Cytosolic phospholipase A2 (cPLA2) cleaves membrane phospholipids to release arachidonic acid, initiating lipoygenase and cyclooxygenase pathways. Mice lacking a gene for cPLA2 suggested important roles of the protein in allergic responses, fertility, and neural cell death. Here we show that cPLA2 negatively regulates c-Myc expression in a B-Myb-dependent manner. Overexpression of cPLA2 protein but not a mutant cPLA2 protein that lacks in vitro binding ability with B-Myb inhibits B-Myb-dependent c-myc gene expression. The inhibition was associated with physical interaction of B-Myb protein with cPLA2 both in the cytoplasm and the nucleus. Binding site analysis demonstrated that both the N and C termini of cPLA2 interact with B-Myb. Macrophage colony stimulating factor (MCSF) stimulated cPLA2 redistribution into the nucleus and also association with B-Myb in human monocytes. Importantly, macrophages from mice with a disrupted cPLA2 gene demonstrated significantly increased levels of c-Myc protein in the nucleus compared with cells from the wild-type mice, whereas B-Myb levels were similar in the cells from the cPLA2+/+ and cPLA2−/− mice. Moreover, an introduction of cPLA2 into cPLA2−/− mouse macrophages resulted in decreased c-Myc protein levels, and an inhibition of cPLA2 expression by small interfering RNAs or antisense RNA increased the c-myc transcription in macrophage colony stimulating factor-activated human monocytes. These findings provide new insights into the function of cPLA2 in B-Myb-dependent gene expression.

cPLA2 is activated by cytokines, submicromolar concentrations of Ca2+ ions, and mitogen-activated protein kinase-mediated phosphorylation of serine residues in the protein (1, 2). Activated cPLA2 protein distributes preferentially to the perinuclear region of the cell (3–5), where the enzyme is thought to participate in arachidonic acid release. Consistent with this notion, 5-lipoxygenase and cyclooxygenase, two major enzymes downstream of cPLA2, reside in the nuclear envelope and oxidize arachidonic acid (6–10). However, the entry of cPLA2 into the nucleus does not occur in these conditions (5).

cPLA2 localizes in the nucleus of subconfluent endothelial cells (11). cPLA2 can regulate the NF-κB-dependent transcription (12). These findings along with the observations that more downstream enzymes such as lipoygenase and cyclooxygenase reside in the nuclear envelope prompted us to test a hypothesis that cPLA2 can enter the nucleus under some cellular conditions. Because the cPLA2 protein does not harbor any apparent classical nuclear localization signal (NLS), cPLA2 might be directed toward the nucleus by a partner protein(s).

Recent studies using mice lacking a gene for cPLA2 showed that these mice were associated with impairment of allergic responses and fertility and also suggested important contributions of cPLA2 to the pathophysiology of neural cell death (13, 14). These studies imply that cPLA2 may play a more important role than previously thought.

B-Myb is a nuclear transcriptional factor belonging to the Myb family that is ubiquitously expressed in tissues and is involved in cell growth control, differentiation, and cancer (15). Furthermore, B-Myb interacts with other transcriptional factors and modulates their biological functions. Here we demonstrate so-far undefined physical associations of cPLA2 and B-Myb transcriptional factor, which facilitates redistribution of cPLA2-B-Myb complexes into the nucleus. Importantly, we also show that redistribution of cytoplasmic cPLA2 after its specific binding to B-Myb transcriptional factor can regulate B-Myb-dependent c-myc gene expression.

