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Lung mast cells are a source of secreted phospholipases A_2

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Abstract

Background—Secreted phospholipases A_2 ($sPLA_2$s) are released in plasma and other biologic fluids of patients with inflammatory, autoimmune, and allergic diseases.

Objective—We sought to evaluate $sPLA_2$ activity in the bronchoalveolar lavage fluid (BALF) of asthmatic patients and to examine the expression and release of $sPLA_2$s from primary human lung mast cells (HLMCs).

Methods—$sPLA_2$ activity was measured in BALF and supernatants of either unstimulated or anti-IgE–activated HLMCs as hydrolysis of oleic acid from radiolabeled *Escherichia coli* membranes. Expression of $sPLA_2$s was examined by using RT-PCR. The release of cysteinyl leukotriene (LT) C_4 was measured by means of enzyme immunoassay.

Results—Phospholipase A_2 (PLA_2) activity was higher in the BALF of asthmatic patients than in the control group. BALF PLA_2 activity was blocked by the $sPLA_2$ inhibitors dithiothreitol and Me-Indoxam but not by the cytosolic PLA_2 inhibitor AZ-1. HLMCs spontaneously released a PLA_2 activity that was increased on stimulation with anti-IgE. This PLA_2 activity was blocked by dithiothreitol and Me-Indoxam but not by AZ-1. HLMCs constitutively express mRNA for group IB, IIA, IID, IIE, IIF, III, V, X, XIIA, and XIIB $sPLA_2$s. Anti-IgE did not modify the expression of $sPLA_2$s. The cell-impermeable inhibitor Me-Indoxam significantly reduced (up to 40%) the production of LTC_4 from anti-IgE–stimulated HLMCs.

Conclusions—$sPLA_2$ activity is increased in the airways of asthmatic patients. HLMCs express multiple $sPLA_2$s and release 1 or more of them when activated by anti-IgE. The $sPLA_2$s released by mast cells contribute to LTC_4 production by acting in an autocrine fashion. Mast cells can be a source of $sPLA_2$s in the airways of asthmatic patients.

Keywords

Lung mast cells; secreted phospholipase A_2; leukotriene C_4; arachidonic acid

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Clinical implications: HLMCs can be a source of $sPLA_2$s in the airways of asthmatic patients. PLA_2s secreted by mast cells are implicated in LT synthesis and might provide a novel therapeutic target in asthma.

Phospholipases A_2 (PLA_2s) are key enzymes involved in the mobilization of arachidonic acid from membrane phospholipids.[1] This is the initial step in the metabolic cascade, leading to the synthesis of eicosanoids (prostaglandins, leukotrienes [LTs], and other). PLA_2s thought to play a role in arachidonic acid release are currently classified as high-molecular-weight cytosolic phospholipases A_2 ($cPLA_2$s) and low-molecular-weight secreted PLA_2s ($sPLA_2$s).[1]

Ten isoforms of $sPLA_2$s have been identified in human cells and tissues.[2] $sPLA_2$s are released in extracellular fluids during local or systemic inflammation.[3] In addition, it has been previously shown that $sPLA_2$ activity is detectable in the bronchoalveolar lavage fluid (BALF) of healthy individuals.[4] This activity is increased in the airways of patients with inflammatory lung diseases (pneumonia, adult respiratory distress syndrome, and sarcoidosis).[3] Moreover, $sPLA_2$ activity is also increased in the BALF of patients with bronchial asthma[5,6] and in the nasal fluid of patients with allergic rhinitis after local allergen challenge.[7] These observations indicate that $sPLA_2$ enzymes can be released during allergic reactions in both the upper and lower airways. However, these studies did not provide information on the cellular sources of these enzymes.

The role of $sPLA_2$ isoforms in airway inflammation has been investigated in rodents. For example, several $sPLA_2$s, such as GIIA, GIID, GIIE, GV, and GX, are overexpressed in lung biopsy specimens in experimentally induced pulmonary inflammation.[8,9] In particular, GX, the isoform with the highest phospholipolytic activity in mammalian cells *in vitro*,[10,11] is constitutively expressed in the lung.[8,9] Interestingly, GX expression did not change on LPS- or carrageenin-induced lung inflammation,[8] whereas it was significantly increased in the airways of mice with ovalbumin-induced asthma.[9] Moreover, knocking out GX reduced all the histologic and functional features associated with the inflammatory response and airway remodeling in this model of asthma. These studies demonstrated that certain $sPLA_2$s play an important role in the pathogenesis of inflammatory and allergic diseases of the lung.

The expression of $sPLA_2$ isoforms in the upper and lower airways has been examined in patients with chronic rhinosinusitis or pneumonia.[12,13] Immunohistochemistry revealed that low levels of human GIIA (hGIIA) were expressed in the nasal epithelium and submucosal glands of healthy donors, whereas the expression of this $sPLA_2$ was increased in patients with rhinosinusitis.[12] In the lung only human GX (hGX) was detected in bronchial epithelial cells and subepithelial interstitium of both healthy donors and patients with pneumonia.[13] In inflamed, but not normal, lung tissue hGIIAwas found in vascular smooth muscle cells and bronchial chondrocytes, whereas human GIID, GV, and GX were found in epithelial cells and macrophages.[13] Immunostaining analysis of cells from induced sputum demonstrated that hGX was expressed by bronchial epithelial cells and macrophages in healthy donors and patients with asthma.[14] However, in asthmatic patients the expression of hGX was significantly higher than in healthy subjects and was further increased during exercise-induced bronchoconstriction. These observations indicate that $sPLA_2$ expression is upregulated in human airways during inflammatory and allergic disorders and suggest that cells resident in the lung might produce distinct $sPLA_2$s.

Mast cells play a primary role in the pathogenesis of bronchial asthma and rhinitis.[15] These cells can be activated by IgE– and non-IgE–mediated stimuli to release a variety of preformed and *de novo* synthesized proinflammatory mediators.[16] Mast cells are particularly abundant at the body's interface with the external environment, such as the mucosae of the respiratory and gastrointestinal tracts and the skin.[17] This unique location justifies the important role of mast cells in allergic inflammation, as well as innate immunity and host defense against infections.[16–18]

Studies on the expression of $sPLA_2$s in mast cells have been primarily carried out in mice. Enomoto et al[19] showed that bone marrow–derived mast cells (BMMCs) from BALB/cJ and C57BL/6J mice express all members of the group II subfamily of $sPLA_2$s, including GIIC, GIID, GIIE, GIIF, and GV. GIIA is expressed in BALB/cJ but not in C57BL/6J mast cells because the latter strain has a natural disruption of the gene encoding for GIIA. BMMCs from either strains do not express GIB and GX $sPLA_2$s.[19] This and other studies[20] indicate that the expression pattern of $sPLA_2$ isoforms differs in mast cells with different phenotypes and from different animal species.

Marked biochemical and functional differences exist between murine and human mast cells, and in many cases information on cell activation and mediator production obtained in murine models was not confirmed in human mast cells.[21] Data on $sPLA_2$ expression in human mast cells are scarce because of the limited number of cells detectable in biopsy specimens or retrieved from biologic fluids. Immunohistochemistry studies demonstrated that human synovial[22] and gut[23] mast cells express hGIIA. However, there are no data on the expression and function of $sPLA_2$s in mast cells purified *ex vivo* from human tissues. In this study we provide evidence that human lung mast cells (HLMCs) express mRNA for several $sPLA_2$s and release a PLA_2 activity with biochemical and pharmacologic characteristics similar to that of the PLA_2s secreted in the airways of patients with bronchial asthma.

METHODS

Reagents

Percoll, dimethyl sulfoxide, L-glutamine, antibiotic-antimycotic solution (10,000 IU/mL penicillin, 10 mg/mL streptomycin, and 25 μg/mL amphotericin B), and phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma (St Louis, Mo). Dithiothreitol (DTT) was from MP Biomedicals (Solon, Calif). Me-Indoxam and AZ-1 were prepared as previously described.[11,24] Tritiated oleic acid (OA)–labeled *Escherichia coli* membranes were kindly provided by Dr Gianfrancesco Goracci (University of Perugia, Perugia, Italy). The rabbit anti-human Fce antibody was donated by Drs T. Ishizaka and K. Ishizaka (La Jolla Institute for Allergy and Immunology, La Jolla, Calif).

