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Enhancement of Mast Cell Survival: A Novel Function of Some Secretory Phospholipase A_2 Isotypes[1]

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This study tested the hypothesis that certain secretory phospholipase A_2 ($sPLA_2$) isotypes act in a cytokine-like fashion through cell surface receptors to influence mast cell survival. Initial experiments revealed that $sPLA_2$ activity and $sPLA_2$ receptor expression are increased, and mast cells lost their capacity to maintain membrane asymmetry upon cytokine depletion. Groups IB and III, but not group IIA PLA_2, prevented the loss of membrane asymmetry. Similarly, group IB prevented nucleosomal DNA fragmentation in mast cells. Providing putative products of $sPLA_2$ hydrolysis to cytokine-depleted mast cells did not influence survival. Furthermore, catalytic inactivation of $sPLA_2$ did not alter its capacity to prevent apoptosis. Inhibition of protein synthesis using cycloheximide or actinomycin reversed the antiapoptotic effect of $sPLA_2$. Additionally, both wild-type and catalytically inactive group IB PLA_2 induced IL-3 synthesis in mast cells. However, adding IL-3-neutralizing Ab did not change Annexin V^{FITC} binding and only partially inhibited thymidine incorporation in $sPLA_2$-supplemented mast cells. In contrast, IL-3-neutralizing Ab inhibited both Annexin V^{FITC} binding and thymidine incorporation in mast cells maintained with IL-3. $sPLA_2$ enhanced phosphoinositide 3′-kinase activity, and a specific inhibitor of phosphoinositide 3′-kinase reversed the antiapoptotic effects of $sPLA_2$. Likewise, $sPLA_2$ increased the degradation of I-κBα, and specific inhibitors of nuclear factor κ activation (NF-κB) reversed the antiapoptotic effects of $sPLA_2$. Together, these experiments reveal that certain isotypes of $sPLA_2$ enhance the survival of mast cells in a cytokine-like fashion by activating antiapoptotic signaling pathways independent of IL-3 and probably via $sPLA_2$ receptors rather than $sPLA_2$ catalytic products. *The Journal of Immunology,* **2001, 167: 4161–4171.**

Mast cells are an important component of allergic inflammation by virtue of their ability to release preformed mediators, newly synthesized mediators, as well as cytokines upon Ag activation (1–5). The size of any cell population represents a balance between cell division and programmed cell death (apoptosis). In the case of mast cells, two cytokines, IL-3 and stem cell factor (SCF),[3] produced in extravascular tissues appear to promote mast cell proliferation and maturation and prevent apoptosis (6–12). For example, IL-3 supports early mast cell proliferation from bone marrow cells by enhancing cell division and preventing apoptosis, while apoptotic changes observed after IL-3 withdrawal can be eliminated by the addition of SCF, supporting the concept that SCF promotes mast cell survival by preventing apoptosis (9, 11, 13–18). Studies within the last few years have begun to unravel some of the molecular events associated with apoptosis induced by IL-3 or SCF removal. Activation of tyrosine kinases and phosphoinositide 3′-kinase (PI3′-K) has been implicated in the induction of mast cell growth (19–21). Other studies have shown a decrease in the protooncogene *bcl*-2 after removal of IL-3; moreover, overexpression of *bcl*-2 prolongs the survival of mast cells after IL-3 removal (22–29). In contrast, SCF does not appear to influence *bcl*-2 expression (14). More recent studies also reveal that the tumor suppressor p53 is not required for mast cell entry into apoptosis after growth factor deprivation (12, 30).

An alternative mechanism of preventing apoptosis is suggested by recent evidence showing enzymes that participate in lipid metabolism play critical roles in regulating growth and death in some cell types (31). Importantly, levels of some of these enzymes are often altered when these cells are treated with specific cytokines or cytokine combinations (32). For example, a large number of studies indicate that the regulation of cyclooxygenase isotypes is an important event that influences cancer cell growth and apoptosis (33, 34). Other studies show that unesterified arachidonic acid (AA) is an important signal leading to ceramide generation and apoptosis (7, 35).

The role of phospholipase A_2 (PLA_2) isotypes in cell proliferation and apoptosis has also been the focus of several studies. Cytosolic PLA_2 ($cPLA_2$) has been implicated as having a critical role in the induction of apoptosis in several cells (36–39). We have shown previously that a combination of IL-3 and SCF results in an increase in $cPLA_2$ levels with a concomitant decrease in secretory PLA_2 ($sPLA_2$) levels (40). Further evidence that $sPLA_2$ plays a role in mitogenesis stems from studies in the *Min* mouse model of adenomatous polyposis, in which $sPLA_2$ has been identified as a candidate gene that modifies the *Apc* gene (41–43). Additionally,

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[3] Abbreviations used in this paper: SCF, stem cell factor; AA, arachidonic acid; BMMC, bone marrow-derived mast cell; BPB, *p*-bromophenylacylbromide; CAPE, caffeic acid phenethyl ester; $cPLA_2$, cytosolic PLA_2; $[^2H_3]SA$, trideuterated stearic acid; $[^2H_8]$, octadeuterated; HSA, human serum albumin; JAK, Janus kinase; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; MAP, mitogen-activated protein; NGF, nerve growth factor; PI, L-α-phosphatidylinositol; PIP_3, L-α-phosphatidylinositol 3,4,5-triphosphate; PLA_2, phospholipase A_2; PTX, pertussis toxin; $sPLA_2$, secretory PLA_2.

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serum levels of some sPLA$_2$ isotypes are increased in patients with certain cancers, and group IB PLA$_2$ stimulates the growth of pancreatic cancer cells (44–47).

The overall objective of the current study was to determine whether sPLA$_2$ isotypes play a role in mast cell survival and proliferation. Our data suggest that sPLA$_2$ released from cells or exogenously provided to cells binds to sPLA$_2$ receptors on the surface of mast cells, and that this event, not the catalytic activity of sPLA$_2$, prolongs mast cell survival by attenuating apoptosis. The current study implies a novel role for sPLA$_2$, that of enhancing the survival of mast cells, thereby potentially slowing resolution of allergic inflammation.

Materials and Methods

Materials

Essentially fatty acid-free human serum albumin (HSA), essential and nonessential amino acids, heat-inactivated FBS, RPMI 1640 cell culture medium, and HBSS were purchased from Life Technologies (Grand Island, NY). Herbimycin A, LY294002, *p*-bromophenylacylbromide (BPB), group IB PLA$_2$ from *Naja naja*, group III PLA$_2$ from bee venom, pertussis toxin (PTX), actinomycin, and cycloheximide were purchased from Sigma (St. Louis, MO). Human group IIA PLA$_2$ was provided by L. Marshal (GlaxoSmithKline, King of Prussia, PA). Caffeic acid phenethyl ester (CAPE) and (*E*)-3-[(4-methylphenylsulfonyl]-2-propenenitrile (BAY 117082) were purchased from Biomol (Plymouth Meeting, PA). Egg lysophosphatidylcholine (LPC) and lysophosphatidic acid (LPA) were purchased from Avanti Polar Lipids (Alabaster, AL). [^{3}H]Inositol (20 Ci/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO). Octadeuterated (5, 6, 8, 9, 11, 12, 14, 15–2)H) AA ([$^2H_8$]AA) and trideuterated stearic acid ([$^2H_3$]SA) were purchased from Biomol. HPLC-grade organic solvents were purchased from Fisher Scientific (Norcross, GA). Guinea pig polyclonal Ab raised against purified rabbit M-type 180-kDa sPLA$_2$ receptor was provided by G. Lambeau (National Center for Scientific Research, Valbonne, France). Mouse rSCF was a generous gift by J. Arm (Harvard Medical School, Boston, MA). Mouse rIL-3 was purchased from Genzyme (Cambridge, MA). I-κBα polyclonal Ab was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse IL-3-neutralizing Ab was purchased from R&D Systems (Minneapolis, MN).

Mast cell culture

Mouse bone marrow-derived mast cells (BMMC) were obtained from CBA/J mice (The Jackson Laboratory, Bar Harbor, ME) and grown in RPMI 1640 culture medium (Life Technologies) supplemented with 10% (v/v) FCS, 50 μM 2-ME, 1% essential amino acids, 1% nonessential amino acids, 2 mM L-glutamine, 5 μg/ml gentamicin, and 1% (v/v) penicillin/streptomycin. The culture medium was enriched twice per week with a 50% WEHI-3 (myelomonocytic cell line; American Type Tissue Collection, Manassas, VA) cell culture supernatant fluid as a source of IL-3 and other growth factors for 3 wk. The method for extracting of bone marrow from CBA/J mice was approved by the Institutional Review Board of Wake Forest University School of Medicine. CFTL-15 mast cells were grown in RPMI 1640 cell culture medium supplemented with 1% penicillin/streptomycin and 20% WEHI-3 supernatant as a source of growth factors. Cell viability (>95%) was determined by trypan blue exclusion.

Determination of sPLA$_2$ activity within cells and in cell culture medium

BMMC or CFTL-15 cells were maintained with or without cytokines for different periods of time. Cells were removed from culture medium by centrifugation (400 × *g*, 10 min), and sPLA$_2$ was extracted from cell pellets using 0.18 M H_2SO_4 for 24 h at 4°C, as previously described (48, 49). PLA$_2$ activities in the acid extract of cells or in 100 μl of culture medium were determined using 0.02 μCi (9.7 nmol) of [^{3}H]AA-labeled *Escherichia coli* membranes (New England Nuclear, Boston, MA) as substrate in 50 mM Tris-HCl, pH 8.3, containing 5 mM $CaCl_2$, 50 mM NaCl, and 0.2 mg of HSA (49). The PLA$_2$ reaction was stopped after 90 min at 37°C by extracting lipids by the method of Bligh and Dyer (50). Free fatty acids were isolated from phospholipids by TLC on silica gel G plates developed in hexane/ethyl ether/formic acid (90:60:6 v/v). The radioactivity in lipids was located using a radiochromatogram imaging system (Bioscan, Washington, DC). Free AA and phospholipids were isolated using TLC zonal scraping, and the quantity of radioactivity was determined using liquid scintillation counting. PLA$_2$ activity was calculated and expressed as picomoles of AA released per milligram of protein per hour.

RNA extraction and determination of sPLA$_2$ receptor expression by real-time PCR

Receptor mRNA levels were determined by extracting RNA using Ultrapure TRIzol reagent (Life Technologies) and generating cDNA copies using an RT-PCR First Strand Synthesis kit (RETROscript kit; Ambion, Austin, TX). Quantitative PCR was performed using Taqman polymerase with detection of Syber Green fluorescence on an ABI Prism 7700 Sequence detector (Applied Biosystems, Foster City, CA) (51–53). sPLA$_2$ receptor mRNA levels were normalized using the expression of GAPDH as a housekeeping gene. Relative quantitation of both sPLA$_2$ and GAPDH mRNA was based on standard curves prepared from serially diluted mouse mast cell cDNA.

Determination of sPLA$_2$ receptor expression by Western analysis

BMMC were cultured without cytokines or with IL-3 or with different concentrations of group IB PLA$_2$. Amounts of sPLA$_2$ receptor expressed in mast cells were determined using total cell lysates (75 μg of protein), followed by immunodetection, as previously described (54).