**EXPERIMENTAL PROCEDURES**

**Cell Culture—** African Green monkey kidney cells (CV-1) and human kidney cells (293T) were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (FBS), 4 mMAT-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. Human monocytes were isolated from the peripheral blood of healthy volunteers by Ficoll-Paque separation followed by adherence for 1 h and removal of non-adherent cells. The adherent cells were collected and washed 3 times with culture medium. The resultant cell population consisted of >90% monocytes as judged by morphological examination and α-naphthyl acetate esterase staining (16). Monocytes were cultured in RPMI1640 medium supplemented with 10% FBS, 4 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. The monocytes were treated with 10 ng/ml MCSF (Leukoplast; Morinaga Co., Tokyo, Japan). Thioglycolate-elicited peritoneal cells were collected from cPLA2−/− and cPLA2+/− mice and seeded at 4×10^5 cells/ml in RPMI1640 supplemented with 10% FBS. To obtain mature macrophages, the collected cells were allowed to adhere to the plastic dish bottom for 2 h in culture medium, and adherent cells were harvested after washing the dish with phosphate-buffered saline (PBS). Lung fibroblasts were isolated from cPLA2−/− and cPLA2+/− mice by digesting...
the minced lung tissue for 4 h in Dulbecco's modified Eagle's medium supplemented with 0.5% collagenase and 2% FBS. Obtained cells were cultured in Dulbecco's modified Eagle's medium supplemented with 20% FBS.

**Plasmid Construction and Transfection**—The FLAG-tagged cPLA₂ fragments 1–749 (full-length), 1–524, 1–294, 1–202, and 202–749 were constructed by subcloning restriction fragments of cPLA₂ into a pact vector. A FLAG-tagged C-terminal truncated mutant cPLA₂-(498–749) and FLAG-tagged cPLA₂, mutated in nuclear export signal (NES) were created by PCR and were also subcloned into the same vector. We carried out DNA transfection by LipofectAMINE reagents (Invitrogen) or by calcium phosphate precipitation method. We used 3 and 65 μg of plasmids, respectively, for LipofectAMINE and calcium phosphate precipitation. The transfection of murine macrophages from cPLA₂−/− mice was carried out according to a commercially available protocol using a Nucleofector transfection system and Mouse Neuron Nucleofector Solution (Axam Biosystems). We transfected 4 × 10⁶ peritoneal macrophages with 0.5 μg of pact plasmid containing full-length cPLA₂.

**Immunofluorescence Staining and Confocal Microscopy**—Cells were fixed for 10 min by 4% paraformaldehyde in PBS at room temperature, then permeabilized by 0.1% Triton X-100 in PBS, and blocked with 1.5% skimmed milk and 1.5% bovine serum albumin in PBS. Primary antibodies (10 μg/ml) were prepared in 2% bovine serum albumin in PBS. Cells were then incubated with a fluorescein isothiocyanate-conjugated goat anti-mouse antibody (1:20; Zymed Laboratories Inc.) and Cy3-conjugated goat anti-rabbit antibody (1:100; Amersham Biosciences). Confocal imaging was performed using a Leica TCS SP2 AOBS laser-scanning microscope.

**Cell Fractionation**—Cells were suspended in ice-cold hypotonic buffer (10 mM Hepes, pH 7.4, 1.5 mM MgCl₂, 10 mM KCl) supplemented with 0.25% Nonidet P-40, 0.5 mM dithiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride and incubated for 5 min on ice. After centrifugation at 16,000 × g for 10 min at 4 °C, the supernatant (cytoplasmic fraction) was collected. The pellets were washed twice with ice-cold buffer. The pellets were resuspended in an appropriate volume of high salt buffer (20 mM Hepes, 25% glycerol, 0.42 mM KCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride) and incubated for 15 min on ice. After centrifugation at 16,000 × g for 10 min at 4 °C, the supernatants were collected (nuclear fraction).

**Immunoprecipitation and Western Blotting**—For the immunoprecipitation experiments, we used monoclonal antibodies specific for cPLA₂ (Santa Cruz) or FLAG (Eastman Kodak Co.) or polyclonal antibodies for B-Myb (Santa Cruz). Immunoprecipitates were then electrophoresed in an SDS-PAGE gel and transferred to a polyvinylidene difluoride filter (Millipore), and the protein–protein interaction was confirmed by Western blotting using the above antibodies.

