nion of MOG-specific T cells was not affected by the defect
in cPLA2α. The defective production of Th1-type cytokines
could be restored, in part, by the addition of IL-12 to the
cultures. Addition of IL-12 also enhanced the encepha
tigeneic potential of cells that were isolated from cPLA2α−/−
mice. When IL-12 was omitted from the cultures, cells that
were isolated from cPLA2α−/− mice had an even lower abil
ity to induce EAE. Conversely, in vivo administration of IL-
12 during the immunization period restored the ability of
these mice to develop EAE, which further suggests that at
least part of the defect in developing EAE in these mice may
be related to the defective differentiation toward Th1-type
responses. Our preliminary experiments have shown that
adoptive transfer of up to 25 million CD4+ T cells from
cPLA2α+/+ mice into cPLA2α−/− mice does not render
cPLA2α−/− mice susceptible to EAE. Further experiments
are required to determine which cells need to produce
PLA2α for Th1-type responses to develop. Our results in
dicate that CD4+ cells from cPLA2α−/− mice do not have an
intrinsic defect in the production of Th1-type cytokines. In-
stead, the defect was acquired during T cell priming in vivo.

The mechanism by which cPLA2α-mediated AA release
may enhance development of Th1 responses is not known.
cPLA2α-mediated AA release was shown to be critical in
MCP-1−/−, MCP-3−/−, regulated on activation, normal T cell
expressed and secreted−, and monocyte inflammatory pro-
tein-1α−mediated activation and migration of human monocytes (48); therefore, it is possible that macrophase re-
cruitment and/or activation is impaired in cPLA2α−/− mice.
Macrophages are believed to be a major effector cell in the
EAE inflammatory response, and the severity of EAE corre-
lates with the number of macrophages detected in the CSF
of mice that have EAE (25, 49, 50). Mice that are deficient
in MCP-1 or its receptor, CCR2, have reduced numbers of
macrophage infiltrates and are resistant to EAE development
when injected with WT encephalitogenic T cells (51, 52).
It is noteworthy that serum levels of IFN-γ were reduced sig-
nificantly in MCP-1−/− mice at the time of peak disease,
which indicated an impaired Th1 response that was analo-
gous to that seen in the cPLA2α−/− mice (52). Unlike cells
that were isolated from cPLA2α−/− mice, MCP-1−/− T cells
were fully encephalitogenic when transferred to WT mice.

In conclusion, we demonstrated that cPLA2α is necessary
for EAE development and that this enzyme plays a role during
the induction and the effector phases of EAE. In the absence
of cPLA2α, induction of Th1-type responses is impaired; we
suggest that cPLA2α may represent a novel therapeutic target
for treatment of MS and other Th1-mediated diseases.

MATERIALS AND METHODS

Mice. cPLA2α−/− mice on C57BL/6 background were generated by
backcrossing cPLA2α−/− mice of mixed B6/129 background (21) to B6
background for 12 generations and then intercrossing cPLA2α−/− mice
to obtain cPLA2αWT, cPLA2α+/−, and cPLA2α−/− mice, or crossing
cPLA2α+/− and cPLA2α−/− mice to obtain cPLA2α−/− and cPLA2α−/− lriternates.
All cPLA2α mice were bred at Taconic Farms and were used at
6–10 wk of age. In all experiments, age- and sex-matched littermates
were used to compare cPLA2α−/− mice with cPLA2α−/− and/or cPLA2αWT mice.
For some experiments, WT female C57BL/6 mice were purchased from
Jackson ImmunoResearch Laboratories or Taconic Farms and used at 6–10
wk of age. These mice were used only when no direct comparison was
made to cPLA2α−/− mice. Wyeth Institutional Animal Care and Use Com-
mittee was the overseeing body that approved the animal studies.

EAE induction. For EAE induction using immunization with MOG, all
mice were injected s.c. at two sites with a total of 200 μg of MOG peptide
35–55 in CFA containing 6 mg/ml killed Mycobacterium tuberculosi. On the
same day, the mice received 500 ng pertussis toxm (List Laboratories) i.p. For
EAE induction in the adoptive transfer model, recipient mice were irradiated sublethally (500 R) and within 16 h were injected i.p. with encephalitogenic cells, prepared as described below. Paralysis (clinical evidence of EAE) was assessed daily, starting on day 5 after immunization or adoptive transfer, when all the mice were still clinically normal. Clinically, animals were scored as follows: 1, limp tail; 2, partial hind leg paraparesis; 3, complete hind leg paralysis or partial hind and front leg paraparesis; 4, complete hind and partial front leg paralysis; 5, complete hind and partial or complete front paralysis with severely reduced responsiveness to external stimuli. Mice were killed immediately if they scored 5, or if they scored 4 on two consecutive days.

Preparation of cells for EAE induction in adoptive transfer model. To prepare MOG-specific cells that were able to induce EAE in the adoptive transfer model, mice were immunized with MOG/CFA in the same fashion as when inducing EAE, but no pertussis toxin was administered. Spleens were collected 10 d later, single-cell suspension was prepared and red blood cells were lysed. Stimulation with MOG was performed in T150 flasks, using 6 × 10^6 cells/ml and 20 μg MOG35–55/ml in T cell medium (RPMI) with 10% FBS, 2 mM L-glutamine, and various amounts of MOG peptide or anti-CD3 antibodies. 48–54 h after the initiation, the culture supernatants were collected for cytokine analysis and the cultures were pulsed with 0.5 μCi of [3H]thymidine/well and harvested 14–18 h later.

To assess proliferation and cytokine production of splenic T cells against MOG, mice were immunized with MOG/CFA in the same fashion as when inducing EAE, but no pertussis toxin was administered. Spleens were collected 10 d later, single-cell suspensions were prepared, and red blood cells were lysed. Stimulation with MOG was performed as described above for LN cells. Culture supernatants were collected 48 h later for cytokine analysis and cultures were pulsed to determine [3H]thymidine incorporation. Concentrations of IL-4, IL-5, IFN-γ, and TNF in the supernatants were quantified using a cytometric bead array kit obtained from BD Biosciences.

Statistical analysis. For statistical analysis, a Poisson distribution was used to model the inflammatory foci parameter. A square root transformation was applied to stabilize the variance, and then the transformed data were analyzed with a one-way analysis of variance. Severity scores were analyzed using the mean score Mantel-Haenszel statistic. Clinical scores were compared using analysis of variance. Statistical significance of differences in cytokine production and lipid mediator presence in spinal cords was determined using Student’s t test.

Online supplemental material. Table S1 shows that effector cells from spleen or lymph node induce EAE in sublethally irradiated, but not in non-irradiated, recipient mice. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20050665/DC1.

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M. Marusic, M.W. Leach, J.W. Pelker, J. Cui, C.M. DeClercq, M.W.H. Shen, J.S. Miyashiro, B.A. Carito, P. Thakker, D.L. Simmons, and J.D. Clark work for Wyeth and own Wyeth stock and/or Wyeth stock options. Wyeth has a cPLA2 inhibitor currently in clinical trials.

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