Inactivation of sPLA$_2$

Group IB PLA$_2$ (1 mg/ml) in 50 mM HEPES buffer (pH 7) containing 150 mM NaCl was treated with 1 mM BPB for 4 h, as previously described (55). Excess BPB was removed by gel filtration using a Spherogel TSK 3000SW column (Altex Scientific, Berkeley, CA) eluted with sPLA$_2$ suspension buffer at 0.4 ml/min. sPLA$_2$ activity in each fraction was determined as described above. Active fractions and corresponding fractions from inactivated sPLA$_2$ were concentrated using a Centricon 3 filtration device (Amicon, Beverly, MA). Protein concentration was determined and sPLA$_2$ filtered before application to BMMC in cell culture medium. The inactivation procedure irreversibly reduced the sp. act. of sPLA$_2$ from 43.8–84.7 μmol/mg/min to 0–4.4 μmol/mg/min. When applied to fatty acid-enriched medium for 24 h, 20 nM active sPLA$_2$ hydrolyzed unsaturated fatty acids (38 ± 6 pmol/ml linoleic acid and 559 ± 81 pmol/ml oleic acid (n = 4)), whereas the same amount of inactivated sPLA$_2$ did not hydrolyze any of these fatty acids.

Assessment of apoptosis in mast cells

Mast cells (BMMC or CFTL-15) were cultured with or without cytokines or various isotypes of sPLA$_2$ (group IB, group IIA, group III) at concentrations from 0 to 20 nM. Cells were then removed from supernatant fluids by centrifugation at various time points between 12 and 96 h. Apoptosis was determined in these studies by examining the loss of phospholipid asymmetry as measured by the appearance of phosphatidylserine in the outer envelope of the plasma membrane, as assessed by Annexin V^{FITC} binding or measuring the fragmentation of internucleosomal DNA.

Annexin V^{FITC} binding

The reorientation of plasma membrane phospholipids was monitored using the TACS Annexin V^{FITC}-binding kit from Trevigen (Gaithersburg, MD). Briefly, ~1 million mast cells were treated with cytokines or 0–20 nM sPLA$_2$ or with WEHI supernatant fluid as a source of IL-3 alone or in combination with 10 μg/ml IL-3-neutralizing Ab. Mast cells were collected by centrifugation (400 × *g*, 10 min) and then washed once in ice-cold (4°C) PBS without Ca^{2+} and Mg^{+}. The cells were resuspended in 100 μl of binding buffer (100 mM HEPES, pH 7.4, containing 1.5 M NaCl, 50 mM KCl, 10 mM $MgCl_2$, and 18 mM $CaCl_2$) containing Annexin V^{FITC}, and 5 μg of propidium iodide for 15 min at room temperature in the dark. Binding buffer was then added to the mixture, and flow cytometry was performed using a Coulter Epics (Hialeah, FL) XL-MCL flow cytometer. The percentage of total cells that did not bind propidium iodide or Annexin V^{FITC} (viable cells), cells that bound Annexin V^{FITC} alone (early apoptosis), or cells that bound both propidium iodide and Annexin V^{FITC} (late apoptosis or necrosis) was determined, and the results were presented in the form of a dot plot.

DNA fragmentation

To monitor internucleosomal DNA fragmentation, mast cells were cultured with or without cytokines or various sPLA$_2$ isotypes. Cellular DNA was then extracted using the DNA extraction kit (Gentra System, Minneapolis, MN). Briefly, mast cells (1–2 million) were placed in lysis buffer, and the lysate was treated with RNase for 1 h at 37°C. Proteins were removed by microcentrifugation after the addition of 100 μl of precipitation buffer.

Subsequently, DNA was precipitated by centrifugation (12,000 × *g*, 10 min) after the addition of 0.6 ml of cold 2-isopropanol and 0.1 ml of NaCl (5 M). The DNA was resolved by electrophoresis using a 1.4% agarose gel, and DNA fragments were detected after ethidium bromide staining and UV visualization.

[^{3}H]Thymidine incorporation into mast cells

Mast cells were cultured with or without cytokines or with different concentrations of group IB PLA_2 with or without 10 μg/ml IL-3-neutralizing Ab. Thymidine incorporation into 1×10^6 mast cells was determined by incubating 1 μCi of [^{3}H]thymidine with one million cells for 1 h at 37°C. Unincorporated label was removed by washing (twice) the cells with HBSS containing 0.25 mg/ml HSA. Lysis of the cell pellet was achieved using 0.2 N NaOH (0.25 ml for 1 h). DNA was precipitated using 15% TCA (1 ml) overnight at 4°C. Cellular DNA was then trapped on glass microfiber GF/C filters (Whatman International, Kent, U.K.). Free cellular [^{3}H]thymidine was removed from the filters by washes (4 ml), and the amount of radioactivity in DNA was determined by liquid scintillation counting.

Influence of $sPLA_2$ isotypes on cell cycle progression in mast cells

In some experiments, we determined whether $sPLA_2$ isotypes that had been shown to increase thymidine incorporation had any effect on cell cycle progression. Briefly, mast cells were maintained in culture without $sPLA_2$ or with different concentrations of $sPLA_2$ for different periods of time. The DNA content of cells was determined by staining the cell with propidium iodide and measuring the red fluorescence per cell after excitation (488 nm) with an argon ion laser using a Coulter Epics XL-MCL flow cytometer. At least 20,000 cells were analyzed per experiment, and the resulting DNA histograms were analyzed for cell cycle kinetics using MODFIT (Verity Software House, Topsham, ME).

Determination of fatty acid levels

BMMC (1×10^6/ml) in cell culture medium were incubated without IL-3, with IL-3, or with 20 nM $sPLA_2$ active and inactive isotypes for 24 h. After the addition of [$^2H_8$]AA (100 ng) and [$^2H_3$]SA as internal standards, lipids were extracted (50). Fatty acids were then converted to pentafluorobenzylesters, and the mole quantities of free fatty acids were determined by negative ion chemical ionization gas chromatography/mass spectrometry using a Hewlett-Packard (Palo Alto, CA) model 5989 instrument (56). Carboxylate anions (*m/z*) at 279, 281, 286, 303, and 311 for linoleic acid, oleic acid, [$^2H_3$]SA, AA, and [$^2H_8$]AA, respectively, were monitored, and mole quantities of fatty acids were calculated from standard curves.

Determination of IL-3 production

BMMC were cultured without cytokines, with IL-3, or with 20 nM active or inactivated group IB PLA_2 for 24 h. Cells were removed from the culture medium by centrifugation (400 × *g*, 10 min). Cell pellets were resuspended in sonication buffer (10 mM HEPES, pH 7.4, containing 80 mM KCl, 1 mM EDTA, 1 mM EGTA, 40 μg/ml leupeptin, 25 μg/ml pepstatin, 1 mM PMSF, 10 mM NaF, 0.2 mM Na_2VO_3, and 4 mM DTT). Sonication was performed using a probe sonicator (Heat System, Farmingdale, NY) at a power setting of 2 and 10% output. IL-3 in culture medium (100 μl) or in sonicates was determined using the ChemiKine Mouse IL-3 enzyme immunoassay kit following the instructions of the manufacturer (Chemicon International, Temecula, CA).

Determination of PI3′-K activity in BMMC

BMMC were labeled with [^{3}H]inositol (1 μCi/10^7 BMMC) for 30 min in HBSS and subsequently maintained in mast cell culture medium for 24 h. Unincorporated label was removed by washing (three times) the cells using HBSS containing 0.25 mg/ml HSA. BMMC (5×10^6) were then incubated with different concentrations of group IB PLA_2 or with 100 nM group IB PLA_2 for different periods of time in the presence or absence of 20 μM specific PI3′-K inhibitor, LY294002. Short-term stimulation with $sPLA_2$ was necessary because BMMC did not survive in inositol-free medium and the unlabeled inositol in the mast cell medium reduced the sensitivity of the assay. For both short-term and long-term stimulations, the ratio of the highest $sPLA_2$ concentration/mast cell number was kept constant. Lipids were extracted, and L-α-phosphatidylinositol (PI) and L-α-phosphatidylinositol 3,4,5-triphosphate (PIP_3) were isolated by TLC using oxalate-impregnated silica gel G plates developed in chloroform/acetone/methanol/acetic acid/water (80:30:26:24:14 v/v) (57). The amount of radioactivity in PI and PIP_3 was determined by zonal scraping and liquid scintillation counting, and the PI3′-K activity was expressed as the percentage of total count incorporated into PIP_3.

Effects of $sPLA_2$ on NF-κB

Activation of NF-κB was monitored by examining I-κBα degradation (58) after BMMC were stimulated with $sPLA_2$. Briefly, BMMC (5×10^6/ml) were stimulated with $sPLA_2$ for different periods of time, and cell lysates were resolved on a 4–20% polyacrylamide gel, as previously described (7). After transfer of proteins to polyvinylidene difluoride membranes, free sites on membranes were blocked using 5% (w/v) fat-free Carnation milk in PBS containing 0.05% Tween (PBS-T). The blots were then incubated in block solution containing I-κBα polyclonal Ab (1:500) overnight at 4°C with gentle rocking. Membranes were washed three times (1×10 min, 2×5 min) using PBS-T buffer and then incubated with HRP-conjugated anti-rabbit IgG (1:1000 in PBS-T containing 1% fat-free Carnation milk) for 1 h at room temperature. Membranes were washed as described above, and immunodetection was accomplished using the Super Signal chemiluminescence detection system (Pierce, Rockford, IL). I-κBα was quantitated by densitometry, and levels after stimulation with $sPLA_2$ were expressed as a percentage of I-κBα levels found in unstimulated BMMC.

Statistical analysis

All data are expressed as the mean ± SEM of separate experiments. Statistical significance was determined using Student's *t* test for paired samples. Notations used on figures and legends are * or ** to denote $p < 0.05$.

Results

Activities of $sPLA_2$ and expression of $sPLA_2$ receptor in mast cells undergoing apoptosis

Mast cells depend on a variety of cytokines (IL-3, IL-4, SCF, IL-10) to survive and proliferate in culture. Cytokine depletion (removing WEHI-containing medium) for 24 h from either BMMC or CFTL-15 cells causes a marked decrease in cell survival (as measured by Annexin V^{FITC} binding to the surface of mast cells or DNA fragmentation) and proliferation (as measured by thymidine incorporation) (7). A previous study in our laboratory indicated that $sPLA_2$ levels were significantly attenuated when mast cells were placed in IL-3- or SCF-enriched medium (40). We postulated that the reduction of $sPLA_2$ in the presence of IL-3 or SCF was due to a reduced need for $sPLA_2$ for cell survival under these conditions. To test this hypothesis, $sPLA_2$ activity was determined during mast cell apoptosis. Basal acid-stable PLA_2 activity (162.9 ± 21.6 pmol/h, $n = 6$) increased >2-fold when BMMC were placed in cytokine-depleted medium for 24 h. Likewise, $sPLA_2$ activity in medium from cytokine-depleted BMMC (126.4 ± 19.8 pmol/h) was higher than in medium obtained from BMMC maintained in IL-3 (80.1 ± 13.2 pmol/h, $n = 7$) for 24 h. Acid-stable PLA_2 activity was also consistently higher (~25%) when CFTL-15 were placed in cytokine-depleted medium for 24–48 h (data not shown). These data indicated that cytokine depletion results in an increase in $sPLA_2$ activity within mast cells and in cell culture medium. However, the activity of $sPLA_2$ in culture medium determined using radiolabeled *E. coli* substrate is most likely underestimated because 10% FCS is very rich in PLA_2 substrates and products.