**Reporter Gene Activity Assays**—CV-1 cells were co-transfected with empty vector or a pact plasmid containing B-Myb (1–700) or pact plasmid (cPLA₂−/−) or both. The reporter gene activity was measured as described previously (17). The chloramphenicol acetyltransferase (CAT) reporter plasmid pmycCAT was used for the c-myc transcription assays in CV-1 cells expressing B-Myb and/or wild-type or mutant cPLA₂. The amount of cell extracts used for chloramphenicol acetyltransferase assays was normalized with respect to cotransfected chloramphenicol acetyltransferase (CAT) reporter plasmid pmycCAT used for the c-myc expression in MCSF-activated human monocytes was assessed by reverse transcription-PCR using a commercially available kit (LightCycler RNA amplification kit SYBR Green I, Roche Diagnostics). The level of c-myc mRNA was expressed as a relative value to glyceraldehyde 3-phosphate dehydrogenase mRNA.

**cPLA₂ Ablation by RNA-mediated Interference and Antisense Oligonucleotide**—The RNA-mediated ablation of endogenous cPLA₂ in M-CSF-activated human monocytes was performed essentially as described as previously (19). A 21-nucleotide small interfering RNA duplex with 3′-dTdT overhangs corresponding to human cPLA₂ mRNA (5′-CCUGACGUUUAGCAGCGUATT and 5′-TGGAAGUCGAAAUGCUGACU) was synthesized (Japan Bio Services). RNA-mediated interference transfection was performed using Oligofectamine reagent (Invitrogen). Harvested human peripheral blood monocytes were stimulated with M-CSF for 24 h and then transfected once using the manufacturer’s protocol (Invitrogen). The ablation of endogenous cPLA₂ in human monocytes was also performed using antisense oligonucleotide, which was complementary to nucleotide 219–238 of human cPLA₂ (5′-GTCTGCTTAAGGATCTAT) (20). The sense-sense oligomer was used for the control (5′-GTCTCTCTTAAGGATCTAT). The phosphorothioate-modified oligonucleotides were synthesized and purified by high performance liquid chromatography (Sigma).

**RESULTS**

**Subcellular Localization of cPLA₂**—To identify the sequence(s) regulating subcellular distribution of cPLA₂, we created serial deletion mutants and assayed their localization by indirect immunostaining and confocal microscopy. Deletions of the C-terminal amino acids up to but not including the putative NES (PLLLLTP), which resides in the N-terminal region of the PH-like domain, did not affect the distribution of cPLA₂ (Fig. 1, A–D). Further deletion of the C-terminal 547 amino acids, cPLA₂-(1–202), resulted in nuclear localization of the protein (Fig. 1, A and E). cPLA₂ distributed diffusely in the nucleus and cytoplasm when CV-1 cells were transfected with a C-terminal-truncated cPLA₂ (amino acids 493–749) (Fig. 1, F) and a truncation of the amino acid 202, cPLA₂-(202–749), however, resulted in exclusively cytoplasmic localization (Fig. 1, G). We obtained similar results with 293T cells.

Replacement of all four leucines with alanines in amino acids 263–269 in a truncated (1–269) (Fig. 1, A and H) and a full-length (1–794) (Fig. 1, A and D) cPLA₂ or complete deletion of this sequence (not shown) resulted in predominant nuclear localization of the proteins in CV-1 cells. Collectively, these findings suggest that the midportion of cPLA₂ protein directs the protein to the cytoplasm, probably via the nuclear export signal (PLLLLTP).

**In Vivo Interaction of cPLA₂ and B-Myb**—Because the cPLA₂ protein does not harbor any apparent classical NLS, cPLA₂ might be directed toward the nucleus by a partner protein(s). We, therefore, investigated a possible adapter protein(s) that binds to cPLA₂ and drives the protein into the nucleus. Physical or functional interactions have been suggested between cPLA₂ and proteins involved in transcriptional regulation (12, 21). Therefore, we tested the possibility that B-Myb, another protein that regulates transcription of several genes, may play a role as an adaptor protein for nuclear transport of cPLA₂. B-Myb is a potential candidate for this adapter protein, since it is ubiquitously expressed in tissues and is involved in cell growth control, differentiation, and cancer (15). Furthermore, B-Myb interacts with other transcriptional factors and modulates their biological functions (22).

