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Objective— Although lipoprotein-associated phospholipase A_2 (Lp-PLA_2) has received recent attention as a biomarker of inflammation and risk for acute coronary events, its relative expression in coronary plaque phenotypes, including unstable lesions, has not been established.

Methods and Results— Coronary segments (n=30) were prospectively collected from 25 sudden coronary death patients for immunolocalization of Lp-PLA_2. Lesion morphologies were classified as pathologic intimal thickening, fibroatheromas, thin-cap fibroatheromas (fibrous cap thicknesses <65 μm), and rupture. The expression of Lp-PLA_2 was detected using a specific monoclonal antibody. Apoptosis was identified by DNA end-labeling using terminal deoxynucleotidyl transferase (TdT). Lp-PLA_2 staining in early plaques was absent or minimally detected. In contrast, thin-cap fibroatheromas and ruptured plaques showed intense Lp-PLA_2 expression within necrotic cores and surrounding macrophages including those in the fibrous cap. The degree of macrophage apoptosis was greater in thin-cap fibroatheroma and ruptures compared with less advanced plaques with additional double labeling studies showing Lp-PLA_2 present in apoptotic cells in regions of high macrophage density.

Conclusions—Lp-PLA_2 is strongly expressed within the necrotic core and surrounding macrophages of vulnerable and ruptured plaques, with relatively weak staining in less advanced lesions. These findings together with the association of Lp-PLA_2 in apoptotic macrophages suggest a potential role in promoting plaque instability. (***Arterioscler Thromb Vasc Biol*. 2006;26:2523-2529.)**

Key Words: lipoprotein-associated phospholipase A_2 ■ sudden coronary death ■ plaque rupture ■ apoptosis ■ cardiovascular risk

The natural history of atherosclerosis in humans is a dynamic process involving the progression of early lesions to more complex plaques that are responsible for the majority of acute ischemic coronary and stroke events. Throughout lesion progression, there are transitional plaque phenotypes ranging from early lipid pools to those characterized by a dense fibrous cap of connective tissue and a strong collagen matrix overlying a core of lipids and necrotic debris, and ultimately, to plaques with large necrotic cores and thin fibrous caps invaded by macrophages, referred to as thin-cap fibroatheromas (TCFAs).[1] TCFAs are characterized by a thin fibrous cap (<65 μm), a large necrotic core, an abundance of macrophages, and limited luminal narrowing.[2] It is widely held that the instability of the TCFA gives rise to the main clinical complications associated with rupture and thrombosis; however, there are important morphological differences between TCFA and ruptured plaques.[2] Ruptured plaques demonstrate even thinner fibrous caps (23 ± 19 μm), larger necrotic cores, and greater macrophage infiltrates compared with TCFAs.[2] In this context a better understanding of the biology of rupture-prone plaques has the potential to reduce the morbidity and mortality associated with atherothrombotic disease.

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Inflammation plays a primary role in the progression of human atheroma based on the local and systemic inflammatory responses observed throughout the spectrum of atherosclerotic disease—from initial lesion formation to plaque destabilization and rupture.[3] The key role of inflammation in atherosclerosis also is evidenced by numerous epidemiology studies indicating an association between inflammatory markers (eg, C-reactive protein [CRP], interleukin [IL]-6) and risk of future cardiovascular events. However, considerably less is known about whether the various inflammatory markers represent similar pathophysiologic processes or

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From CVPath Institute, Inc (F.D.K., A.P.B., K.S.S., E.L., R.K., R.V.), Gaithersburg, Md; and the Department of Pathology (A.T.M.), Georgetown University, Washington, DC.

Correspondence to Renu Virmani, MD, Medical Director, CVPath Institute Inc, 19 Firstfield Road, Gaithersburg, MD 20878. E-mail rvirmani@cvpath.org

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unique biological events, and whether they are causally involved in the atherosclerotic process, or merely a marker that correlates with disease progression. In the absence of a convincing animal model of human plaque rupture, studying the expression of inflammatory markers in various plaque morphologies may help focus future research efforts on specific inflammatory markers of greater relevance to clinical events, rather than atherosclerosis per se.

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is a novel inflammatory marker that has been the recent focus of several epidemiology studies involving populations with varying degrees of baseline risk (ie, primary and secondary prevention populations). Most,[4–14] but not all,[15] studies have indicated that Lp-PLA_2 measured in plasma predicts future cardiovascular events, and the association appears to be independent of traditional cardiovascular risk factors and various other novel inflammatory markers. Previous work using combined in situ hybridization and immunocytochemistry, detected Lp-PLA_2 mRNA and protein in macrophages in both human and rabbit noncoronary atherosclerotic lesions. Reverse transcriptase–polymerase chain reaction (RT-PCR) analysis indicated an increased expression of Lp-PLA_2 mRNA in human atherosclerotic lesions.[16] As the majority of events related to CV mortality and morbidity are accompanied by the changes in coronary plaque biology, the purpose of the present study was to explore the localization and distribution of Lp-PLA_2 protein in human coronary atheroma.