Study population

Bronchoalveolar lavage was performed in 14 patients with mild persistent asthma and 19 nonasthmatic subjects (see the Methods section in this article's Online Repository at www.jacionline.org). The study protocol was approved by the Ethical Committee of the University of Naples Federico II, and informed consent was obtained from each subject before bronchoscopy.

Bronchoalveolar lavage procedure

Bronchoscopy and bronchoalveolar lavage were performed according to a standardized protocol based on current National Heart, Lung, and Blood Institute guidelines (see also the Methods section in this article's Online Repository).[25]

Cell isolation

The study protocol involving the use of human lung tissue was approved by the Ethical Committee of the University of Naples Federico II, and informed consent was obtained from patients undergoing thoracic surgery. Human mast cells were obtained from the lungs of patients undergoing thoracic surgery and were purified (>98%) by means of immunomagnetic selection, as previously described (see also the Methods section in this article's Online Repository).[26]

Cell incubation

Mast cells suspended in PCG buffer[26] (10^6/mL) were incubated (at 37°C for 15–120 minutes) with anti-IgE (0.03–1 μg/mL). For LTC_4 production, the cells (10^5/mL) were preincubated (at 37°C for 15 minutes) with increasing concentrations (0.01–10 μmol/L) of Me-Indoxam or AZ-1 before stimulation (at 37°C for 30 minutes). The reactions were stopped by means of centrifugation (at 800*g* for 5 minutes at 4°C), and the cell-free supernatant was stored at −80° C for determination of PLA_2 activity, histamine release, LTC_4 production, or β-hexosaminidase release. The cell pellets were lysed with freeze-thaw cycles in distilled water, and aliquots were stored at −80°C for determination of total content of histamine or β-hexosaminidase.

PLA_2 assay

PLA_2 activity in BALF and HLMC supernatants was measured as previously described[27] by using tritiated OA–labeled *E coli* membranes. PLA_2 activity was determined in 50 mmol/L Tris HCl (pH 7.5) and 10 mmol/L $CaCl_2$ in a total volume of 1.0 mL. The reaction was initiated by the addition of 0.1 μCi of tritiated OA–labeled *E coli* membranes. At the end of incubation (90 minutes at 37°C), the reaction was stopped by adding 2 mL of methanol, 1 mL of chloroform, and 50 μL of 9% formic acid, and lipids were extracted and separated by means of thin-layer chromatography. Tritiated OA was measured by means of liquid scintillation counting (Tri-Carb 2800 TR; PerkinElmer, Waltham, Mass), and PLA_2 activity was expressed as picomoles of tritiated OA released per minute per milliliter of BALF or HLMC supernatants. Aliquots of BALF or HLMC supernatants were incubated (for 1 hour at 37°C) with 10 mmol/L DTT, 10 μmol/L Me-Indoxam, 10 μmol/L AZ-1, or 2 mmol/L PMSF before PLA_2 assay to examine the effect of various inhibitors on PLA_2 activity.

Mediator release assays

Histamine was measured in duplicate determinations by using a commercially available enzyme immunoassay (Immunotech, Praha, Czech Republic). β-Hexosaminidase was measured in duplicate determinations by using a colorimetric assay.[28] LTC_4 was measured in mast cell supernatants in duplicate determinations with a commercially available enzyme immunoassay (GE Healthcare, Fairfield, Conn). The linearity range of this assay was 15 to 1,000 pg/mL. Inhibition of LTC_4 production was expressed as a percentage of maximum response calculated as follows: $(R-R_b)/(R_{max}-R_b)\times 100$, where R is the release in samples treated with the inhibitor, R_b is the release in unstimulated samples, and R_{max} is the release in samples stimulated in the absence of the inhibitor.

RT-PCR for $sPLA_2$s

Total RNA from HLMCs was extracted by using the SV total RNA isolation system (Promega, Madison, Wis), treated with RNase-free DNase I, and suspended in diethylpyrocarbonate-treated (DEPC) water. RNA concentration and quality were assessed by means of spectroscopy. One microgram of total RNA was reverse transcribed with 25 mmol/L $MgCl_2$, 50 μmol/L oligo (dT), and 200 U of Superscript III Reverse Transcriptase (Invitrogen, Carlsbad, Calif). Semiquantitative and real-time quantitative PCR (qPCR) were performed as previously described (see the Methods section and Table E1 in this article's Online Repository at www.jacionline.org).[29,30]

Statistical analysis

Data are expressed as means ± SEs of the indicated number of experiments. *P* values were determined with the Student unpaired or paired *t* tests. Correlation was assessed by using the linear regression function of Microsoft (Redmond, Wash) Excel software.

RESULTS

Characterization of PLA_2 activity in BALF

Initial experiments were performed to verify the presence of PLA_2 activity in the airways of patients with bronchial asthma. PLA_2 enzymatic activity was determined in the cell-free unconcentrated BALF of 14 asthmatic patients and 19 control subjects. Fig 1, *A*, shows that low levels of PLA_2 activity were found in the BALF of control subjects (9.3 ± 0.9 pmol of tritiated OA/min/mL of BALF). PLA_2 activity was significantly increased in patients with asthma (28.3 ± 2.2 pmol of tritiated OA/min/mL of BALF, $P < .01$ vs control subjects). There was no significant correlation between the PLA_2 activity and the protein content of the BALF of asthmatic patients (see Fig E1, *A*, in this article's Online Repository at www.jacionline.org), whereas a positive correlation was found in the control group (see Fig E1, *B*, in this article's Online Repository).

To verify that the activity detected in the airways of asthmatic patients was due to the presence of $sPLA_2$s, we incubated the BALF with 2 different inhibitors of $sPLA_2$s (DTT and Me-Indoxam),[11] an inhibitor of $cPLA_2$s (referred to as AZ-1),[31] or an inhibitor of platelet-activating factor acetylhydrolase (PMSF).[32] In particular, DTT is a reducing agent that alters the secondary structure of all $sPLA_2$s by reducing disulfide bonds, whereas Me-Indoxam is an active, site-directed, reversible, competitive inhibitor of $sPLA_2$ enzymatic activity.[11] The latter compound binds to $sPLA_2$ catalytic sites, thereby preventing the interaction of the enzyme with its substrate. Because the various $sPLA_2$ isoforms display structural differences in their catalytic sites,[2] the affinity and inhibitory effect of Me-Indoxam are different for the various human $sPLA_2$s. In particular, Me-Indoxam shows different potencies on the various $sPLA_2$ isoforms in *in vitro* assays with an inhibitory concentration of 50% (IC_{50}) of less than 100 nmol/L for hGIIA, hGIIE, and hGV; an IC_{50} of between 200 and 600 nmol/L for hGIB and hGX; and an IC_{50} of greater than 2 μmol/L for hGIID, hGIIF, hGIII, and hGXIIA.[11] Fig 1, *B*, shows that the PLA_2 activity in the BALF of asthmatic patients was almost completely suppressed by means of incubation with either DTT or Me-Indoxam, whereas it was not affected by AZ-1 or PMSF. These results indicate that 1 or more $sPLA_2$s account for most of the enzymatic activity in the airways of asthmatic patients.