Previous data from our laboratory suggest exogenous $sPLA_2$ (group IB and III) may activate signal transduction pathways (Ras/mitogen-activated protein (MAP)/extracellular signal-related kinase kinase/extracellular signal-related kinase) leading to $cPLA_2$ activation and the selective release of AA via cell surface receptors (56). This raised the question of whether there was a change in $sPLA_2$ receptor expression during cytokine depletion. Therefore, $sPLA_2$ receptor levels were examined in BMMC undergoing apoptosis induced by cytokine depletion. Quantitative PCR data show that depletion of cytokine results in a time-dependent increase in $sPLA_2$ receptor mRNA expression by BMMC (Fig. 1*A*). At >6 h, $sPLA_2$ receptor mRNA expression increased (>12-fold) in BMMC maintained in cytokine-depleted medium, whereas only modest increases (<2-fold) were measured in IL-3-cultured or $sPLA_2$-treated BMMC. In agreement with the PCR data, Western

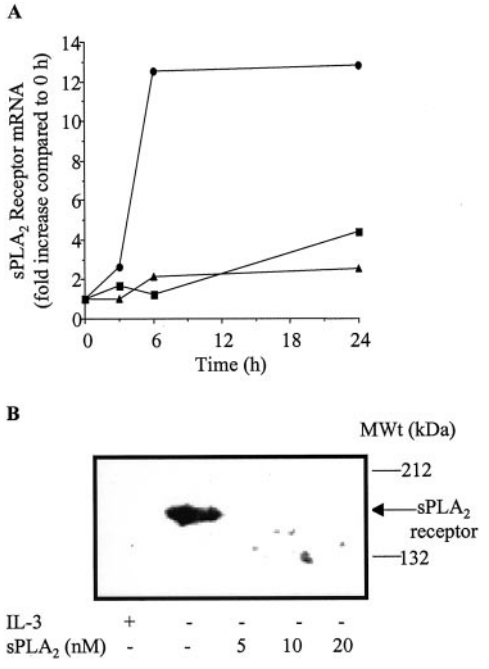

FIGURE 1. Effects of IL-3 and sPLA$_2$ on sPLA$_2$ receptor expression. *A*, mRNA expression: BMMC were cultured with IL-3 (▲), without cytokines (●), or with 20 nM group IB PLA$_2$ (■) for different periods of time. sPLA$_2$ receptor RNA expression was determined by real-time PCR, as described in *Materials and Methods*. *B*, Protein expression. BMMC were cultured with IL-3, without IL-3, or with different concentrations of group IB PLA$_2$ for 24 h. sPLA$_2$ receptor expression was determined using total lysate (75 μg of protein) of cells by immunodetection using a guinea pig anti-rabbit receptor polyclonal Ab and peroxidase-conjugated anti-guinea pig IgG. These data are representative of four separate experiments.

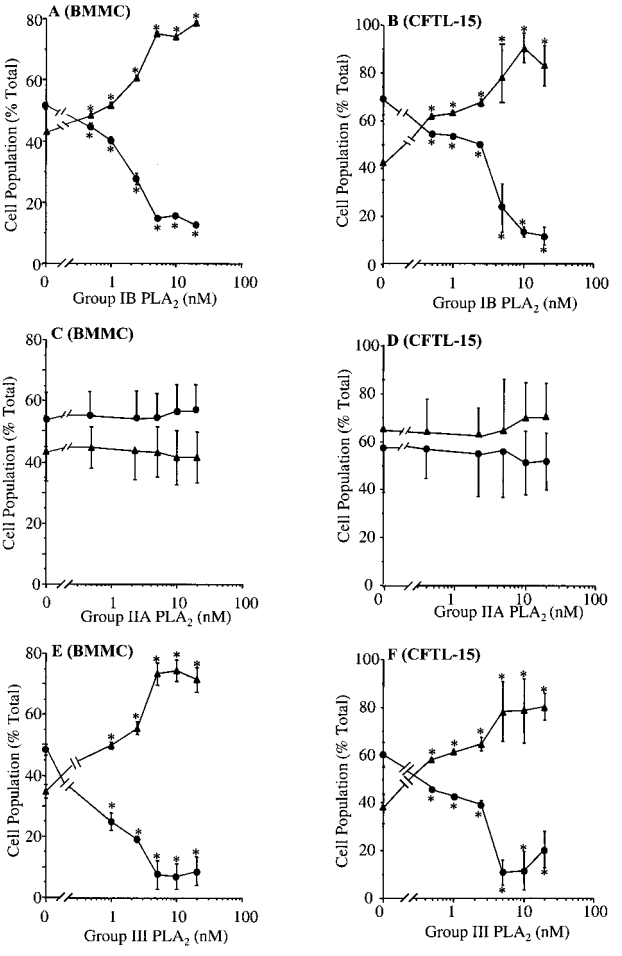

FIGURE 2. Influence of sPLA$_2$ on Annexin binding. BMMC or CFTL-15 mast cells were incubated with different concentrations of group IB PLA$_2$ (*A* and *B*, respectively), group IIA PLA$_2$ (*C* and *D*, respectively), or group III PLA$_2$ (*E* and *F*, respectively) for 24 h. The percentages of live cells (▲) and apoptotic cells (●) were determined by Annexin V^{FITC} binding. These data are the means ± SEM of five separate experiments (*, $p <$ 0.05 compared with 0 nM sPLA$_2$).

analysis (Fig. 1*B*, *lane 2*) shows that there is an increase in sPLA$_2$ receptor level when BMMC are cultured in cytokine-depleted medium for 24 h.

Influence of sPLA$_2$ on Annexin V^{FITC} binding to mast cells

To determine whether sPLA$_2$ could influence cell death by apoptosis, mast cells were incubated with various sPLA$_2$ isotypes, and the percentage of cells undergoing apoptosis was determined by Annexin V^{FITC} binding. When BMMC were place in cytokine-depleted medium for 24 h, ~38–60% of the cells bound Annexin V^{FITC}, indicating early apoptosis. The percentage of apoptotic cells was significantly reduced when BMMC are cultured with low (nanomolar) concentrations of group IB PLA$_2$ (Fig. 2*A*). At 20 nM group IB PLA$_2$, the percentages of apoptotic or live cells were similar to those obtained when mast cells were cultured with WEHI as a source of IL-3 (16.2 ± 0.6% and 78.7 ± 0.8, respectively, $n = 6$). Higher concentrations of sPLA$_2$ (>100 nM) did not significantly decrease the percentages of Annexin V^{FITC}-binding cells, suggesting that saturation binding of sPLA$_2$ to mast cells is attained at ~5–20 nM. Group IB PLA$_2$ also prevented apoptosis of CFTL-15 mast cells placed in cytokine-depleted medium (Fig. 2*B*). In contrast to group IB PLA$_2$, group IIA PLA$_2$ did not influence the percentage of live or apoptotic BMMC or CFTL-15 (Fig. 2, *C* and *D*, respectively). However, group III PLA$_2$ showed the same effect as did group IB PLA$_2$ by preventing apoptosis of BMMC and CFLT-15 mast cells (Fig. 2, *E* and *F*, respectively). These data revealed that groups IB and III PLA$_2$, which bind to mast cell sPLA$_2$ receptors, are as effective as the cytokine IL-3 in preventing early apoptotic events in mast cells, but group IIA PLA$_2$ that does not bind to mast cell sPLA$_2$ receptors lacks the capacity to prevent apoptosis (54, 56).

Influence of sPLA$_2$ DNA fragmentation

A subsequent set of experiments examined the effects of sPLA$_2$ isotypes on a late event of apoptosis, the fragmentation of internucleosomal DNA. No DNA laddering was observed when BMMC were cultured with cytokines. However, BMMC placed in cytokine-depleted medium for 24 h displayed laddering typical of DNA fragmentation when cells are undergoing apoptosis. Incubation of BMMC with group IB PLA$_2$ prevented DNA fragmentation. In contrast, group IIA PLA$_2$ did not influence DNA fragmentation of BMMC. As with BMMC, group IB PLA$_2$ prevented DNA fragmentation of CFTL-15 mast cells. However, it required 96 h of cytokine depletion for DNA fragmentation to be induced in a mast cell line, CFTL-15, which is less dependent on IL-3 for survival. Interestingly, DNA fragmentation could be reversed if group IB PLA$_2$ was added to CFTL-15 cell medium up to 72 h after IL-3 removal. These data further suggest that group IB PLA$_2$ can rescue mast cells from apoptosis induced by cytokine depletion.

Influence of sPLA$_2$ isotypes on cell growth, cell cycle progression, and cell death

The above studies suggest that groups I and III PLA$_2$ have the capacity to prevent apoptosis of mast cells. We next determined whether these same sPLA$_2$ isotypes could induce mast cell growth. Yee and colleagues (12) demonstrated that different concentrations of SCF were required to prevent cell death or drive cell proliferation. For example, low concentrations of SCF prevented apoptosis and maintained mast cells at G_0/G_1 of the cell cycle. However, proliferation of mast cells only occurred at higher concentrations of SCF. To initially determine whether certain sPLA$_2$ isotypes influenced mast cell proliferation, we examined [^{3}H]thymidine incorporation into mast cells incubated with different concentrations of group IB PLA$_2$. Cytokine depletion resulted in decrease in thymidine incorporation into mast cells from 131,462 ± 12,642 DPM/1×10^6 BMMC ($n = 8$ performed in duplicates) cultured in IL-3 medium to 8,989 ± 1,794 DPM/1×10^6 BMMC ($n = 8$). Group IB PLA$_2$ at 5–20 nM induced a small increase (1.5- to 2-fold) in thymidine incorporation into mast cells compared with cells grown without cytokines. However, the maximum amount of thymidine incorporation induced by group IB PLA$_2$ represented only 5–10% of that induced by IL-3 or SCF. Higher concentrations of sPLA$_2$ (>100 nM) did not further enhance thymidine incorporation into BMMC most likely because saturation binding of sPLA$_2$ to mast cells is attained at concentrations >5 nM (56).

The ploidy status of mast cells was also examined by flow cytometry. In agreement with studies by Yee and colleagues, most resting mast cells (>80%) resided in G_0/G_1 of the cell cycle. BMMC or CFTL-15 maintained in IL-3-enriched medium had 12.3 ± 1.2% and 22.9 ± 2.6% ($n = 6$) of total cell population, respectively, in the S phase. When BMMC were placed in cytokine-depleted medium for 24 h, there was a significant decrease in the S phase to 3.59 ± 0.63% ($n = 4$, $p < 0.05$). When group IB PLA$_2$ was added to the culture medium, there was no significant change in the percentage of cells in the S phase when compared with cytokine-depleted mast cells. These data suggest that in contrast to its antiapoptotic effects, group IB PLA$_2$ has only a modest effect on BMMC growth.