To test this hypothesis we first determined the subcellular distribution of cPLA₂ after co-transfection with vectors containing either wild-type or mutant B-Myb protein. As described above, the wild-type full-length cPLA₂-(1–749) predominantly localizes in the cytoplasm (Fig. 1B). Surprisingly, cPLA₂ redistributes into the nucleus and colocalizes with B-Myb in the nucleus when the wild-type B-Myb is co-expressed (Fig. 2A). In contrast, the wild-type cPLA₂ remains in the cytoplasm of cells
expressing NLS-deleted B-Myb (B-Myb NLS) (Fig. 2B), suggesting that the NLS of B-Myb is required for the nuclear entry of cPLA₂-B-Myb complexes. Similarly, truncated cPLA₂ that is cytoplasmic when expressed alone translocates into the nucleus after co-expression with the wild-type B-Myb but not with NLS-mutated B-Myb (not shown). In contrast, co-expression of c-Myb, another member of the Myb family functioning as a transcriptional transactivator, does not direct cPLA₂ into the nucleus (Fig. 2C), indicating that interaction between cPLA₂ and B-Myb is specific.

Fig. 1. C terminus and NES, but not CaLB domain, of the cPLA₂ molecule cooperatively compartmentalize the protein in the cytoplasm. A, intramolecular domains of cPLA₂ protein and subcellular localization of wild-type and truncated or mutated cPLA₂ proteins exogenously expressed in CV-1 cells. NES(L/A), all leucines in the NES replaced by alanine. Cytoplasmic (C) or nuclear (N) localization of the proteins is indicated on the right column. B–I, confocal microphotographs showing subcellular localization of wild-type (B) and truncated (C–G) or mutated (H and I) cPLA₂ proteins in CV-1 cells. The nuclei are indicated by 4,6-diamidino-2-phenylindole (DAPI) staining.
We next examined in vivo interaction between cPLA2 and B-Myb by immunoprecipitating cPLA2 followed by Western blotting with monoclonal antibody specific for B-Myb after transfection of 293T cells with the wild-type cPLA2 tagged with FLAG (Fig. 2F). In this condition, cPLA2 immunoprecipitated with endogenous B-Myb. A reciprocal experiment using FLAG-specific antibodies for immunoprecipitation of the full-length cPLA2, followed by Western blotting for B-Myb confirmed in vivo interaction of cPLA2 and B-Myb (Fig. 2G). We detected B-Myb protein in cPLA2 immunoprecipitates from 293T cells overexpressed with the N- or C-terminal-truncated cPLA2 (Fig. 2H), suggesting that the B-Myb protein can interact with both the N- and C-terminal regions of cPLA2.

Nuclear Colocalization of cPLA2 and B-Myb in Human Macrophages—We ask next whether the specific interaction between cPLA2 and B-Myb and subsequent entry of cPLA2 into the nucleus might have any biological relevance. To address this question, we examined cPLA2-B-Myb interaction and the subcellular distributions of these proteins in human monocytes. MCSF up-regulates cPLA2 protein levels and intrinsic enzymatic activity in human monocytes (16). In resting human monocytes, cPLA2 was exclusively cytoplasmic, and the endogenous B-Myb protein level was very low in the nucleus (Fig. 3, A and C). MCSF treatment resulted in a significant nuclear redistribution of cPLA2, which colocalizes with B-Myb (Fig. 3, B and C). The interaction was confirmed by Western blotting using antibodies specific for cPLA2 of immunoprecipitates isolated by antibodies specific for B-Myb (Fig. 3D). A reciprocal experiment using antibodies specific for cPLA2 with immunoprecipitation and antibodies specific for B-Myb with Western blotting also showed an in vivo interaction of these proteins (not shown). In vitro cPLA2 enzyme assay showed that the nuclear fraction of resting monocytes contained a greater activity than the cytoplasmic fraction (Fig. 3E), indicating that a significant part of cPLA2 resides in the cytoplasmic fraction. The nuclear redistribution of cPLA2 was associated with a 2.6-fold increase in cPLA2 enzymatic activity in the nuclear fraction from MCSF-activated monocytes. In contrast, MCSF treatment resulted in only a 40% increase in cPLA2 activity in the cytoplasmic fraction, consistent with the notion that MCSF-induced terminal differentiation of human monocytes is associated with selective induction of cPLA2 activity in the nucleus.