Release of $sPLA_2$s from HLMCs

The detection of $sPLA_2$ activity in the airways of patients with asthma led us to investigate the role of lung mast cells as a potential source of these enzymes. In this group of experiments, we explored the capacity of primary human mast cells purified from the lung parenchyma (HLMCs) to release $sPLA_2$ activity on immunologic activation. Fig 2, *A*, shows that HLMCs spontaneously released PLA_2 activity (23.6 ± 2.4 pmol of tritiated OA/min/mL of HLMC supernatant). Stimulation of HLMCs with anti-IgE (0.03–1 μg/mL) concentration-dependently increased the release of PLA_2 activity, with a maximum at 1 μg/mL (75.6 ± 5.7 pmol of tritiated OA/min/mL of HLMC supernatant). To understand whether the PLA_2 activity released by lung mast cells was from an $sPLA_2$ and had the same biochemical properties as those present in the BALF of asthmatic patients, supernatants of anti-IgE–activated HLMCs were preincubated with the PLA_2 inhibitors described in the previous section. Fig 2, *B*, shows that the PLA_2 activity released by HLMCs was completely blocked by the reducing agent DTT and by the site-directed $sPLA_2$ inhibitor Me-Indoxam at concentrations known to inhibit most of the human $sPLA_2$s *in vitro*.[11] In contrast, AZ-1 and PMSF had no effect on PLA_2 activity released from mast cells. These data indicate that immunologically activated HLMCs released a PLA_2 activity with biochemical and pharmacologic properties of an $sPLA_2$.

In the next group of experiments, we examined the kinetics of release of $sPLA_2$ activity and histamine from anti-IgE–stimulated HLMCs. In 3 different preparations of HLMCs, the release

of the $sPLA_2$ activity was detectable already after 15 minutes of stimulation and peaked at 30 minutes (Fig 3, *A*). The kinetics of $sPLA_2$ release were similar to those of histamine (half-time of $sPLA_2$ release, 15.3 ± 3.2 minutes; half-time of histamine release, 12.8 ± 2.6 minutes). Data obtained with HLMCs from 9 different donors indicated that there was a significant correlation between maximal release of $sPLA_2$s (expressed as biologic activity) and of histamine (expressed as the percentage of the total cellular content) when mast cells were stimulated with an optimal concentration of anti-IgE (1 μg/mL; Fig 3, *B*). These data suggest that the $sPLA_2$ is stored as a preformed mediator within mast cells and is rapidly released on immunologic activation.

Expression of mRNA for $sPLA_2$s in HLMCs

The results obtained thus far have indicated that human mast cells release 1 or more isoforms of $sPLA_2$s. We therefore examined constitutive gene expression of the known human isoforms of $sPLA_2$s in resting HLMCs by means of RT-PCR by using target-specific primers for the various $sPLA_2$s (see Table E1 in this article's Online Repository). Fig 4 shows the PCR amplification signals of 2 experiments representative of 4 different preparations of HLMCs. PCR fragments of the expected size encoding for hGIB (341 bp), hGIIA (434 bp), hGIID (294 bp), hGIIE (120 bp), hGIIF (211 bp), hGIII (500 bp), hGV (358 bp), hGX (370 bp), hGXIIA (105 bp), and hGXIIB (141 bp) were amplified in all HLMC preparations at subsaturating cycle numbers (35 cycles). As previously reported in human neutrophils, the primers used to evaluate hGV mRNA expression in HLMCs also generated a 251-bp PCR product that was identical to the hGV mRNA from nucleotides 24 to 381 but lacked the untranslated region corresponding to exon 4.[24] These data indicate that primary HLMCs constitutively express mRNA for most human $sPLA_2$s.

To investigate whether anti-IgE challenge of HLMCs modifies the expression of $sPLA_2$s, we next examined mRNA for the major $sPLA_2$ isoforms (hGIIA, hGIID, hGIIE, hGIIF, hGIII, hGV, and hGX) in both resting and anti-IgE–activated HLMCs. To this end, we carried out qPCR in 3 different preparations of HLMCs incubated (at 37°C for 3 hours) in the absence (unstimulated) or presence of anti-IgE (1 μg/mL). These experiments allowed an accurate quantification of the $sPLA_2$s constitutively expressed by HLMCs. Table I shows that human mast cells express high levels of hGIII, hGV, and hGX; intermediate levels of hGIID and hGIIF; and low levels of hGIIA and hGIIE. Stimulation with anti-IgE did not enhance the expression of any $sPLA_2$s examined. We rather observed a tendency toward a reduction in the expression of all $sPLA_2$s, but these results did not reach statistical significance.

Role of endogenous $sPLA_2$s in the generation of LTC_4 from HLMCs

$sPLA_2$s contribute to the generation of eicosanoids in murine mast cells[19,27,33,34] and macrophages.[10,35] However, it is still debated whether this contribution is due to intracellular or extracellular actions of $sPLA_2$s. Our experiments demonstrated that human mast cells secrete 1 or more $sPLA_2$s based on results obtained with Me-Indoxam. It is worth noting that, being cell impermeable, Me-Indoxam is able to inhibit the activity of $sPLA_2$s only when they are secreted in the extracellular space.[36] Thus we were able to evaluate the role of endogenous PLA_2s on LTC_4 production once these enzymes have been released by immunologically activated mast cells. In these experiments mast cells were stimulated with anti-IgE in the presence of increasing concentrations (0.1–10 μmol/L) of Me-Indoxam or AZ-1, a potent and cell-permeable inhibitor of GIV-$cPLA_2$. At the end of incubation, LTC_4 production was determined in the supernatants. Fig 5 shows that the GIV inhibitor AZ-1 caused a complete suppression of LTC_4 synthesis (IC_{50}, 40.3 ± 7.9 nmol/L). However, Me-Indoxam also inhibited, in a concentration-dependent fashion, up to 40% of anti-IgE–induced LTC_4 release. Neither Me-Indoxam nor AZ-1 significantly influenced anti-IgE–induced degranulation of mast cells, as assessed by means of β-hexosaminidase release (data not shown). These results

indicate that LTC_4 synthesis in mast cells is primarily due to the GIV-$cPLA_2$. However, $sPLA_2$s, once secreted, significantly contribute to this process by augmenting IgE-mediated cysteinyl LT production.

DISCUSSION

Patients with mild persistent asthma have higher levels of $sPLA_2$ activity in BALF than nonasthmatic control subjects. Primary lung mast cells constitutively express mRNA for several $sPLA_2$s and release, on immunologic activation, $sPLA_2$ activity with biochemical characteristics similar to those of the $sPLA_2$s detected in the BALF of asthmatic patients. Endogenous $sPLA_2$s released by mast cells significantly contribute to IgE-mediated production of cysteinyl LTs.

Our results indicate that low levels of $sPLA_2$s are detectable in the airways of control subjects and that this activity is increased in patients with mild asthma. A significant correlation between $sPLA_2$ activity and protein content exists in the BALF of control subjects. This correlation is lost in asthmatic patients, suggesting that $sPLA_2$ enzymes are selectively released in the airways of these patients. These results are in line with previous studies showing that bronchial antigen challenge in asthmatic patients increases $sPLA_2$ activity in the BALF 3- to 5-fold during the late-phase reaction (ie, 4–20 hours after challenge).[5,6]

Several cells involved in the pathogenesis of asthma, including eosinophils, basophils, T_H2 cells, epithelial cells, macrophages, and fibroblasts, express $sPLA_2$s.[20] Our work provides the first characterization of $sPLA_2$s in human mast cells and indicates that they might be a source of these proinflammatory molecules in patients with asthma. An interesting observation of this study is that, in contrast to other inflammatory cells involved in asthma, mast cells not only express $sPLA_2$s but also release 1 or more of these enzymes on stimulation. Epithelial cells, macrophages, and eosinophils in induced sputum of asthmatic patients express various $sPLA_2$s[14]; the ability of these cells to release $sPLA_2$s was not investigated. This is a relevant issue because eosinophils,[37] basophils,[38] and macrophages[29] synthesize 1 or more $sPLA_2$s but fail to release them, at least *in vitro*. Thus mast cells are rather unique among effector cells in bronchial asthma because they secrete $sPLA_2$s when activated by IgE.

The release of $sPLA_2$s from stimulated mast cells is rapid and coincident with that of histamine. However, stimulation with anti-IgE does not modify the mRNA expression of any $sPLA_2$s. These findings suggest that $sPLA_2$s are synthesized and stored as pre-formed mediators. The correlation between the release of $sPLA_2$ activity and histamine by activated mast cells supports the hypothesis that these mediators are stored together within secretory granules. Early studies demonstrated that $sPLA_2$s are contained within granules of rodent mast cells.[19,39] Moreover, unpublished data from our laboratory also indicate that $sPLA_2$s of the group II subfamily, identified by means of immunohistochemistry, colocalize with tryptase in skin mast cells from patients with mastocytosis.