Effects of AA and lysophospholipids on apoptosis

Our previous study indicated that groups IB and III PLA$_2$, but not human group IIA PLA$_2$, induced the selective release of AA from mast cells (52). IL-3 removal resulted in an increase in AA levels in cell culture medium from 0.61 ± 0.03 to 2.05 ± 0.25 μM ($n = 5$, $p < 0.05$). Addition of 20 nM groups IB, IIA, and III PLA$_2$ isotypes resulted in a significant increase in the mole quantities of AA in the cell culture medium compared with BMMC in IL-3-supplemented medium (5.38 ± 0.53, 2.22 ± 0.20, and 4.11 ± 0.12 μM, respectively, $n = 5$). To determine whether catalytic products of the sPLA$_2$ reaction (AA and lysophospholipids) could be involved in the antiapoptotic effects, different concentrations of products were added exogenously to BMMC cell culture medium. AA did not prevent or increase apoptosis of BMMC (Fig. 3*A*). Likewise, LPA at concentrations that have been shown to enhance cell proliferation did not enhance survival or decrease apoptosis of BMMC (Fig. 3*B*). In the same experiment, LPC increased apoptosis of BMMC concomitant with a decrease in the live cell population. These data suggest that the generation of AA or lysophospholipids via the hydrolytic activity of sPLA$_2$ is not linked to its antiapoptotic effects.

To confirm that the catalytic activity of sPLA$_2$ is not required for its antiapoptotic effects, BMMC were treated with group IB PLA$_2$ that was irreversibly inactivated. BMMC treated with

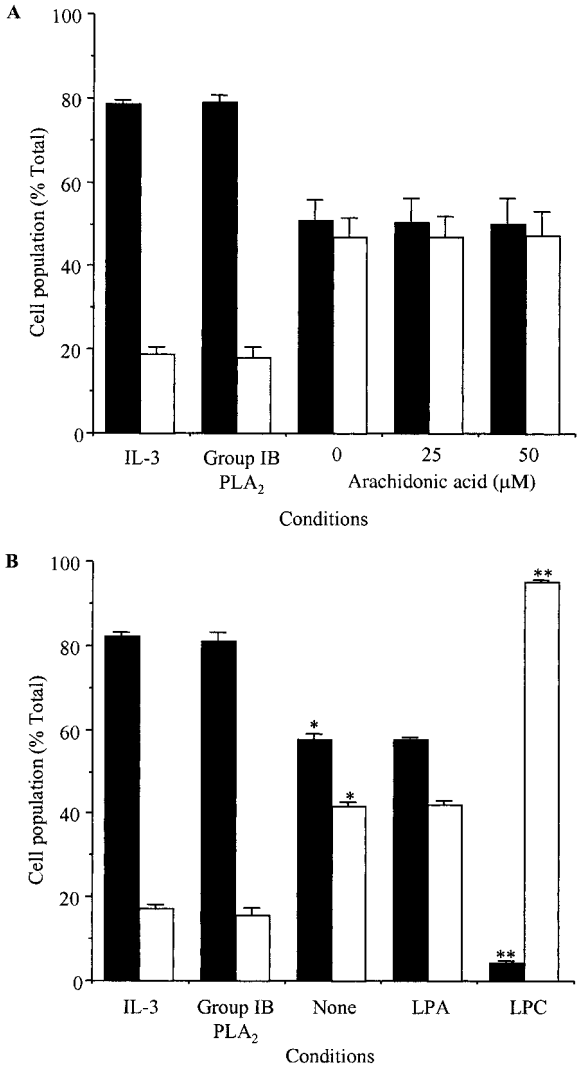

FIGURE 3. Influence of putative sPLA$_2$ products on BMMC survival. BMMC were grown in culture medium supplemented with IL-3 or with different concentrations of AA for 24 h (*A*). Likewise, BMMC were grown in medium supplemented with lysophospholipids (50 μM LPA, 50 μM LPC) (*B*). The percentages of live (■) and apoptotic cells (□) were determined by Annexin V^{FITC} binding. These data are the means ± SEM of three separate experiments (*, $p < 0.05$ compared with None; **, $p < 0.05$ compared with sPLA$_2$).

IL-3 or SCF maintained cell viability of 78.5 ± 2.2% and 75.3 ± 4.6%, respectively. After removal of cytokines, there was a significant decrease in the percentage of live cells to 50.7 ± 0.6% ($p < 0.05$, $n = 4$). Incubation of BMMC with catalytically inactive sPLA$_2$ resulted in a significant increase in the percentage of live cells concomitant with a decrease in the apoptotic cell population (Fig. 4). The inactive sPLA$_2$ completely restored BMMC viability to levels found when cells are cultured with cytokines or active sPLA$_2$. These data reveal that the hydrolytic activity of sPLA$_2$ is not required for its antiapoptotic effects. However, it required ~10 nM to obtain maximal effect using inactive sPLA$_2$ vs ~5 nM for active sPLA$_2$. We suspect that the 2-fold difference to achieve maximal survival between inactive and active sPLA$_2$ is most likely due to structural modifications caused by the bulky BPB that irreversibly inactivates the enzyme.

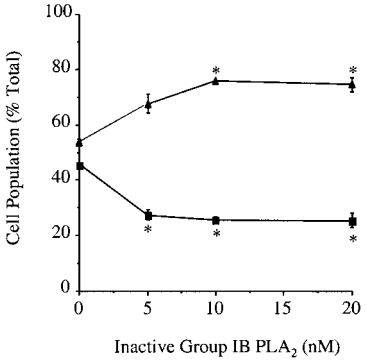

FIGURE 4. Effects of catalytically inactive sPLA$_2$ on mast cell survival. Cytokine-depleted BMMC were incubated with different concentrations of inactive sPLA$_2$ for 24 h. The percentages of live (▲) cells or apoptotic cells (■) were determined by Annexin V^{FITC} binding. These data are the means ± SEM of three separate experiments (*, $p < 0.05$ compared with 0 nM sPLA$_2$).

Role of mRNA and protein synthesis on the antiapoptotic effects of sPLA$_2$

A potential mechanism that might account for the antiapoptotic properties of sPLA$_2$ is induction of synthesis of proteins that regulate cellular apoptosis. This hypothesis was tested using an inhibitor of mRNA synthesis (actinomycin) or an inhibitor of protein synthesis (cycloheximide). As shown in Fig. 5, actinomycin significantly attenuated the antiapoptotic effects of sPLA$_2$. Likewise, incubation of BMMC with sPLA$_2$ in combination with cycloheximide resulted in a decrease in the percentage of live cells. Actinomycin enhanced apoptosis (48.6 ± 6.2% to 92.5 ± 3.1%, $n = 3$) in cells cultured without IL-3. Likewise, cycloheximide increased the percentage of apoptotic cells from 53.4 ± 5.6 to 67.6 ± 9.4 ($n = 3$) in cells cultured without IL-3. These data are in agreement with our initial observation that survival of mast cells in cytokine-depleted medium depends on enhanced protein (sPLA$_2$ and sPLA$_2$ receptor) expression (Fig. 1). Cycloheximide also increased the percentage of apoptotic cells in IL-3 medium from 24.4 ± 5.8 to 67.5 ± 9.4 ($n = 3$). Together, these data suggest that new protein synthesis is involved in both the antiapoptotic effects of sPLA$_2$ or IL-3 and in the induction of apoptosis induced by cytokine withdrawal.

During antigenic stimulation of BMMC, there is an increase in cytokine mRNA expression and protein synthesis (2, 4, 59). Because BMMC survival is supported by IL-3, sPLA$_2$ may be enhancing mast cell survival by inducing IL-3 production. Indeed, whereas removal of WEHI medium resulted in a decrease in IL-3 in cell culture medium (from 765 ± 50.2 to 199.8 ± 23.5 pg/ml, $n = 6$), the addition of active or inactivated group IB PLA$_2$ significantly increased IL-3 levels in BMMC medium (270.7 ± 40.1 pg/ml and 303.3 ± 33.5 pg/ml, respectively, $n = 6$). However, the increase in IL-3 by sPLA$_2$ represented only 15–20% of that required for cell survival and proliferation using WEHI supernatant as a source of IL-3. These data suggest that newly synthesized IL-3 may be a key protein that is responsible for the capacity of sPLA$_2$ to block apoptosis or induce modest increases in thymidine incorporation into mast cells. Further studies are required to determine whether sPLA$_2$ induces the expression of other cytokines that have been implicated in mast cell survival and growth (12, 19, 60).

Influence of IL-3-neutralizing Ab on the antiapoptotic effects of IL-3 and group IB PLA$_2$

In subsequent studies, we examined the effects of IL-3-neutralizing Ab on the effects of sPLA$_2$ and IL-3 on mast cells to determine whether the antiapoptotic effects of sPLA$_2$ were mediated totally through the induction of IL-3. IL-3 prevented apoptosis of BMMC, as shown by reduced Annexin V^{FITC} binding. IL-3-neutralizing Ab (10 μg/ml) restored apoptosis, as evidenced by the increase in Annexin V^{FITC} binding to BMMC (Fig. 6). In contrast, this neutralizing Ab did not affect the percentages of live or apoptotic cells when BMMC were cultured with 20 nM group IB PLA$_2$ for 24 h. In a similar set of studies, we examined the effects of neutralizing Ab on thymidine incorporation in BMMC cultured without cytokines, with IL-3, or with group IB PLA$_2$. Incubation of BMMC with neutralizing Ab resulted in a 98.9 ± 0.1%, 5.6 ± 4.5%, and

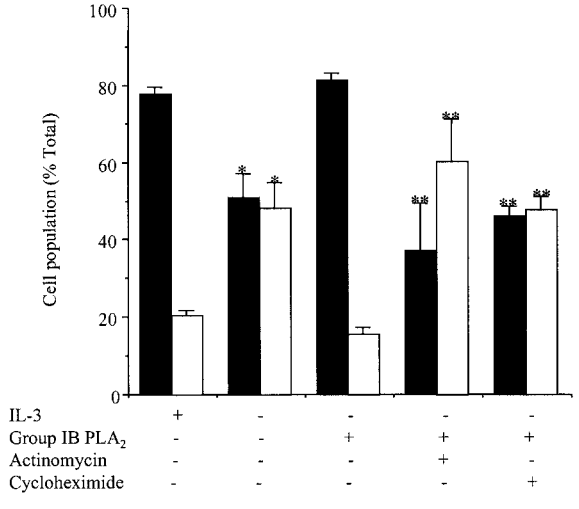

FIGURE 5. Effects of inhibitors of macromolecule synthesis on the antiapoptotic effects of sPLA$_2$. BMMC were cultured with or without IL-3, 20 nM group IB PLA$_2$, group IB PLA$_2$, actinomycin (0.5 μg/ml), or cycloheximide (5 μg/ml), as indicated. The percentages of live (■) and apoptotic cells (□) were determined by flow cytometry using an Annexin V^{FITC}-binding kit. These data are the mean ± SEM of three separate experiments (*, $p < 0.05$ compared with 20 nM sPLA$_2$; **, $p < 0.05$ compared with sPLA$_2$).