Regulation of B-Myb-dependent Gene Expression by cPLA2—B-Myb participates in regulation of several genes, each of which has an important role in cell growth. For example, the c-myc gene bears a putative B-Myb-responsive element on its promoter (23). Therefore, we tested whether nuclear cPLA2 could affect the B-Myb-dependent c-myc gene expression. To this end we measured the B-Myb-dependent reporter gene activity in CV-1 cells transiently expressing the pmycCAT reporter in the presence or absence of coexpressed wild-type cPLA2 (Fig. 4A). As expected, transient expression of B-Myb activated the reporter gene activity. Interestingly, coexpression of the full-length cPLA2-(1–749) greatly inhibits the B-Myb-dependent reporter gene activity to the control level. These findings suggest that cPLA2 negatively regulates c-myc gene expression in a B-Myb-dependent manner. However, it is unclear how the interaction between cPLA2 and B-Myb

(B-Myb NLS) (B) or with c-Myb (C). Control stains show specificity of the secondary antibodies for cPLA2 (D) and B-Myb (E). F and G, immunoprecipitation (IP) assay shows the interaction between cPLA2 and B-Myb in 293T cells (lane 3). H, B-Myb interacts with the N (1–294) and C (493–749)-terminal regions of cPLA2. WB, Western blot; DAPI, 4,6-diamidino-2-phenylindole.
Fig. 3. cPLA₂ and B-Myb interaction in human macrophages. A and B, cPLA₂ colocalizes with B-Myb in the nucleus of MCSF-activated macrophages (B), whereas cPLA₂ is exclusively cytoplasmic in resting human peripheral monocytes (A). C, Western blotting shows that cPLA₂ is present in the nucleus of MCSF-activated human macrophages (lane 2) but not in the nucleus of resting monocytes (lane 1). Note that B-Myb is also found in the nucleus of macrophages (lane 2), but it is at low levels in the nucleus of resting monocytes (lane 1). Effective separations of cytoplasmic and nuclear extracts were evaluated by Western blotting using antibodies specific for actin (for cytoplasmic fraction) and lamin A/C and B (both for nuclear fraction). DAPI, 4,6-diamidino-2-phenylindole. D, cPLA₂ coimmunoprecipitates with B-Myb in the nuclear extracts from MCSF-activated human macrophages. IP, immunoprecipitation; WB, Western blot. E, nuclear redistribution of cPLA₂ in MCSF-activation of human macrophages is associated with increased cPLA₂ enzyme activity in the nucleus. Each bar represents the average ± S.D. of three experiments.
would be effective. To explore this, we next asked whether the action of B-Myb would be influenced by changes in levels of cPLA2. To test this, we transfected CV-1 cells with varying doses of plasmid containing full-length cPLA2 and then monitored the B-Myb-dependent activation of c-myc reporters. We found that cPLA2 inhibited B-Myb-dependent c-myc expression in a dose-dependent manner. Increasing doses (0.2–1 μg) of plasmid containing full-length cPLA2 (1–749) or empty vector (control) were transfected into CV-1 cells along with pmycCAT reporter plasmids. Relative activities were quantified using a BAS 1500 imagers.

**Fig. 4.** cPLA2 inhibits B-Myb-dependent c-myc gene expression. A, co-expression of full-length cPLA2 (1–749) with B-Myb inhibits transcriptional activity of B-Myb-dependent gene expressions of c-myc (pmycCAT reporter plasmid). Each bar represents the average ± S.D. of three experiments. B, cPLA2 inhibits B-Myb-dependent c-myc gene expression in a dose-dependent manner. Increasing doses (0.2–1 μg) of plasmids containing full-length cPLA2 (1–749) or empty vector (control) were transfected into CV-1 cells along with pmycCAT reporter plasmids. Relative activities were quantified using a BAS 1500 imager. CAT, chloramphenical acetyltransferase.