Another unique feature of human mast cells is the expression of mRNA for a number of $sPLA_2$s. This is at variance with most human inflammatory cells, which express a restricted profile of $sPLA_2$ isoforms.[20] The amount of mRNA for the various $sPLA_2$s is rather different because HLMCs constitutively express high levels of hGIII, hGV, and hGX; medium levels of hGIID and hGIIF; and low levels of hGIIA and hGIIE. These results suggest that these cells might synthesize different quantities of the various $sPLA_2$s. The detection of $sPLA_2$ proteins in mast cells by using conventional techniques (eg, Western blotting) is limited by the low number of cells retrieved from specimens of lung tissue. Thus although our data indicate that mast cells produce messages for all $sPLA_2$s, they do not define which isoforms are translated into proteins, secreted, or both. However, information on the $sPLA_2$s secreted by stimulated

mast cells can be inferred from the data obtained with Me-Indoxam. This compound inhibits hGIB, hGIIA, hGIIE, hGV, and hGX with an IC_{50} of less than 600 nmol/L and hGIID, hGIIF, hGIII, hGXIIA, and hGXIIB with an IC_{50} of greater than 2 μmol/L. Thus it is conceivable that HLMC supernatants contain those $sPLA_2$s that can be blocked by Me-Indoxam (hGIB, hGIIA, hGIIE, hGV, and hGX) rather than those $sPLA_2$s that are poorly sensitive or nonsensitive to the inhibitory effect of this compound (hGIID, hGIIF, hGIII, hGXIIA, and hGXIIB). Further studies with more sensitive and specific techniques for $sPLA_2$ detection will define which $sPLA_2$ proteins are synthesized and released by human mast cells.

The role of $sPLA_2$s in asthma is still under investigation. Some of these molecules, such as hGX,[9,14] can participate in airway inflammation and remodeling through at least 3 mechanisms.

First, $sPLA_2$s can contribute to prostaglandin and LT biosynthesis by potentiating the effect of $cPLA_2$s. The results shown in Fig 5 indicate that LTC_4 production in stimulated HLMCs is primarily dependent on GIV-$cPLA_2$. Nevertheless, the observation that the cell-impermeable $sPLA_2$ inhibitor Me-Indoxam reduces LTC_4 production by 40% indicates that the $sPLA_2$s released by HLMCs contribute to LT production by cross-talking with GIV-$cPLA_2$. These data are reminiscent of those obtained with murine mast cells and macrophages showing that $sPLA_2$s alone do not initiate LTC_4 production but potentiate the eicosanoid-forming capacity of GIV-$cPLA_2$.[27,33,35] The mechanisms of the cross-talk between $cPLA_2$s and $sPLA_2$s are still unclear, but it is currently believed that $sPLA_2$-induced intracellular signals might increase the activation of the GIV-$cPLA_2$.[34] Several isoforms of $sPLA_2$s (GIB, GIIA, GV, and GX) bind to a specific M-type receptor, which generates intracellular signals leading to proinflammatory responses in target cells.[40] Me-Indoxam blocks not only the catalytic activity of $sPLA_2$s[11] but also the receptor-mediated activation of inflammatory cells[30,40] by preventing the binding of $sPLA_2$s to the M-type receptor.[41] Of relevance to the present study, GIB, GIIA, and GV activate the M-type receptor expressed on murine BMMCs, thereby activating GIV-$cPLA_2$ and promoting arachidonate mobilization and eicosanoid production.[42] Therefore the enhancement of LT production by $sPLA_2$s in HLMCs might be due to an autocrine effect on the M-type receptor expressed on these cells.

A second mechanism by which $sPLA_2$s might promote inflammation in asthma is through their nonenzymatic, receptor-mediated activation of inflammatory cells.[40] We and others demonstrated that several $sPLA_2$s (GIB, GIIA, and GX) activate cytokine and chemokine production[30,43–45] by interacting with the M-type or other receptors expressed on human inflammatory cells.

Finally, $sPLA_2$s might contribute to the pathogenesis of asthma *in vivo* through the degradation of surfactant phospholipids. Alterations of the physicochemical properties of surfactant occur in asthma and are associated with airway obstruction and hyperreactivity.[46] $sPLA_2$s hydrolyze surfactant phospholipids, generating lysophospholipids that, in turn, alter surfactant properties and induce proinflammatory effects.[47] Together, these observations help explain why knocking out just 1 $sPLA_2$ isoform (GX) dramatically reduces allergic inflammation.[9]

The capacity of mast cells to secrete $sPLA_2$s might also be relevant to the role of these cells in innate immunity.[16–18] Several $sPLA_2$s have potent bactericidal activity.[48,49] In addition, GIII $sPLA_2$ inhibits HIV replication by blocking viral entry into the cells.[50] Our results raise the interesting hypothesis that $sPLA_2$s are mediators supporting the role of mast cells in innate immunity.

In conclusion, $sPLA_2$s released by immunologically activated mast cells have biochemical properties similar to those of the enzymes secreted in the airways of asthmatic patients, indicating that mast cells might be a major source of $sPLA_2$s in asthma. The demonstration

that $sPLA_2$s are released by mast cells further reinforces the concept that these molecules have an important role in inflammation and tissue remodeling in asthma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BALF	Bronchoalveolar lavage fluid
BMMC	Bone marrow–derived mast cell
$cPLA_2$	Cytosolic phospholipase A_2
Ct	Cycle threshold
DTT	Dithiothreitol
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
hGIIA	Human GIIA
hGX	Human GX
HLMC	Human lung mast cell
IC_{50}	Inhibitory concentration of 50%
LTC_4	Leukotriene C_4
OA	Oleic acid
PLA_2	Phospholipase A_2*
PMSF	Phenylmethylsulfonyl fluoride
qPCR	Real-time quantitative PCR
$sPLA_2$	Secreted phospholipase A_2

References

1. Schaloske RH, Dennis EA. The phospholipase A2 superfamily and its group numbering system. Biochim Biophys Acta 2006;1761:1246–59. [PubMed: 16973413]
2. Lambeau G, Gelb MH. Biochemistry and physiology of mammalian secreted phospholipases A2. Annu Rev Biochem 2008;77:495–520. [PubMed: 18405237]

*The Roman numeral after the letter *G* indicates the group, and the uppercase letter after the numeral indicates the subgroup (eg, *GIB* indicates *group IB PLA_2*).