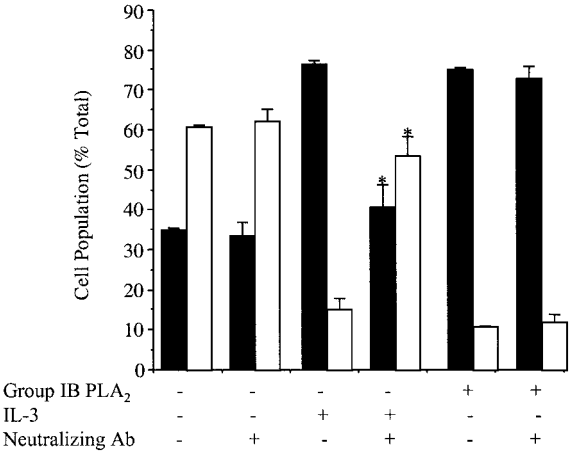

FIGURE 6. Influence of IL-3-neutralizing Ab on apoptosis. BMMC were incubated without IL-3, with 20 nM group IB PLA$_2$, or with IL-3 in the absence or presence of 10 μg/ml IL-3-neutralizing Ab. The percentages of live (■) and apoptotic cells (□) were determined by Annexin V^{FITC} binding to BMMC, as described in *Materials and Methods*. These data are the mean ± SEM of three separate experiments performed in duplicates (*, $p < 0.05$ compared with IL-3 or sPLA$_2$ alone; **, $p < 0.05$ compared with sPLA$_2$).

35 ± 3.8% decrease in thymidine incorporation in BMMC cultured with IL-3, without IL-3, or with 20 nM group IB PLA_2. Likewise, PTX inhibited thymidine incorporation into mast cultured in IL-3- and $sPLA_2$-supplemented medium by 65.3 ± 2.9% ($n = 3$) and 27 ± 9.9% ($n = 3$), respectively. These data suggest that the antiapoptotic effect of group IB PLA_2 is not due to IL-3 expression, while the enhancement of thymidine incorporation by group IB PLA_2 may be partially due to the induction of IL-3 expression.

Signaling pathways responsible for the antiapoptotic effects of group IB PLA_2

Phosphorylation of tyrosine residues on various receptors has been implicated in the effects of cytokines on mast cells (19, 21, 61). To determine whether the antiapoptotic effects of $sPLA_2$ might be mediated by tyrosine phosphorylation, BMMC were incubated with group IB PLA_2 and an inhibitor of tyrosine phosphorylation, herbimycin A, and binding of Annexin V^{FITC} was determined. Herbimycin A significantly reduced the percentage of live cells while increasing the percentage of dead cells after $sPLA_2$ treatment (Table I). Because the PI3′-K pathway has been implicated in the survival of mast cells (20), we examined the effects of $sPLA_2$ on PI3′-K activity in BMMC. As shown in Fig. 7*A*, $sPLA_2$ dose dependently increased PIP_3 formation by mast cells. Likewise, $sPLA_2$ time dependently enhanced PIP_3 formation in mast cells (Fig. 7*B*). These data showed that $sPLA_2$ activated PI3′-K in mast cells. Subsequent studies examined the influence of a PI3′-K inhibitor (LY294002) on the antiapoptotic effects of $sPLA_2$. LY294002 reversed the antiapoptotic effects of $sPLA_2$, suggesting that PI3′-K activation was involved in the antiapoptotic effects of $sPLA_2$ (Table I). In contrast, PTX did not affect the capacity of $sPLA_2$ to increase mast cell survival, suggesting that other products of $sPLA_2$ receptor activation that can activate G protein-coupled receptors are not involved in the antiapoptotic effects of $sPLA_2$. LY294002 also inhibited thymidine incorporation when mast cells were cultured with $sPLA_2$ (82.1 ± 2.4%, $n = 4$) and with IL-3 (98.4 ± 0.1%, $n = 3$). Together, these data suggest that the antiapoptotic effects of $sPLA_2$ and the capacity of $sPLA_2$ to increase thymidine incorporation are mediated by PI3′-K activation.

During Ag activation of mast cells, the production of cytokines has been linked to NF-κB activation, and various cytokines promote cell survival by activating NF-κB (62, 63). Initial studies examined NF-κB activation in BMMC by examining the degradation of I-κBα after BMMC had been activated with $sPLA_2$. As shown in Fig. 8, stimulation of BMMC with $sPLA_2$ resulted in a time-dependent decrease in I-κBα levels. At 2 h after stimulation with $sPLA_2$, levels of I-κBα were reduced to 54.8 ± 7.5 ($p <$ 0.05, $n = 6$) of that found in unstimulated BMMC (Fig. 8, *lower panel*). These data suggest that $sPLA_2$ activates NF-κB pathways. Additional studies examined the effects of two NF-κB inhibitors (CAPE and BAY 11-7082) (64, 65) on the antiapoptotic effects of $sPLA_2$. As shown in Fig. 9*A*, CAPE reversed the antiapoptotic effects of group IB PLA_2 ($IC_{50} > 20$ μg/ml). Likewise, BAY 11-7082, which prevents the phosphorylation of I-κB, potently reversed the antiapoptotic effects of group IB PLA_2 with an IC_{50} of ~7.5 μM (Fig. 9*B*). A similar inhibitory profile was obtained when mast cells cultured with IL-3 were treated with 20 μg/ml CAPE (37.4 ± 3.5% and 62.5 ± 3.6% live and apoptotic cells, respectively) or 10 μM BAY 11-7082 (32.1 ± 3.2% and 68.1 ± 3.4% live and apoptotic cells, respectively, $n = 3$). Taken together, these data suggest that the antiapoptotic effects of some $sPLA_2$ isotypes are most likely mediated via $sPLA_2$ receptor-dependent phosphorylation of tyrosine residues, and the activation of antiapoptotic signaling pathways involving PI3′-K and NF-κB pathways.

Table I. *Effects of inhibitors on the anti-apoptotic effects of group IB PLA_2*[a]

Conditions	Live Cells (% total)	Apoptotic Cells (% total)
IL-3	80.6 ± 3.7	15.7 ± 2.6
None	51.25 ± 4.85*	37.72 ± 2.77*
$sPLA_2$	84.49 ± 4.67	12.05 ± 1.78
$sPLA_2$ + Herbimycin A	58.29 ± 4.28*	20.64 ± 2.85*
$sPLA_2$ + LY294002	52.84 ± 9.61*	28.26 ± 3.56*
$sPLA_2$ + PTX	72.61 ± 3.69	15.78 ± 2.29

[a] BMMC were incubated without (none) or with 20 nM group IB PLA_2 in the presence of 5 μM herbimycin A, 20 μM LY294002, or 0.5 μg/ml PTX for 24 h. Annexin V^{FITC} binding to BMMC was then determined as described in *Materials and Methods*. These data are the mean ± SEM of nine separate experiments. *, $p < 0.05$ compared to $sPLA_2$

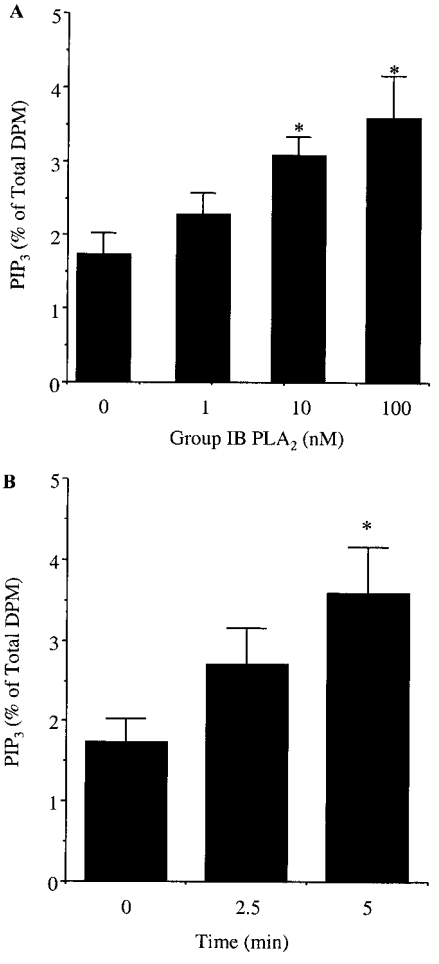

FIGURE 7. Influence of $sPLA_2$ on PI3′-K activity. BMMC (5×10^6/ml) labeled with [^{3}H]inositol were incubated with different concentrations of group IB PLA_2 at 37°C for 5 min (*A*) or with 100 nM group IB PLA_2 for different periods of time (*B*). PI and PIP_3 were resolved by TLC, and the percentage of total radioactivity in PIP_3 was determined as described in *Materials and Methods*. These data are the mean ± SEM of six separate experiments performed (*, $p < 0.05$ compared with unstimulated BMMC).

Discussion

The present study demonstrates that $sPLA_2$ may play an important cytokine-like role in mast cells by enhancing survival without inducing proliferation. Several lines of evidence support this novel

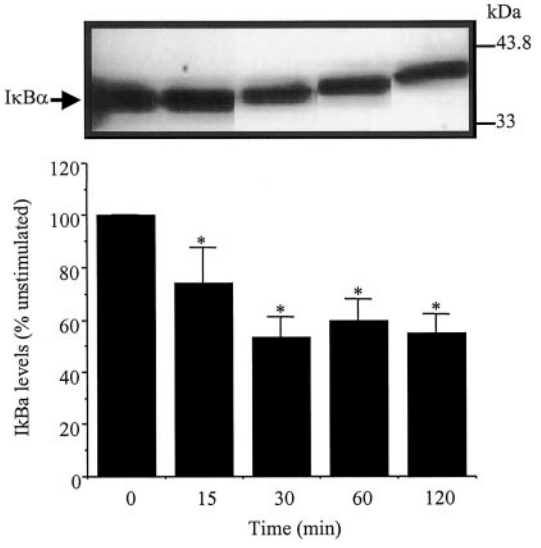

FIGURE 8. sPLA$_2$ increase I-κBα degradation. BMMC (5×10^6/ml) were incubated without or with 100 nM group IB PLA$_2$ for different periods of time. Cell lysates were obtained, and I-κBα levels (*upper panel*, representative of six separate experiments) were determined by Western analysis, as described in *Materials and Methods*. Levels of I-κBα were determined by laser densitometry and expressed as a percentage of levels found in unstimulated BMMC (*lower panel*, ∗, $p < 0.05$, $n = 6$).