Materials and Methods

Case Selection

Hearts of patients who had died suddenly of coronary causes were obtained as described previously.[17] Cases were identified by the presence of an intracoronary thrombus or at least one major epicardial coronary artery with >75% cross-sectional area luminal narrowing, in the absence of a noncoronary cause of death. Sudden deaths were classified by the presumed mechanism of death: acute thrombus attributable to acute plaque rupture, stable plaque (≥75% cross-sectional area luminal narrowing) with healed infarct in the absence of an acute thrombus, and stable plaque without evidence of infarction. In most cases, major epicardial arteries were serially sectioned at 3- to 4-mm intervals, and all segments with ≥50% cross-sectional luminal narrowing were processed for histology. In a limited number of cases, a complete sampling of epicardial coronary arteries was performed, irrespective of the severity of cross-sectional luminal narrowing.

A dataset of 72 hearts to include 488 paraffin sections was available with measurements of vessel size, luminal stenosis, necrotic core area, and macrophage density; parts of this data were published previously.[18] Immunohistochemical assessment of Lp-PLA_2 was performed in 25 prospective cases where 60 frozen (OCT embedded) coronary segments were available. Of these, 30 coronary segments were chosen to match the distribution of the various parameters of the larger sampling of paraffin-embedded tissues. Only cryosections were selected for detection of Lp-PLA_2 study because standard techniques of antigen retrieval failed to produce consistent results in paraffin sections.

Classification of Lesions

A simplified scheme developed by our laboratory modified from the current American Heart Association recommendations was used to classify atherosclerotic lesions.[1] Coronary plaques were characterized as pathologic intimal thickening, fibroatheromas, thin-cap fibroatheromas, and plaque rupture.

Histological Preparation

Coronary segments at 3- to 4-mm thickness were either fixed in 10% neutral buffered formalin or frozen for cryosectioning. Tissue sections were cut at 6-μm, mounted on charged slides, and stained with hematoxylin and eosin and by the modified Movat pentachrome method. Unstained cyrosections for immunohistochemical markers were cut and stored at −80°C until use.

Immunohistochemistry

Serial cryostat sections were thawed, fixed in 10% neutral buffered formalin, air-dried, and stained with antibodies specific for smooth muscle α-actin (clone 1A4, Sigma), macrophage marker CD68 (KP-1 clone, Dako, Carpinteria, Calif), and the endothelial marker (von Willebrand factor [vWF]). Immunohistochemical detection of Lp-PLA_2 was performed using a monoclonal antibody clone 4B4 (diaDexus, South San Francisco, Calif). The preparation and specificity of the anti–Lp-PLA_2 antibody has been described in detail elsewhere.[19] All primary antibodies were labeled with a biotinylated link antibody directed against mouse antigen with the use of a peroxidase-based kit (LSAB, Dako) and visualized with the use of a 3-amino-9-ethylcarbazole substrate; the sections were counterstained with Gill hematoxylin (Sigma-Aldrich). Omission of a primary antibody served as a specificity control.

Measurement of Apoptosis

Apoptotic nuclei were visualized by in situ end labeling (ISEL) DNA fragmentation staining using terminal deoxyribonucleotide transferase (TdT)-mediated nick *end-labeling* (TACS; Trevigen, Gaithersburg, Md) according to previously published methods.[20] Only positive nuclei with morphological features of apoptotic cell death (cell shrinkage, aggregation of chromatin into dense masses, and cell fragmentation) were counted. Overall apoptosis data for coronary lesions were obtained by counting total and ISEL-positive nuclei in macrophages ($CD\text{-}68^+$)/Lp-PLA_2-rich areas in 3 different high power fields (×400 magnification) and related to the total cell number.

Apoptosis and Lp-PLA_2 Expression

Colocalization of apoptosis in cells expressing Lp-PLA_2 was assessed by combined ISEL and immunohistochemistry. Tissue sections were initially stained for DNA fragmentation (as described above) substituting diaminobenzidine (DAB) as the chromogenic substrate with enhancement by nickel salts (brown-black reaction product). Immunostaining of Lp-PLA_2 was visualized with a red streptavidin-alkaline phosphatase substrate (Vector, Burlingame, Calif); slides were counterstained with methyl green.

Evaluation of Coronary Lesions

Morphometric measurements of coronary sections were performed using image-processing software (IPLabs, Scanalytics, Rockville, Md) on slides stained with Movat Pentachrome. Quantitative planimetery included areas analysis of the internal elastic lamina (IEL), lumen, and necrotic core size. The percent stenosis was derived from the formula (1-lumen area/IEL area) × 100. In cases with acute plaque rupture, the area of the thrombus was not included for the calculation of percent stenosis. Computer-assisted color image analysis segmentation with background correction was used to quantify immunohistochemical stains of macrophages, smooth muscle cells, and Lp-PLA_2 within regions of interest. The percentage of positive staining as a function of total plaque area was determined.