3. Granata F, Balestrieri B, Petraroli A, Giannattasio G, Marone G, Triggiani M. Secretory phospholipases A2 as multivalent mediators of inflammatory and allergic disorders. Int Arch Allergy Immunol 2003;131:153–63. [PubMed: 12876405]
4. Samet JM, Madden MC, Fonteh AN. Characterization of a secretory phospholipase A2 in human bronchoalveolar lavage fluid. Exp Lung Res 1996;22:299–315. [PubMed: 8792123]
5. Bowton DL, Seeds MC, Fasano MB, Goldsmith B, Bass DA. Phospholipase A2 and arachidonate increase in bronchoalveolar lavage fluid after inhaled antigen challenge in asthmatics. Am J Respir Crit Care Med 1997;155:421–5. [PubMed: 9032172]
6. Chilton FH, Averill FJ, Hubbard WC, Fonteh AN, Triggiani M, Liu MC. Antigen-induced generation of lysophospholipids in human airways. J Exp Med 1996;183:2235–45. [PubMed: 8642333]
7. Stadel JM, Hoyle K, Naclerio RM, Roshak A, Chilton FH. Characterization of phospholipase A2 from human nasal lavage. Am J Respir Cell Mol Biol 1994;11:108–13. [PubMed: 8018333]
8. Hamaguchi K, Kuwata H, Yoshihara K, Masuda S, Shimbara S, Ohishi S, et al. Induction of distinct sets of secretory phospholipase A2 in rodents during inflammation. Biochim Biophys Acta 2003;1635:37–47. [PubMed: 14642775]
9. Henderson WR Jr, Chi EY, Bollinger JG, Tien YT, Ye X, Castelli L, et al. Importance of group X-secreted phospholipase A2 in allergen-induced airway inflammation and remodeling in a mouse asthma model. J Exp Med 2007;204:865–77. [PubMed: 17403936]
10. Saiga A, Morioka Y, Ono T, Nakano K, Ishimoto Y, Arita H, et al. Group X secretory phospholipase A2 induces potent productions of various lipid mediators in mouse peritoneal macrophages. Biochim Biophys Acta 2001;1530:67–76. [PubMed: 11341959]
11. Singer AG, Ghomashchi F, Le Calvez C, Bollinger J, Bezzine S, Rouault M, et al. Interfacial kinetic and binding properties of the complete set of human and mouse groups I, II, V, X, and XII secreted phospholipases A2. J Biol Chem 2002;277:48535–49. [PubMed: 12359733]
12. Liu Z, Lu X, Wang H, You XJ, Gao QX, Cui YH. Group II subfamily secretory phospholipase A2 enzymes: expression in chronic rhinosinusitis with and without nasal polyps. Allergy 2007;62:999–1006. [PubMed: 17578498]
13. Masuda S, Murakami M, Mitsuishi M, Komiyama K, Ishikawa Y, Ishii T, et al. Expression of secretory phospholipase A2 enzymes in lungs of humans with pneumonia and their potential prostaglandin-synthetic function in human lung-derived cells. Biochem J 2005;387:27–38. [PubMed: 15509193]
14. Hallstrand TS, Chi EY, Singer AG, Gelb MH, Henderson WR Jr. Secreted phospholipase A2 group X overexpression in asthma and bronchial hyperresponsiveness. Am J Respir Crit Care Med 2007;176:1072–8. [PubMed: 17901411]
15. Marone, G.; Lichtenstein, LM.; Galli, SJ. Mast cells and basophils. San Diego: Academic Press; 2000.
16. Mekori YA, Metcalfe DD. Mast cells in innate immunity. Immunol Rev 2000;173:131–40. [PubMed: 10719674]
17. Williams CM, Galli SJ. The diverse potential effector and immunoregulatory roles of mast cells in allergic disease. J Allergy Clin Immunol 2000;105:847–59. [PubMed: 10808163]
18. Galli SJ, Grimbaldeston M, Tsai M. Immunomodulatory mast cells: negative, as well as positive, regulators of immunity. Nat Rev Immunol 2008;8:478–86. [PubMed: 18483499]
19. Enomoto A, Murakami M, Valentin E, Lambeau G, Gelb MH, Kudo I. Redundant and segregated functions of granule-associated heparin-binding group II subfamily of secretory phospholipases A2 in the regulation of degranulation and prostaglandin D2 synthesis in mast cells. J Immunol 2000;165:4007–14. [PubMed: 11034411]
20. Triggiani M, Granata F, Giannattasio G, Marone G. Secretory phospholipases A2 in inflammatory and allergic diseases: not just enzymes. J Allergy Clin Immunol 2005;116:1000–6. [PubMed: 16275367]
21. Bischoff SC. Role of mast cells in allergic and non-allergic immune responses: comparison of human and murine data. Nat Rev Immunol 2007;7:93–104. [PubMed: 17259966]
22. Jamal OS, Conaghan PG, Cunningham AM, Brooks PM, Munro VF, Scott KF. Increased expression of human type IIa secretory phospholipase A2 antigen in arthritic synovium. Ann Rheum Dis 1998;57:550–8. [PubMed: 9849315]

23. Lilja I, Gustafson-Svard C, Franzen L, Sjodahl R, Andersen S, Johansen B. Presence of group IIA secretory phospholipase A2 in mast cells and macrophages in normal human ileal submucosa and in Crohn's disease. Clin Chem Lab Med 2000;38:1231–6. [PubMed: 11205686]
24. Degousee N, Ghomashchi F, Stefanski E, Singer A, Smart BP, Borregaard N, et al. Groups IV, V, and X phospholipases A2s in human neutrophils: role in eicosanoid production and gram-negative bacterial phospholipid hydrolysis. J Biol Chem 2002;277:5061–73. [PubMed: 11741884]
25. Busse WW, Wanner A, Adams K, Reynolds HY, Castro M, Chowdhury B, et al. Investigative bronchoprovocation and bronchoscopy in airway diseases. Am J Respir Crit Care Med 2005;172:807–16. [PubMed: 16020805]
26. Triggiani M, Giannattasio G, Balestrieri B, Granata F, Gelb MH, de Paulis A, et al. Differential modulation of mediator release from human basophils and mast cells by mizolastine. Clin Exp Allergy 2004;34:241–9. [PubMed: 14987304]
27. Fonteh AN, Bass DA, Marshall LA, Seeds M, Samet JM, Chilton FH. Evidence that secretory phospholipase A2 plays a role in arachidonic acid release and eicosanoid biosynthesis by mast cells. J Immunol 1994;152:5438–46. [PubMed: 8189063]
28. Feng C, Beller EM, Bagga S, Boyce JA. Human mast cells express multiple EP receptors for prostaglandin E2 that differentially modulate activation responses. Blood 2006;107:3243–50. [PubMed: 16357326]
29. Giannattasio G, Lai Y, Granata F, Mounier CM, Nallan L, Oslund R, et al. Expression of phospholipases A2 in primary human lung macrophages Role of cytosolic phospholipase A2-alpha in arachidonic acid release and platelet activating factor synthesis. Biochim Biophys Acta 2009;1791:92–102. [PubMed: 19130898]
30. Granata F, Petraroli A, Boilard E, Bezzine S, Bollinger J, Del Vecchio L, et al. Activation of cytokine production by secreted phospholipases A2 in human lung macrophages expressing the M-type receptor. J Immunol 2005;174:464–74. [PubMed: 15611272]
31. Connolly S, Bennion C, Botterell S, Croshaw PJ, Hallam C, Hardy K, et al. Design and synthesis of a novel and potent series of inhibitors of cytosolic phospholipase A2 based on a 1,3-disubstituted propan-2-one skeleton. J Med Chem 2002;45:1348–62. [PubMed: 11882004]
32. Triggiani M, De Marino V, Sofia M, Faraone S, Ambrosio G, Carratu L, et al. Characterization of platelet-activating factor acetylhydrolase in human bronchoalveolar lavage. Am J Respir Crit Care Med 1997;156:94–100. [PubMed: 9230731]
33. Murakami M, Yoshihara K, Shimbara S, Lambeau G, Singer A, Gelb MH, et al. Arachidonate release and eicosanoid generation by group IIE phospholipase A2. Biochem Biophys Res Commun 2002;292:689–96. [PubMed: 11922621]
34. Kikawada E, Bonventre JV, Arm JP. Group V secretory PLA2 regulates TLR2-dependent eicosanoid generation in mouse mast cells through amplification of ERK and cPLA2alpha activation. Blood 2007;110:561–7. [PubMed: 17369491]
35. Satake Y, Diaz BL, Balestrieri B, Lam BK, Kanaoka Y, Grusby MJ, et al. Role of group V phospholipase A2 in zymosan-induced eicosanoid generation and vascular permeability revealed by targeted gene disruption. J Biol Chem 2004;279:16488–94. [PubMed: 14761945]
36. Mounier CM, Ghomashchi F, Lindsay MR, James S, Singer AG, Parton RG, et al. Arachidonic acid release from mammalian cells transfected with human groups IIA and X secreted phospholipase A2 occurs predominantly during the secretory process and with the involvement of cytosolic phospholipase A2-alpha. J Biol Chem 2004;279:25024–38. [PubMed: 15007070]
37. Blom M, Tool AT, Wever PC, Wolbink GJ, Brouwer MC, Calafat J, et al. Human eosinophils express, relative to other circulating leukocytes, large amounts of secretory 14-kD phospholipase A2. Blood 1998;91:3037–43. [PubMed: 9531617]
38. Hundley TR, Marshall LA, Hubbard WC, MacGlashan DW Jr. Characteristics of arachidonic acid generation in human basophils: relationship between the effects of inhibitors of secretory phospholipase A2 activity and leukotriene C4 release. J Pharmacol Exp Ther 1998;284:847–57. [PubMed: 9495842]
39. Chock SP, Schmauder-Chock EA, Cordella-Miele E, Miele L, Mukherjee AB. The localization of phospholipase A2 in the secretory granule. Biochem J 1994;300:619–22. [PubMed: 8010941]