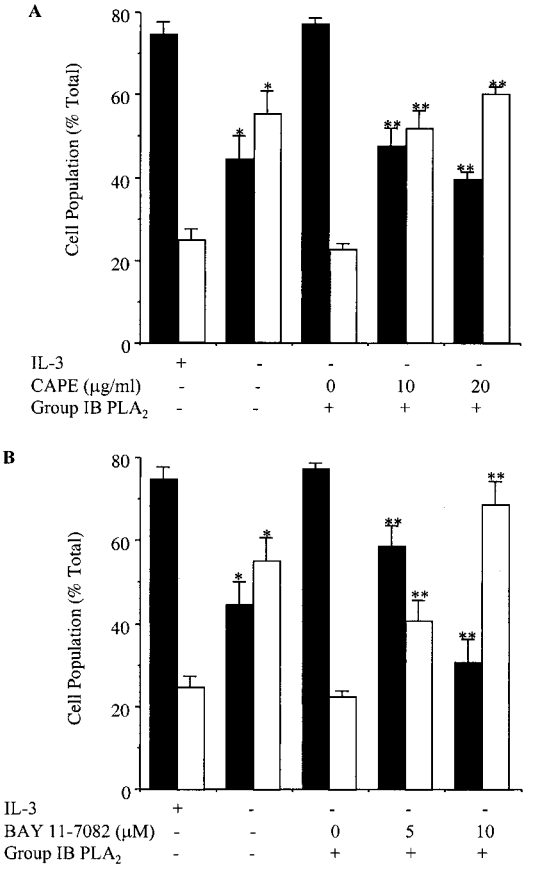

FIGURE 9. Effects of NF-κB inhibitors on the antiapoptotic effects of group IB PLA$_2$. BMMC were incubated with IL-3, without cytokines, or with 20 nM group IB PLA$_2$ in the presence of 0–20 μg/ml CAPE (*A*) or 0–10 μM BAY-117082 (*B*) for 24 h, as indicated. The percentages of live (■) and apoptotic cells (□) were determined by Annexin V^{FITC} binding to BMMC, as described in *Materials and Methods*. These data are the mean ± SEM of three separate experiments performed in duplicate (∗, $p <$ 0.05 compared with IL-3 or sPLA$_2$ alone; ∗∗, $p < 0.05$ compared with sPLA$_2$).

role for sPLA$_2$: 1) When mast cells are placed in cytokine-depleted medium, sPLA$_2$ and sPLA$_2$ receptor levels are increased within the cells. We propose that these cells are producing more sPLA$_2$ and sPLA$_2$ receptors in an attempt to counteract the absence of cytokines. 2) Incubation of mast cells with group IB or III PLA$_2$ supports mast cell survival in the absence of cytokines, suggesting that these sPLA$_2$ isotypes can substitute for cytokines by promoting BMMC survival. Both early (Annexin V^{FITC} binding) and late (DNA fragmentation) events of apoptosis are prevented by the addition of groups IB or III PLA$_2$ to the growth medium. 3) Group IB PLA$_2$ modestly enhances thymidine incorporation into DNA of mast cells, but does not alter the percentage of cells in S phase of the cell cycle. This suggests that whereas sPLA$_2$ enhances mast cell survival, it only modestly enhances cell growth. 4) The sPLA$_2$ isotypes that bind sPLA$_2$ receptors on mast cells (56) are those that prevent mast cells from undergoing apoptosis, whereas human group IIA PLA$_2$ that does not bind to mouse sPLA$_2$ receptors does not. This suggests that a PLA$_2$ receptor-dependent pathway mediates the effects of sPLA$_2$. 5) Catalytically inactive sPLA$_2$ has the same antiapoptotic characteristics as its catalytically active counterpart, suggesting that the catalytic activity of sPLA$_2$ is not required for the cytokine-like effects. 6) A key product of the sPLA$_2$ reaction, AA, does not prevent mast cell apoptosis. Likewise, other putative PLA$_2$ products with mitogenic potential (66), LPA and LPC, do not prevent apoptosis of BMMC. This suggests that sPLA$_2$ itself, and not a putative sPLA$_2$ product, is responsible for enhancing mast cell survival. 7) Inhibitors of mRNA and protein synthesis attenuate the antiapoptotic effects of sPLA$_2$, and active and catalytically inactive group IB PLA$_2$ increase the synthesis of IL-3 by BMMC. 8) sPLA$_2$ enhances PI3′-K activity and I-κBα degradation, and inhibitors of PI3′-K and NF-κB reversed the antiapoptotic effects of sPLA$_2$, suggesting that the effects of sPLA$_2$ are most likely mediated by the activation of these cell survival signaling pathways. 9) An IL-3-neutralizing Ab did not inhibit mast cell survival induced by sPLA$_2$, suggesting that IL-3 does not contribute to the antiapoptotic effects of sPLA$_2$. However, sPLA$_2$-induced radiolabel thymidine incorporation into mast cells was attenuated by IL-3-neutralizing Ab, suggesting that the modest induction of DNA synthesis was most likely due to the production of IL-3 by sPLA$_2$-stimulated mast cells.

There are several potential mechanisms that may account for the effects of sPLA$_2$ on mast cell survival. Perhaps the most likely mechanism supported by these data is that certain sPLA$_2$ isotypes bind to cell surface receptors on mast cells and activate signals that are responsible for enhanced mast cell survival. Our previous study suggested that mast cells express a receptor that tightly binds groups IB and III PLA$_2$ (56). The cloned sPLA$_2$ receptor is composed of an N-terminal cysteine-rich domain, a fibronectin-like type II domain, eight carbohydrate recognition domains (C1-C8), a membrane-spanning domain (M), and intracellular tail region (I) (67). Although no signaling motif is known for the sPLA$_2$ receptor, treatment of mast cells or astrocytes with certain sPLA$_2$ isotypes induces tyrosine phosphorylation of phospholipase Cγ, the mobilization of cytosolic calcium, and the activation of MAP kinase (p44/p42 MAP kinase) (68). Products of sPLA$_2$ receptor activation may also interact with G protein-coupled receptors to initiate survival signaling pathways (66). Many cytokines are known to induce cell survival or induce cell proliferation through receptor tyrosine-dependent phosphorylation and the activation of PI3′-K and its downstream effectors (59, 62). Kawamoto and colleagues

(19) have shown that nerve growth factor (NGF) prevents apoptosis of peritoneal mast cells by activating tyrosine receptor kinases. Likewise, Besmer and colleagues (20) have shown that signaling through PI3′-K and Src kinase is important in *kit* receptor-mediated mast cell survival and proliferation. Whereas NGF and $sPLA_2$ prevent apoptosis without inducing proliferation, SCF and insulin-like growth factor-1 have been shown to be mitogenic when provided to mast cells (12, 20, 69). Our data show that $sPLA_2$ activates PI3′-K in mast cells and that an inhibitor of PI3′-K reversed the antiapoptotic effects of $sPLA_2$. Likewise, $sPLA_2$ enhanced the degradation of the NF-κB inhibitor (I-κBα), and two different inhibitors of NF-κB attenuated the antiapoptotic effects of $sPLA_2$. Similarly, these same inhibitors induced apoptosis of mast cells cultured with IL-3. Together, our data suggest that $sPLA_2$ prevents the apoptosis of mast cells by activating survival pathways similar to that reported for cytokines such as SCF, IL-3, or NGF. There are several cytokine-dependent pathways that are activated in mast cells and are reported to support survival or induce proliferation. Janus kinases (JAK) associate with the β units of cytokine receptors, and the activated JAK phosphorylate STAT (70). Although activation of the Ras/MAP kinase and the JAK2/Stat 5 pathways and induction of pim-1 by IL-3 and similar pathways induce proliferation in some mast cell lines, activation of these same pathways is insufficient in mediating proliferation in other mast cell lines (20, 71, 72). More studies are currently underway in our laboratory to more specifically elucidate the complete receptor-mediated signaling pathways by which $sPLA_2$ prevent apoptosis of mast cells and to compare these pathways with those mediated by SCF or IL-3.

Another mechanism by which $sPLA_2$ might prevent apoptosis of mast cells is by inducing the formation of bioactive lipid products that act on mast cells to prevent apoptosis. For example, studies by Fourcade and colleagues (73) showed that $sPLA_2$ induces the formation of LPA and this product may induce cell proliferation. Our data suggest that the generation of such products is not responsible for the antiapoptotic effects of $sPLA_2$. First, the hydrolytic capacity of $sPLA_2$ isotypes is not required to affect apoptosis of mast cells. Second, major products of the hydrolytic reaction of $sPLA_2$ (AA and lysophospholipids) have no effect or enhance apoptosis (LPC) due to solubilizing effects on mast cell membranes rather than protect these cells. Several reports support this data by showing that AA induces apoptosis in many cases (7, 35, 74–77). Additionally, higher levels of $sPLA_2$ that nonspecifically hydrolyze outer plasma membrane phospholipids may induce rather than prevent apoptosis of cell (78). These data clearly indicate that the catalytic activity of $sPLA_2$ is not linked to their antiapoptotic effects.

During antigenic stimulation of BMMC, there is an increase in the mRNA and protein levels of various cytokines (2, 59). Because BMMC depend on cytokines for survival, induction of the synthesis of new proteins is a potential mechanism by which $sPLA_2$ may prevent apoptosis of BMMC. This hypothesis was supported by data that inhibitors of mRNA and protein synthesis reversed the antiapoptotic effects of $sPLA_2$. The fact that $sPLA_2$ enhanced IL-3 production provided more direct evidence that $sPLA_2$ receptor occupancy leads to the formation of important mast cell cytokines. $sPLA_2$ isotypes have also been shown to induce secretion and IL-6 production in human macrophages independent of catalytic activity (79). Thus, it is likely that antiapoptotic, proliferative, or inflammatory effects of $sPLA_2$ are mediated in part by the capacity of $sPLA_2$ to induce the synthesis of growth/survival factors or cytokines. Our data using IL-3-neutralizing Ab suggest that IL-3 is not involved in the early antiapoptotic events of $sPLA_2$, but may contribute to the ability of $sPLA_2$ to induce a modest increase in thymidine incorporation in mast cells. Because low concentrations of cytokines may enhance mast cell survival, whereas higher concentrations are required to induce growth (12), the failure of $sPLA_2$ to induce cell cycle progression may be due to the fact that only a small amount of IL-3 production is induced.

These data provide evidence for a previously unrecognized role of $sPLA_2$ in allergic and inflammatory diseases. Specifically, these in vitro data suggest that $sPLA_2$ may increase the survival of mast cells at sites of inflammation, thereby providing a mechanism through which mast cells can respond to Ags for extended periods of time. In addition to an autocrine role, $sPLA_2$ could have cytokine-like roles in that they are released from one cell type and act on another cell type. It is well documented that high levels of $sPLA_2$ are found in the serum of patients with severe illnesses, such as sepsis, shock, organ injury, and pancreatitis (80, 81). The tools available at the time of those studies suggested that the $sPLA_2$ implicated was group IIA PLA_2. However, many of the Abs used in those studies were nonspecific, recognizing groups IIA, III, V, and X PLA_2. Thus, further studies are necessary to definitively identify the circulating $sPLA_2$ isotypes. We and others have shown that $sPLA_2$ isotypes are released after allergen challenge of asthmatic airways (82, 83), and we postulate that these $sPLA_2$ isotypes have the capacity to influence the recruitment and function of cells that participate in airway disease. A study by Reddy and Herschman (84) showing that mast cells can provide $sPLA_2$ to fibroblasts for PG production supports this hypothesis. Alternatively, cells other than mast cells could be a major source for $sPLA_2$ in airways. For example, human eosinophils contain large quantities of $sPLA_2$ (20- to 100-fold more $sPLA_2$ than human neutrophils, monocytes, lymphocytes, and basophils using an ELISA that recognizes both group IIA and group V $sPLA_2$), and this $sPLA_2$ is rapidly released after cell activation (85, 86). Similarly, Hundley and colleagues (82, 87) have shown that $sPLA_2$ is released from human basophils and most likely participates in leukotriene generation. Finally, human group III and XII PLA_2 have recently been cloned from lung tissues and T cells, respectively (88, 89). Thus, there is potential for $sPLA_2$ from mast cells, eosinophils, and basophils, or from lung tissues to have both autocrine and cytokine-like effects in airway diseases.