Next, we tested whether the restoration of cPLA2 expression could affect the c-Myc protein level in cPLA2−/− mouse peritoneal macrophages. We obtained low levels of cPLA2 after transfection in these primary cells, whereas the c-Myc expression was inhibited to 69% of the levels in non-transfected cPLA2−/− cells (Fig. 5B). We further examined the effects of cPLA2 inhibition in normal human peripheral blood monocytes. When the cPLA2 expression was specifically inhibited by using antisense oligonucleotide or small interfering RNA in MCSF-activated human monocytes, the c-myc expression was up-regulated in these cells (Fig. 5, C–E). Collectively, these results suggest that cPLA2 interacts directly with B-Myb to enter into the nucleus and thereby functions as an inhibitory factor for the c-Myc activity.

A causative relationship was found between cPLA2 activation and UV irradiation and apoptosis (24, 25). Therefore, we assessed the lack of cPLA2 on cell survival after DNA-damaging agents using lung fibroblasts isolated from cPLA2+/+ and cPLA2−/− mice. The growth rate was similar between cPLA2+/+ and cPLA2−/− cells without treatment (data not shown). However, compared with cPLA2+/+ cells, cPLA2−/− cells showed decreased numbers of cells 48 h after treatment with UV irradiation or H2O2 (Table I). In contrast, cPLA2−/− cells were more resistant to ionizing radiation than cPLA2+/+ cells, as assessed 96 h after irradiation. Taken together these results suggest that the lack of cPLA2 may affect cell growth and/or apoptosis after DNA damage, depending on the types of cells and damaging.

**DISCUSSION**

Our report has demonstrated thus far undefined physical associations of cPLA2 and B-Myb transcriptional factor that facilitates redistribution of cPLA2–B-Myb complexes into the nucleus. cPLA2 protein does not harbor any apparent intrinsic NLS, whereas B-Myb protein has multiple functional NLSs (26). Furthermore, a deletion of the NLS from the B-Myb protein results in a mutant protein that cannot direct cPLA2 protein into the nucleus. Therefore, B-Myb protein may serve as an adapter protein for the nuclear entry of cPLA2. We also found that the physical interaction of cPLA2 and B-Myb proteins is specific and c-Myb, which is another Myb family transcriptional factor, does not direct cPLA2 into the nucleus. More importantly, our results suggest that, after binding to B-Myb protein, nuclear cPLA2 may participate in the transcriptional regulation of B-Myb-dependent expression of c-myc gene.

It is surprising that cPLA2 is not confined to the cytoplasm but is redistributed into the nucleus to serve as a regulator for B-Myb-dependent gene expression. Originally, cPLA2 was considered to translocate onto the cytoplasmic membrane after appropriate stimuli to release arachidonic acid from membrane phospholipids (3). However, accumulating evidence suggests that cPLA2 can translocate from the cytosolic compartment to the nuclear membrane (2, 4). The ATP depletion may contribute to the nuclear redistribution of cPLA2 (27). Although the localization of downstream enzyme 5-lipoxygenase and cyclooxygenase in the nuclear membrane (6–10) may explain in part the physiologic relevance of the redistribution of cPLA2 to the nuclear membrane, the definite role of the “nuclear” cPLA2 is not well understood. Recent findings that an acetyltransferase Tip60 interacted and colocal-
ized with cPLA_2 in the nucleus and that the introduction of protein complexes into the nucleus was associated with stimulation of apoptosis (28) strongly support the notion that cPLA_2 plays additional roles in the nucleus other than its enzymatic activity at the nuclear membrane.