40. Triggiani M, Granata F, Frattini A, Marone G. Activation of human inflammatory cells by secreted phospholipases A2. Biochim Biophys Acta 2006;1761:1289–300. [PubMed: 16952481]
41. Boilard E, Rouault M, Surrel F, Le Calvez C, Bezzine S, Singer A, et al. Secreted phospholipase A2 inhibitors are also potent blockers of binding to the M-type receptor. Biochemistry 2006;45:13203–18. [PubMed: 17073442]
42. Fonteh AN, Atsumi G, LaPorte T, Chilton FH. Secretory phospholipase A2 receptor-mediated activation of cytosolic phospholipase A2 in murine bone marrow-derived mast cells. J Immunol 2000;165:2773–82. [PubMed: 10946309]
43. Triggiani M, Granata F, Balestrieri B, Petraroli A, Scalia G, Del Vecchio L, et al. Secretory phospholipases A2 activate selective functions in human eosinophils. J Immunol 2003;170:3279–88. [PubMed: 12626587]
44. Jo EJ, Lee HY, Lee YN, Kim JI, Kang HK, Park DW, et al. Group IB secretory phospholipase A2 stimulates CXC chemokine ligand 8 production via ERK and NF-kappaB in human neutrophils. J Immunol 2004;173:6433–9. [PubMed: 15528384]
45. Beck G, Yard BA, Schulte J, Haak M, van Ackern K, van der Woude FJ, et al. Secreted phospholipases A2 induce the expression of chemokines in microvascular endothelium. Biochem Biophys Res Commun 2003;300:731–7. [PubMed: 12507511]
46. Hohlfeld JM. The role of surfactant in asthma. Respir Res 2002;3:4. [PubMed: 11806839]
47. Graler MH, Goetzl EJ. Lysophospholipids and their G protein-coupled receptors in inflammation and immunity. Biochim Biophys Acta 2002;1582:168–74. [PubMed: 12069825]
48. Koduri RS, Gronroos JO, Laine VJ, Le Calvez C, Lambeau G, Nevalainen TJ, et al. Bactericidal properties of human and murine groups I, II, V, X, and XII secreted phospholipases A2. J Biol Chem 2002;277:5849–57. [PubMed: 11694541]
49. Piris-Gimenez A, Paya M, Lambeau G, Chignard M, Mock M, Touqui L, et al. In vivo protective role of human group IIa phospholipase A2 against experimental anthrax. J Immunol 2005;175:6786–91. [PubMed: 16272335]
50. Fenard D, Lambeau G, Valentin E, Lefebvre JC, Lazdunski M, Doglio A. Secreted phospholipases A2, a new class of HIV inhibitors that block virus entry into host cells. J Clin Invest 1999;104:611–8. [PubMed: 10487775]

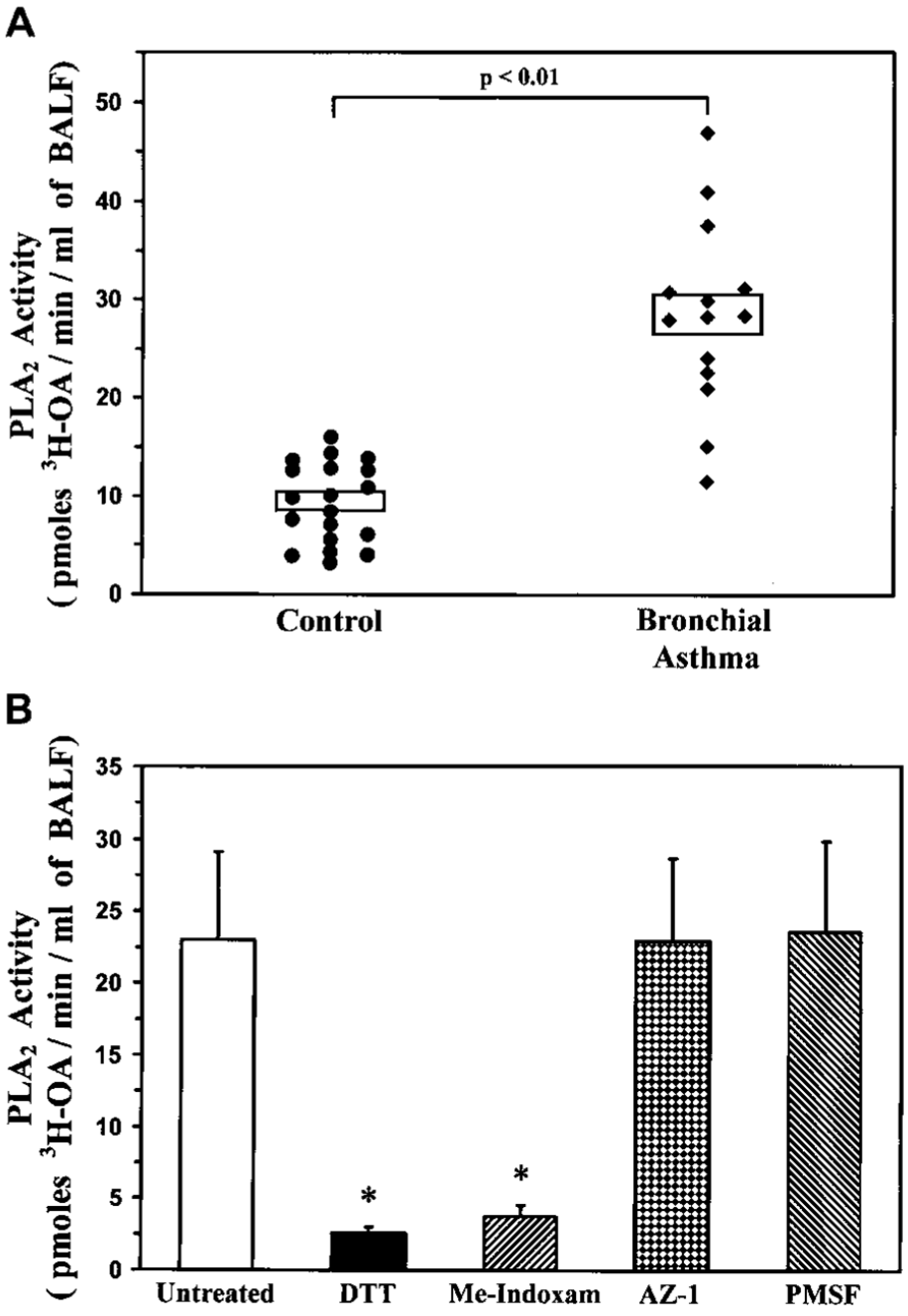

FIG 1.

Characterization of PLA_2 activity in human BALF. **A**, BALF from control subjects (n = 19) and asthmatic patients (n = 14) was assayed for PLA_2 activity, as described in the Methods section. *Boxes* indicate means ± SEs. **B**, BALF was preincubated (for 1 hour at 37°C) with 10 mmol/L DTT, 10 μmol/L Me-Indoxam, 10 μmol/L AZ-1, or 2 mmol/L PMSF before the PLA_2 activity assay. Data are presented as means ± SEs of BALF from 4 asthmatic patients. *$P < .01$ versus untreated.