Acknowledgments

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References

1. Galli, S. J., and J. J. Costa. 1995. Mast-cell-leukocyte cytokine cascades in allergic inflammation. *Allergy 50:851.*
2. Galli, S. J., J. R. Gordon, and B. K. Wershil. 1993. Mast cell cytokines in allergy and inflammation. *Agents Actions Suppl. 43:209.*
3. Chilton, F. H., and L. M. Lichtenstein. 1990. Lipid mediators of the allergic reaction. *Chem. Immunol. 49:173.*
4. Gordon, J. R., P. R. Burd, and S. J. Galli. 1990. Mast cells as a source of multifunctional cytokines. *Immunol. Today 11:458.*
5. Metcalfe, D. D., D. Baram, and Y. A. Mekori. 1997. Mast cells. *Physiol. Rev. 77:1033.*
6. Galli, S. J., A. Iemura, D. S. Garlick, C. Gamba-Vitalo, K. M. Zsebo, and R. G. Andrews. 1993. Reversible expansion of primate mast cell populations in vivo by stem cell factor. *J. Clin. Invest. 91:148.*
7. Fonteh, A. N., T. LaPorte, D. Swan, and M. A. McAlexander. 2001. A decrease in remodeling accounts for the accumulation of arachidonic acid in murine mast cells undergoing apoptosis. *J. Biol. Chem. 276:1439.*
8. Galli, S. J., M. Tsai, B. K. Wershil, S. Y. Tam, and J. J. Costa. 1995. Regulation of mouse and human mast cell development, survival and function by stem cell factor, the ligand for the c-*kit* receptor. *Int. Arch. Allergy Immunol. 107:51.*
9. Haig, D. M., J. F. Huntley, A. MacKellar, G. F. Newlands, L. Inglis, R. Sangha, D. Cohen, A. Hapel, S. J. Galli, and H. R. Miller. 1994. Effects of stem cell factor (*kit*-ligand) and interleukin-3 on the growth and serine proteinase expression of rat bone-marrow-derived or serosal mast cells. *Blood 83:72.*

10. Iemura, A., M. Tsai, A. Ando, B. K. Wershil, and S. J. Galli. 1994. The c-*kit* ligand, stem cell factor, promotes mast cell survival by suppressing apoptosis. *Am. J. Pathol. 144:321.*
11. Mekori, Y. A., C. K. Oh, and D. D. Metcalfe. 1993. IL-3-dependent murine mast cells undergo apoptosis on removal of IL-3: prevention of apoptosis by c-*kit* ligand. *J. Immunol. 151:3775.*
12. Yee, N. S., I. Paek, and P. Besmer. 1994. Role of *kit*-ligand in proliferation and suppression of apoptosis in mast cells: basis for radiosensitivity of white spotting and steel mutant mice. *J. Exp. Med. 179:1777.*
13. Lantz, C. S., J. Boesiger, C. H. Song, N. Mach, T. Kobayashi, R. C. Mulligan, Y. Nawa, G. Dranoff, and S. J. Galli. 1998. Role for interleukin-3 in mast cell and basophil development and in immunity to parasites. *Nature 392:90.*
14. Mekori, Y. A., C. K. Oh, and D. D. Metcalfe. 1995. The role of c-*Kit* and its ligand, stem cell factor, in mast cell apoptosis. *Int. Arch. Allergy Immunol. 107: 136.*
15. Metcalfe, D. D., J. A. Mekori, and M. Rottem. 1995. Mast cell ontogeny and apoptosis. *Exp. Dermatol. 4:227.*
16. Tsai, M., T. Takeishi, H. Thompson, K. E. Langley, K. M. Zsebo, D. D. Metcalfe, E. N. Geissler, and S. J. Galli. 1991. Induction of mast cell proliferation, maturation, and heparin synthesis by the rat c-*kit* ligand, stem cell factor. *Proc. Natl. Acad. Sci. USA 88:6382.*
17. Dvorak, A. M., R. A. Seder, W. E. Paul, E. S. Morgan, and S. J. Galli. 1994. Effects of interleukin-3 with or without the c-*kit* ligand, stem cell factor, on the survival and cytoplasmic granule formation of mouse basophils and mast cells in vitro. *Am. J. Pathol. 144:160.*
18. Karimi, K., F. A. Redegeld, B. Heijdra, and F. P. Nijkamp. 1999. Stem cell factor and interleukin-4 induce murine bone marrow cells to develop into mast cells with connective tissue type characteristics in vitro. *Exp. Hematol. 27:654.*
19. Kawamoto, K., T. Okada, Y. Kannan, H. Ushio, M. Matsumoto, and H. Matsuda. 1995. Nerve growth factor prevents apoptosis of rat peritoneal mast cells through the *trk* proto-oncogene receptor. *Blood 86:4638.*
20. Timokhina, I., H. Kissel, G. Stella, and P. Besmer. 1998. Kit signaling through PI 3-kinase and Src kinase pathways: an essential role for Rac1 and JNK activation in mast cell proliferation. *EMBO J. 17:6250.*
21. Tsai, M., R. H. Chen, S. Y. Tam, J. Blenis, and S. J. Galli. 1993. Activation of MAP kinases, pp90*rsk* and pp70–S6 kinases in mouse mast cells by signaling through the c-*kit* receptor tyrosine kinase or FcεRI: rapamycin inhibits activation of pp70–S6 kinase and proliferation in mouse mast cells. *Eur. J. Immunol. 23: 3286.*
22. Cervero, C., L. Escribano, J. F. San Miguel, B. Diaz-Agustin, P. Bravo, J. Villarrubia, R. Garcia-Sanz, J. L. Velasco, P. Herrera, M. Vargas, et al. 1999. Expression of Bcl-2 by human bone marrow mast cells and its overexpression in mast cell leukemia. *Am. J. Hematol. 60:191.*
23. Chen, C. Y., and D. V. Faller. 1995. Direction of p21^{ras}-generated signals towards cell growth or apoptosis is determined by protein kinase C and Bcl-2. *Oncogene 11:1487.*
24. Chen, Q., J. Turner, A. J. Watson, and C. Dive. 1997. v-Abl protein tyrosine kinase (PTK) mediated suppression of apoptosis is associated with the up-regulation of Bcl-X$_L$. *Oncogene 15:2249.*
25. Horiuchi, M., W. Hayashida, T. Kambe, T. Yamada, and V. J. Dzau. 1997. Angiotensin type 2 receptor dephosphorylates Bcl-2 by activating mitogen-activated protein kinase phosphatase-1 and induces apoptosis. *J. Biol. Chem. 272: 19022.*
26. Mekori, A. Y. 1997. Mast cell apoptosis: from the DNA to the bedside. *Allerg. Immunol. (Paris) 29:4.*
27. Navarro, P., A. M. Valverde, M. Benito, and M. Lorenzo. 1999. Activated Ha-*ras* induces apoptosis by association with phosphorylated Bcl-2 in a mitogen-activated protein kinase-independent manner. *J. Biol. Chem. 274:18857.*
28. Nemoto, S., J. Xiang, S. Huang, and A. Lin. 1998. Induction of apoptosis by SB202190 through inhibition of p38β mitogen-activated protein kinase. *J. Biol. Chem. 273:16415.*
29. Srivastava, R. K., A. R. Srivastava, S. J. Korsmeyer, M. Nesterova, Y. S. Cho-Chung, and D. L. Longo. 1998. Involvement of microtubules in the regulation of Bcl2 phosphorylation and apoptosis through cyclic AMP-dependent protein kinase. *Mol. Cell. Biol. 18:3509.*
30. Silva, A., A. Wyllie, and M. K. Collins. 1997. p53 is not required for regulation of apoptosis or radioprotection by interleukin-3. *Blood 89:2717.*
31. Watson, A. J., and R. N. Dubois. 1997. Lipid metabolism and APC: implications for colorectal cancer prevention. *Lancet 349:444.*
32. Samet, J. M., M. B. Fasano, A. N. Fonteh, and F. H. Chilton. 1995. Selective induction of prostaglandin G/H synthase I by stem cell factor and dexamethasone in mast cells. *J. Biol. Chem. 270:8044.*
33. Heyden, S. 1974. Polyunsaturated fatty acids and colon cancer. *Nutr. Metab. 17:321.*
34. Reddy, B. S. 1994. Chemoprevention of colon cancer by dietary fatty acids. *Cancer Metastasis Rev. 13:285.*
35. Surette, M. E., A. N. Fonteh, C. Bernatchez, and F. H. Chilton. 1999. Perturbations in the control of cellular arachidonic acid levels block cell growth and induce apoptosis in HL-60 cells. *Carcinogenesis 20:757.*
36. Adam-Klages, S., R. Schwandner, S. Luschen, S. Ussat, D. Kreder, and M. Kronke. 1998. Caspase-mediated inhibition of human cytosolic phospholipase A$_2$ during apoptosis. *J. Immunol. 161:5687.*
37. Lo, H. H., P. Teichmann, G. Furstenberger, I. Gimenez-Conti, and S. M. Fischer. 1998. Suppression or elevation of cytosolic phospholipase A$_2$ alters keratinocyte prostaglandin synthesis, growth, and apoptosis. *Cancer Res. 58:4624.*
38. Luschen, S., S. Ussat, M. Kronke, and S. Adam-Klages. 1998. Cleavage of human cytosolic phospholipase A$_2$ by caspase-1 (ICE) and caspase-8 (FLICE). *Biochem. Biophys. Res. Commun. 253:92.*
39. Wu, Y. L., X. R. Jiang, A. C. Newland, and S. M. Kelsey. 1998. Failure to activate cytosolic phospholipase A$_2$ causes TNF resistance in human leukemic cells. *J. Immunol. 160:5929.*
40. Fonteh, A. N., J. M. Samet, and F. H. Chilton. 1995. Regulation of arachidonic acid, eicosanoid, and phospholipase A$_2$ levels in murine mast cells by recombinant stem cell factor. *J. Clin. Invest. 96:1432.*
41. Kennedy, B. P., C. Soravia, J. Moffat, L. Xia, T. Hiruki, S. Collins, S. Gallinger, and B. Bapat. 1998. Overexpression of the nonpancreatic secretory group II PLA$_2$ messenger RNA and protein in colorectal adenomas from familial adenomatous polyposis patients. *Cancer Res. 58:500.*
42. MacPhee, M., K. P. Chepenik, R. A. Liddell, K. K. Nelson, L. D. Siracusa, and A. M. Buchberg. 1995. The secretory phospholipase A$_2$ gene is a candidate for the Mom1 locus, a major modifier of ApcMin-induced intestinal neoplasia. *Cell 81:957.*
43. Vadas, P., E. Stefanski, B. Grouix, B. D. Schouten, and W. Pruzanski. 1995. Inhibition of human group II phospholipase A$_2$ by C-reactive protein in vitro. *J. Lipid Mediat. Cell Signal. 11:187.*
44. Cirino, G. 1998. Multiple controls in inflammation: extracellular and intracellular phospholipase A$_2$, inducible and constitutive cyclooxygenase, and inducible nitric oxide synthase. *Biochem. Pharmacol. 55:105.*
45. De Marino, V., M. Gentile, F. Granata, G. Marone, and M. Triggiani. 1999. Secretory phospholipase A$_2$: a putative mediator of airway inflammation. *Int. Arch. Allergy Immunol. 118:200.*
46. Pruzanski, W., P. Vadas, and J. Browning. 1993. Secretory non-pancreatic group II phospholipase A$_2$: role in physiologic and inflammatory processes. *J. Lipid Mediators 8:161.*
47. Vadas, P., J. Browning, J. Edelson, and W. Pruzanski. 1993. Extracellular phospholipase A$_2$ expression and inflammation: the relationship with associated disease states. *J. Lipid Mediators 8:1.*
48. Kramer, R. M., C. Hession, B. Johansen, G. Hayes, P. McGray, E. P. Chow, R. Tizard, and R. B. Pepinsky. 1989. Structure and properties of a human nonpancreatic phospholipase A$_2$. *J. Biol. Chem. 264:5768.*
49. Fonteh, A. N., D. A. Bass, L. A. Marshall, M. Seeds, J. M. Samet, and F. H. Chilton. 1994. Evidence that secretory phospholipase A$_2$ plays a role in arachidonic acid release and eicosanoid biosynthesis by mast cells. *J. Immunol. 152:5438.*
50. Bligh, E. A., and W. T. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Physiol. Pharmacol. 37:911.*
51. Dolken, L., F. Schuler, and G. Dolken. 1998. Quantitative detection of t(14;18)-positive cells by real-time quantitative PCR using fluorogenic probes. *BioTechniques 25:1058.*
52. Haugland, R. A., S. J. Vesper, and L. J. Wymer. 1999. Quantitative measurement of *Stachybotrys chartarum conidia* using real time detection of PCR products with the TaqMan(TM) fluorogenic probe system. *Mol. Cell Probes 13:329.*
53. Pongers-Willemse, M. J., O. J. Verhagen, G. J. Tibbe, A. J. Wijkhuijs, V. de Haas, E. Roovers, C. E. van der Schoot, and J. J. van Dongen. 1998. Real-time quantitative PCR for the detection of minimal residual disease in acute lymphoblastic leukemia using junctional region specific TaqMan probes. *Leukemia 12:2006.*
54. Fonteh, A. N., G. Atsumi, T. LaPorte, and F. H. Chilton. 2000. Secretory phospholipase A$_2$ receptor-mediated activation of cytosolic phospholipase A$_2$ in murine bone marrow-derived mast cells. *J. Immunol. 165:2773.*
55. Nair, X., D. Nettleton, D. Clever, K. M. Tramposch, S. Ghosh, and R. C. Franson. 1993. Swine as a model of skin inflammation: phospholipase A$_2$-induced inflammation. *Inflammation 17:205.*
56. Fonteh, A. N., J. M. Samet, M. Surette, W. Reed, and F. H. Chilton. 1998. Mechanisms that account for the selective release of arachidonic acid from intact cells by secretory phospholipase A$_2$. *Biochim. Biophys. Acta 1393:253.*
57. Traynor-Kaplan, A. E., A. L. Harris, B. L. Thompson, P. Taylor, and L. A. Sklar. 1998. An inositol tetrakisphosphate-containing phospholipid in activated neutrophils. *Nature 334:353.*
58. Brown, K., S. Gerstberger, L. Carlson, G. Franzoso, and U. Siebenlist. 1995. Control of IκBα proteolysis by site-specific signal-induced phosphorylation. *Science 267:1485.*
59. Kitaura, J., K. Asai, M. Maeda-Yamamoto, Y. Kawakami, U. Kikkawa, and T. Kawakami. 2000. Akt-dependent cytokine production in mast cells. *J. Exp. Med. 192:729.*
60. Gordon, J. R., and S. J. Galli. 1990. Mast cells as a source of both preformed and immunologically inducible TNF-α/cachectin. *Nature 346:274.*
61. Kawakami, Y., T. Miura, R. Bissonnette, D. Hata, W. N. Khan, T. Kitamura, M. Maeda-Yamamoto, S. E. Hartman, L. Yao, F. W. Alt, and T. Kawakami. 1997. Bruton's tyrosine kinase regulates apoptosis and JNK/SAPK kinase activity. *Proc. Natl. Acad. Sci. USA 94:3938.*
62. Romashkova, J. A., and S. S. Makarov. 1999. NF-κB is a target of AKT in anti-apoptotic PDGF signalling. *Nature 401:86.*
63. Yang, C. H., A. Murti, S. R. Pfeffer, L. Basu, J. G. Kim, and L. M. Pfeffer. 2000. IFNα/β promotes cell survival by activating NF-κB. *Proc. Natl. Acad. Sci. USA 97:13631.*
64. Natarajan, K., S. Singh, T. R. Burke, Jr., D. Grunberger, and B. B. Aggarwal. 1996. Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-κB. *Proc. Natl. Acad. Sci. USA 93:9090.*