Our results suggest that the c-myc gene is one of the major targets of cPLA_2-B-Myb complexes in the nucleus. We also found that cPLA_2-B-Myb complexes in the nucleus inhibit B-Myb-dependent gene expressions of cdc25C, but the effect was minimal (data not shown). cPLA_2 was reported to be involved in the induction of apoptosis caused by tumor necrosis factor (29). Tumor necrosis factor is considered to transduce apoptotic signals from the cytoplasmic membrane to mitochondria. During these processes the 100-kDa cPLA_2 is cleaved in a caspase 3-dependent fashion to a 70-kDa proteolytic fragment that loses its catalytic activity but contributes to apoptosis (30, 31). Although we have shown in the present study that the nuclear cPLA_2 exhibited in vitro enzyme activity, the enzyme activity may be inhibited or masked in the nucleus by an adaptor protein such as B-Myb and Tip60.

The c-myc protooncogene encodes a transcriptional factor that participates in the regulation of cellular proliferation and apoptosis (32, 33). In particular, the Myc protein can induce S-phase entry and apoptosis by independent mechanisms (34). As shown in cells from cPLA_2_/- mice, the absence of cPLA_2 may lead to an increased expression of c-Myc protein. Therefore, cPLA_2 might exert an antiproliferative function in the nucleus by inhibiting aberrant expression of c-Myc and other proteins whose expressions are B-Myb-dependent. Most recently, Pawliczak et al. (12) raised possibility that cPLA_2 may

**Fig. 5.** Lack of cPLA_2 up-regulates c-Myc expression in primary monocytes and macrophages. A, up-regulation of c-Myc expression in peritoneal macrophages from mice with disrupted cPLA_2 genes. Western blot analysis shows increased c-Myc protein levels in the nucleus of peritoneal macrophages from cPLA_2_/- mice. B-Myb protein levels are similar between cPLA_2_/- and cPLA_2_/- mice. Note that cPLA_2 is detectable in the nucleus of macrophages from cPLA_2_/- mice. The left panel shows c-Myc expression in cPLA_2_/- macrophages with (+) or without (−) cPLA_2 transfection. The right panel shows the results of densitometric analysis of the c-Myc protein levels. C and D, ablation of cPLA_2 is associated with up-regulation of c-myc gene. MCSF-activated human peripheral blood monocytes were treated with antisense (AS) or missense (MS) oligonucleotide or small interfering RNA (siRNA) of cPLA_2 (C), and then c-myc expression was assessed by reverse transcription (RT)-PCR (D); c-myc gene expression in 293T cells was used as positive control (lane 2), lane 1, DNA marker; lane 2, 293T cells; lane 3, non-transfected MCSF-treated monocytes; lane 4, missense of cPLA_2; lane 5, small interfering RNAs of cPLA_2 lane 6, antisense of cPLA_2. E, the level of c-myc expression was quantified by real-time PCR and is expressed as a relative value to glyceraldehydes 3-phosphate dehydrogenase mRNA. Data are representative from three separate experiments.

**TABLE I**

Survival and growth rates after treatment with UV irradiation, \( \text{H}_2\text{O}_2 \), or X irradiation

|          | UV | \( \text{H}_2\text{O}_2 \) | X irradiation |
|----------|----|----------------|--------------|
| cPLA_2_/- | 24.9% | 51.2% | 44.7% |
| cPLA_2_/- | 3.5 | 14.4 | 100.0 |

The c-myc protooncogene encodes a transcriptional factor that participates in the regulation of cellular proliferation and apoptosis (32, 33). In particular, the Myc protein can induce S-phase entry and apoptosis by independent mechanisms (34). As shown in cells from cPLA_2_/- mice, the absence of cPLA_2 may lead to an increased expression of c-Myc protein. Therefore, cPLA_2 might exert an antiproliferative function in the nucleus by inhibiting aberrant expression of c-Myc and other proteins whose expressions are B-Myb-dependent. Most recently, Pawliczak et al. (12) raised possibility that cPLA_2 may...
regulate peroxisome proliferator activated receptor-mediated gene transcription, implying further a role in the regulation of genes that participate in differentiation and apoptosis. However, cPLA₂ may exert its regulatory function for peroxisome proliferator-activated receptor-dependent gene transcription in the cytoplasm, whereas the protein plays a role in the inhibition of proliferation and cell death in the nucleus.

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