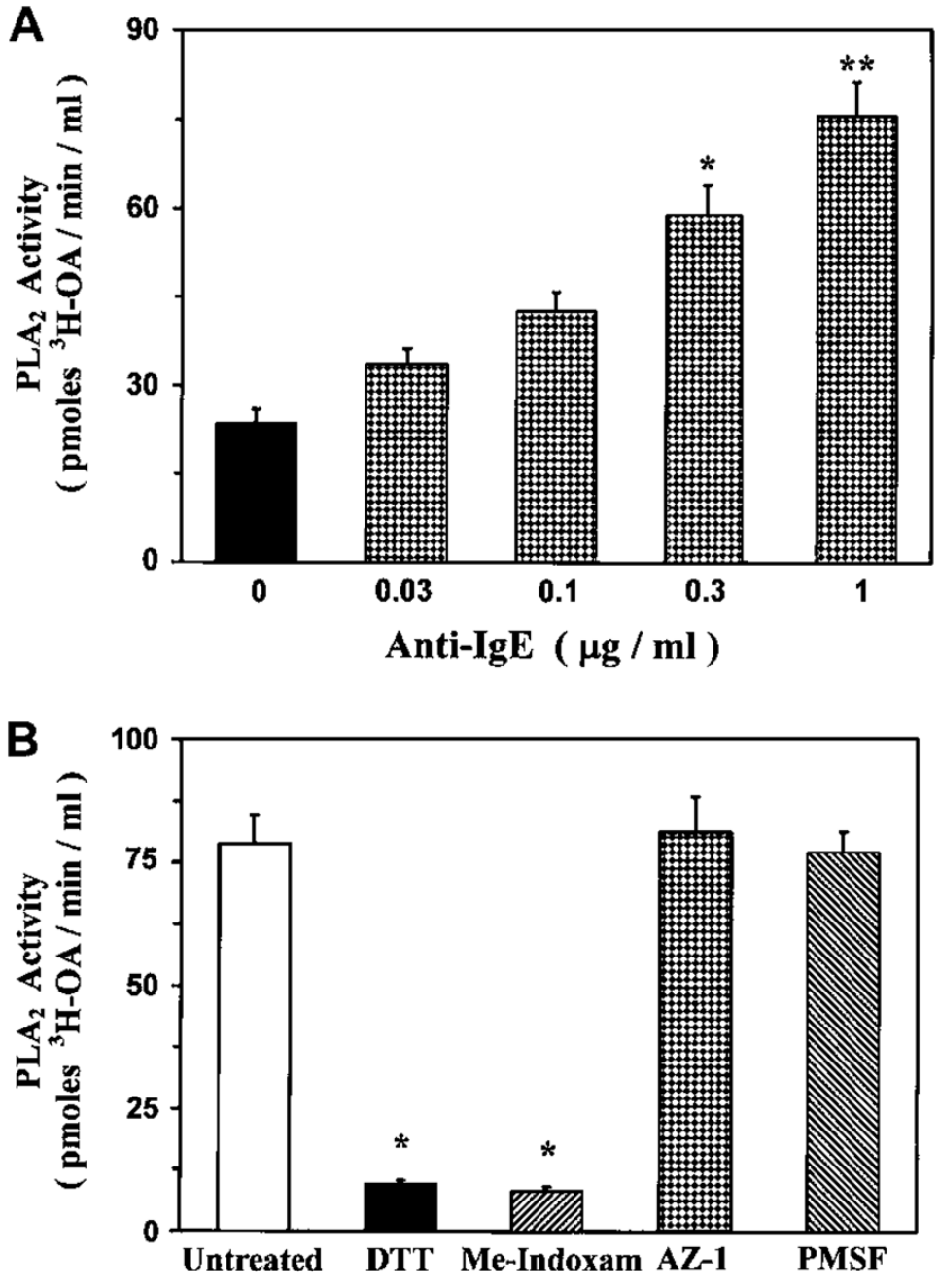

FIG 2.

Characterization of PLA_2 activity in supernatants of HLMCs. **A**, HLMCs were incubated (for 30 minutes at 37°C) with anti-IgE. PLA_2 activity was determined in supernatants, as described in the Methods section. Data are presented as means ± SEs of 4 experiments. **P* < .05 versus unstimulated; ***P* < .01 versus unstimulated. **B**, Supernatants of anti-IgE–stimulated HLMCs were preincubated (for 1 hour at 37°C) with 10 mmol/L DTT, 10 μmol/L Me-Indoxam, 10 μmol/L AZ-1, or 2 mmol/L PMSF before the PLA_2 activity assay. Data are presented as means ± SEs of 4 experiments. * *P* < .01 versus untreated.

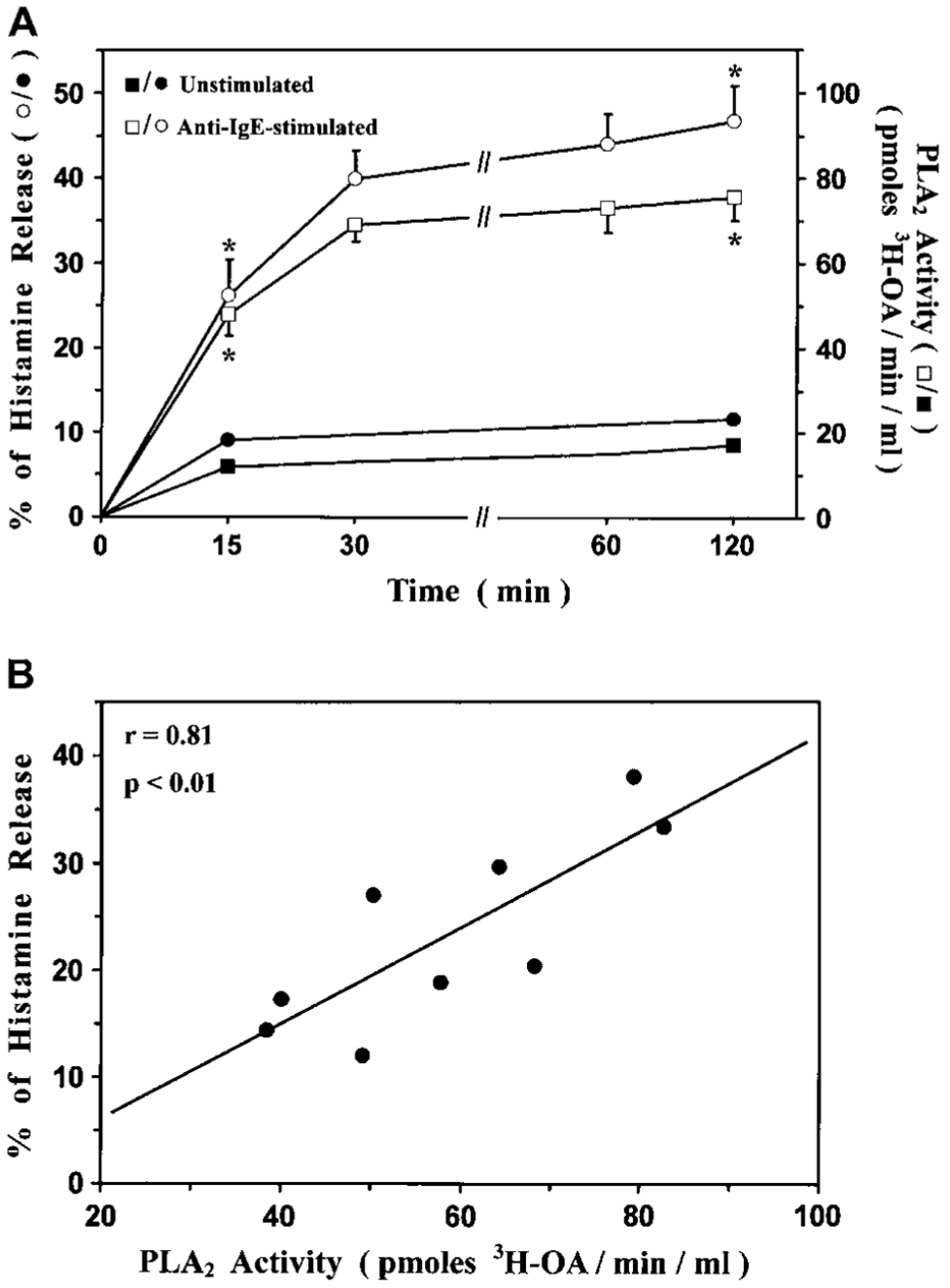

FIG 3.