65. Pierce, J. W., R. Schoenleber, G. Jesmok, J. Best, S. A. Moore, T. Collins, and M. E. Gerritsen. 1997. Novel inhibitors of cytokine-induced IκBα phosphorylation and endothelial cell adhesion molecule expression show anti-inflammatory effects in vivo. *J. Biol. Chem. 272:21096.*
66. Zhang, Y., J. Lemasters, and B. Herman. 1999. Secretory group IIA phospholipase A_2 generates anti-apoptotic survival signals in kidney fibroblasts. *J. Biol. Chem. 274:27726.*
67. Lambeau, G., and M. Lazdunski. 1999. Receptors for a growing family of secreted phospholipases A_2. *Trends Pharmacol. Sci. 20:162.*
68. Hernandez, M., M. J. Barrero, J. Alvarez, M. Montero, C. M. Sanchez, and M. L. Nieto. 1999. Secretory phospholipase A_2 induces phospholipase Cγ-1 activation and Ca^{2+} mobilization in the human astrocytoma cell line 1321N1 by a mechanism independent of its catalytic activity. *Biochem. Biophys. Res. Commun. 260:99.*
69. Rodriguez-Tarduchy, G., M. K. Collins, I. Garcia, and A. Lopez-Rivas. 1992. Insulin-like growth factor-I inhibits apoptosis in IL-3-dependent hemopoietic cells. *J. Immunol. 149:535.*
70. Le, F., F. Stomaki, J. Woodcock, A. Lopex, and T. Gonda. 2000. The role of disulfide-linked dimerization in interleukin-3 receptor signaling and biological activity. *J. Biol. Chem. 275:5124.*
71. O'Farrell, A., M. Ichihara, A. Mui, and A. Miyajima. 1996. Signaling pathways activated in a unique mast cell line where interleukin-3 supports survival and stem cell factor is required for a proliferative response. *Blood 87:3655.*
72. Shirogane, T., T. Fukada, J. Muller, D. Shima, M. Hibi, and T. Hirano. 1999. Synergistic roles for Pim-1 and c-Myc in STAT3-mediated cell cycle progression and anti-apoptosis. *Immunity 11:709.*
73. Fourcade, O., M. F. Simon, C. Viode, N. Rugani, F. Leballe, A. Ragab, B. Fournie, L. Sarda, and H. Chap. 1995. Secretory phospholipase A_2 generates the novel lipid mediator lysophosphatidic acid in membrane microvesicles shed from activated cells. *Cell 80:919.*
74. Surette, M. E., J. D. Winkler, A. N. Fonteh, and F. H. Chilton. 1996. Relationship between arachidonate-phospholipid remodeling and apoptosis. *Biochemistry 35: 9187.*
75. Begin, M. E., U. N. Das, G. Ells, and D. F. Horrobin. 1985. Selective killing of human cancer cells by polyunsaturated fatty acids. *Prostaglandins Leukot. Med. 19:177.*
76. Chajes, V., W. Sattler, A. Stranzl, and G. M. Kostner. 1995. Influence of *n*-3 fatty acids on the growth of human breast cancer cells in vitro: relationship to peroxides and vitamin-E. *Breast Cancer Res. Treat. 34:199.*
77. Hawkins, R. A., K. Sangster, and M. J. Arends. 1998. Apoptotic death of pancreatic cancer cells induced by polyunsaturated fatty acids varies with double bond number and involves an oxidative mechanism. *J. Pathol. 185:61.*
78. Atsumi, G., M. Murakami, M. Tajima, S. Shimbara, N. Hara, and I. Kudo. 1997. The perturbed membrane of cells undergoing apoptosis is susceptible to type II secretory phospholipase A_2 to liberate arachidonic acid. *Biochim. Biophys. Acta 1349:43.*
79. Triggiani, M., F. Granata, A. Oriente, V. de Marino, M. Gentile, C. Calabrese, C. Palumbo, and G. Marone. 2000. Secretory phospholipases A_2 induce β-glucuronidase release and IL-6 production from human lung macrophages. *J. Immunol. 164:4908.*
80. Vadas, P., W. Pruzanski, and V. Farewell. 1991. A predictive model for the clearance of soluble phospholipase A_2 during septic shock. *J. Lab. Clin. Med. 118:471.*
81. Buchler, M., A. Deller, P. Malfertheiner, H. O. Kleine, H. Wiedeck, W. Uhl, M. Samtner, H. Friess, T. Nevalainen, and H. G. Beger. 1989. Serum phospholipase A_2 in intensive care patients with peritonitis, multiple injury, and necrotizing pancreatitis. *Klin. Wochenschr. 67:217.*
82. Chilton, F. H., F. J. Averill, W. C. Hubbard, A. N. Fonteh, M. Triggiani, and M. C. Liu. 1996. Antigen-induced generation of lyso-phospholipids in human airways. *J. Exp. Med. 183:2235.*
83. Bowton, D. L., M. C. Seeds, M. B. Fasano, B. Goldsmith, and D. A. Bass. 1997. Phospholipase A_2 and arachidonate increase in bronchoalveolar lavage fluid after inhaled antigen challenge in asthmatics. *Am. J. Respir. Crit. Care Med. 155:421.*
84. Reddy, S. T., and H. R. Herschman. 1996. Transcellular prostaglandin production following mast cell activation is mediated by proximal secretory phospholipase A_2 and distal prostaglandin synthase. *J. Biol. Chem. 271:186.*
85. Seeds, M. C., D. F. Jones, F. H. Chilton, and D. A. Bass. 1998. Secretory and cytosolic phospholipases A_2 are activated during TNF priming of human neutrophils. *Biochim. Biophys. Acta 1389:273.*
86. Blom, M., A. T. Tool, P. C. Wever, G. J. Wolbink, M. C. Brouwer, J. Calafat, A. Egesten, E. F. Knol, C. E. Hack, D. Roos, and A. J. Verhoeven. 1998. Human eosinophils express, relative to other circulating leukocytes, large amounts of secretory 14-kD phospholipase A_2. *Blood 91:3037.*
87. Hundley, T. R., L. A. Marshall, W. C. Hubbard, and D. W. MacGlashan, Jr. 1998. Characteristics of arachidonic acid generation in human basophils: relationship between the effects of inhibitors of secretory phospholipase A_2 activity and leukotriene C_4 release. *J. Pharmacol. Exp. Ther. 284:847.*
88. Valentin, E., F. Ghomashchi, M. H. Gelb, M. Lazdunski, and G. Lambeau. 2000. Novel human secreted phospholipase A_2 with homology to the group III bee venom enzyme. *J. Biol. Chem. 275:7492.*
89. Ho, I.-C., J. P. Arm, C. O. Bingham, III, A. Choi, K. F. Austen, and L. H. Glimcher. 2001. A novel group of phospholipase A_2s preferentially expressed in type 2 helper T cells. *J. Biol. Chem. 276:18321.*