Release of sPLA$_2$s and histamine from anti-IgE–stimulated HLMCs. **A**, HLMCs were incubated with or without anti-IgE (1 μg/mL). Histamine release and PLA$_2$ activity were determined in supernatants, as described in the Methods section. Data are presented as means ± SEs of 4 experiments. *P < .01 versus unstimulated. **B**, HLMCs were incubated (for 30 minutes at 37°C) with anti-IgE (1 μg/mL). Histamine release and PLA$_2$ activity were determined as described above. Correlation between histamine release and sPLA$_2$ activity was assessed by using the linear regression function of Microsoft Excel.

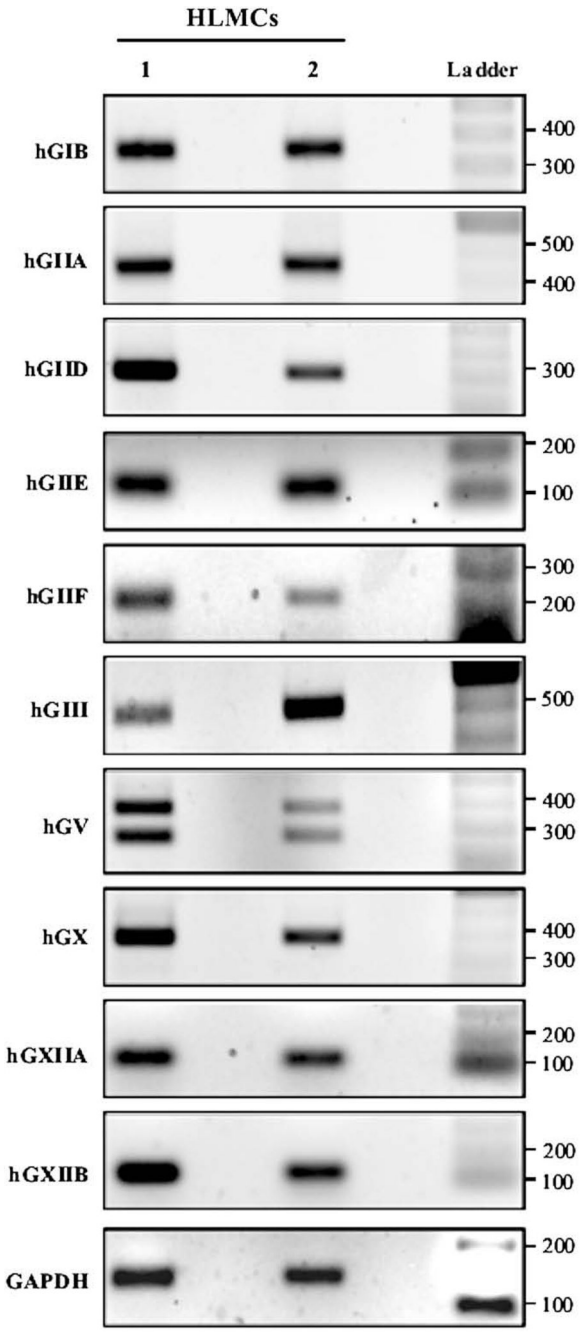

FIG 4.

Expression of sPLA$_2$s in HLMCs. HLMCs from 2 different lung preparations were lysed, and total RNA was extracted. Expression of sPLA$_2$s was evaluated by means of semiquantitative PCR, as described in the Methods section. RT-PCR amplification products were separated on 2% agarose gel, stained with ethidium bromide, and photographed.

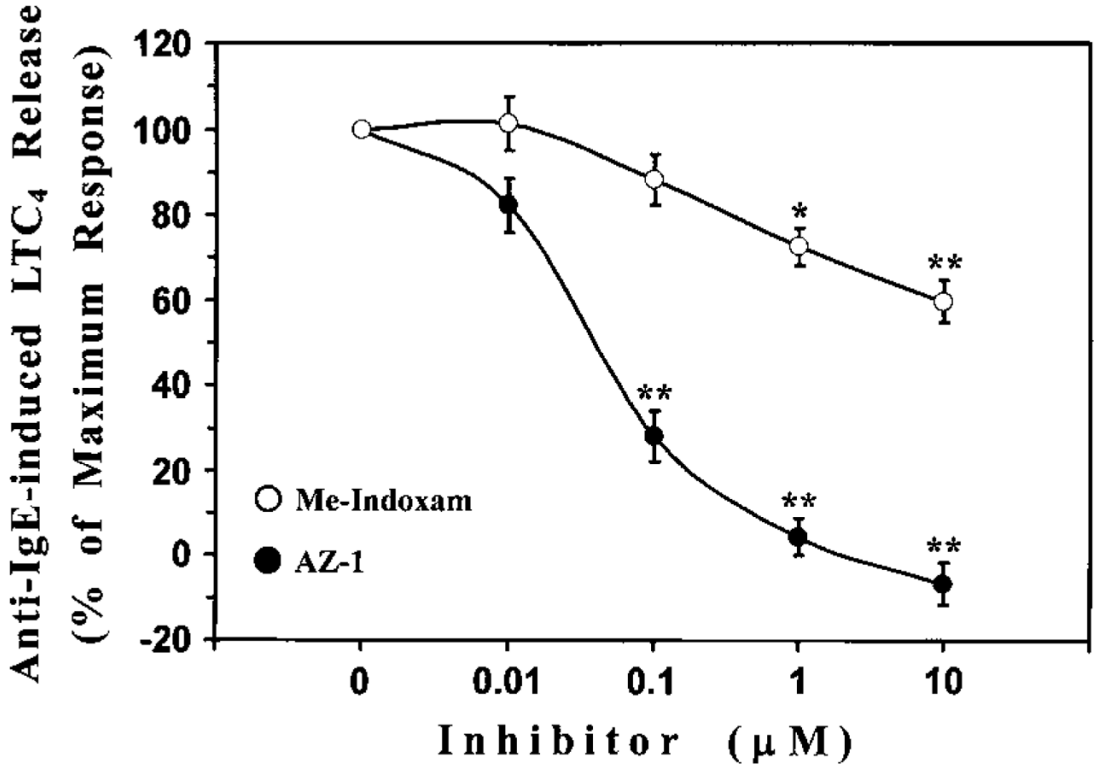

FIG 5.

Effect of $sPLA_2$ and $cPLA_2$ inhibitors on LTC_4 production from anti-IgE–stimulated HLMCs. The cells were preincubated (for 15 minutes at 37°C) with increasing concentrations of Me-Indoxam or AZ-1 before stimulation (for 30 minutes at 37°C) with anti-IgE (1 μg/mL). LTC_4 production was determined in supernatants by using an enzyme immunoassay. Inhibition of LTC_4 production was expressed as a percentage of maximum response. Data are presented as means ± SEs of 4 experiments. **P* < .05 versus anti-IgE; ***P* < .01 versus anti-IgE.

TABLE I

Expression of $sPLA_2$s in human lung mast cells

$sPLA_2$s	mRNA expression (ΔCt)*		*P* value
	Unstimulated†	Anti-IgE†	
GIIA	15.73 ± 1.11	16.34 ± 1.17	.179
GIID	9.36 ± 1.03	9.90 ± 0.47	.225
GIIE	12.06 ± 0.94	13.46 ± 0.64	.155
GIIF	9.09 ± 0.96	9.36 ± 0.54	.322
GIII	4.84 ± 0.38	5.29 ± 0.29	.222
GV	6.81 ± 0.78	7.54 ± 0.99	.211
GX	7.43 ± 1.03	7.99 ± 1.28	.119

*mRNA expression is based on qPCR, and data are expressed as ΔCt (see the Methods section in this article's Online Repository). A ΔCt of less than 10 means high to medium expression, a ΔCt of 10 to 15 means medium to low expression, and a ΔCt of greater than 15 means low expression. Data are presented as the means ± SEs of 3 different donors. *P* values were determined by using the Student paired *t* test.

†The cells were incubated (at 37°C for 3 hours) in the absence (unstimulated) or presence of anti-IgE (1 μg/mL). At the end of incubation, mRNA expression of $sPLA_2$s was evaluated as described above.