Statistical Analysis

Values are expressed as mean±SD. Mean variables between the various stents were compared with the one-way analysis of variance (ANOVA) (JMP software, Cary, NC) followed by all pairs Tukey-HSD test for all differences among means. A value of $P \leq 0.05$ was considered statistically significant.

TABLE 1. Patient Demographics by Data Subsets

Paraffin Sections	Cryosections for Lp-PLA$_2$
Total unique cases (n=72)	Total unique cases (n=25)
Total sections (n=488)	Total sections (n=30)
Pathologic intimal thickening (n=125)	Pathologic intimal thickening (n=7)
Fibroatheromas (n=262)	Fibroatheromas (n=8)
Thin-cap fibroatheromas (n=46)	Thin-cap fibroatheromas (n=8)
Plaque Ruptures (n=55)	Plaque Ruptures (n=7)
Males=64	Male=21
Females=8	Female=4
Mean Patient Age=53.3±11.9	Mean Patient Age=46.7±7.8

The values in parentheses correspond to the number of coronary sections. Of over 350 formalin-fixed hearts from our files, a dataset with 72 patients (488 sections) was available with measurements of vessel size, luminal stenosis, necrotic core areas, and macrophages density. Parts of this data were published previously (Kolodgie et al, *N Engl J Med* 2003;349:231). For immunohistochemical assessment of lipoprotein-associated phospholipase A_2 (Lp-PLA$_2$), an additional 25 cases were prospectively collected to include 30 frozen (OCT embedded) coronary segments with representative morphologies of various lesion types.

Results

Histomorphometric Analysis

A total of 72 sudden coronary death cases (mean age 53±12 years) with formalin-fixed coronary arteries 488 segments with varying lesions were studied (Table 1). Sudden death secondary to plaque rupture and thrombosis occurred in 55 cases (76%) with the remaining cases (24%) associated with severe luminal narrowing. A summary of histomorphometric measurements including IEL, percent stenosis, and macrophage density in various lesion morphologies is shown in Table 2. Coronary artery cross-sectional area was significantly larger in thin-cap fibroatheromas and acute ruptures relative to lesions with pathologic intimal thickening and fibroatheromas, which were comparable in size. Similarly there were no differences in overall lesion size between plaques with thin caps and ruptures. Together with the change in positive remodeling, both thin-cap fibroatheromas and acute ruptures showed greater percent stenosis, necrotic core area, and overall percentage of lesional macrophages when compared with lesions with pathological intimal thickening and fibroatheromas with macrophage densities highest in ruptured plaques.

An additional 25 cases with 30 OCT embedded coronary segments were prospectively collected for immunolocalization of Lp-PLA$_2$. The mean patient age was 48.0±10.2 years with 22 men and 5 women (Table 1). The cause of death was attributed to acute plaque rupture in 7 cases with stable plaque the presumed mechanism of death in the remaining 20 patients. The selected lesion morphologies included pathologic intimal thickening (n=7), fibroatheroma (n=8), thin-cap fibroatheroma (n=8), and plaque rupture (n=7). As shown in Tables 2 and 3, the smaller subset of lesions selected for Lp-PLA$_2$ staining from 25 patients was comparable with that of the larger 72 patient series based on consistent histomorphometric data.

The expression of Lp-PLA$_2$ was relatively low or absent in early lesions defined as pathological intimal thickening (Table 4 and Figure 1). In particular, lesions of pathologic intimal thickening showed little Lp-PLA$_2$ within lipid pools with only occasional staining of surrounding macrophages. Similarly, Lp-PLA$_2$ staining of fibroatheromas generally showed weak staining of necrotic cores and surrounding macrophages such that the percentage of plaque staining for Lp-PLA$_2$ was <3%. In contrast, thin-cap fibroatheromas and ruptured plaques showed intense Lp-PLA$_2$ expression within the necrotic core and surrounding macrophages including those in the fibrous cap (Table 4 and Figure 1). Further, the percentage of plaque positively stained for Lp-PLA$_2$ was significantly greater in lesions with rupture (15.3±11.1%) compared with other lesion morphologies (TFCA=7.1±4.2%, FA=1.7±3.1%, and PIT=0.3±0.5%; $P<0.002$). Although the majority of Lp-PLA$_2$ reactivity was found in macrophages, occasional smooth muscle cells also showed positive staining while endothelial cells were generally negative.

In situ end labeling (ISEL) as a marker of apoptotic cell death was present to a greater degree in lesions classified as thin-cap fibroatheroma or ruptured plaques compared with fibroatheromas or arteries with pathologic intimal thickening (Table 3 and Figure 2). Quantitative analysis of cell types

TABLE 2. Morphometric Assessment of Vessel Area, Stenosis, Necrotic Core Size, and Macrophages Density in a Large Series of Paraffin-Embedded Human Coronary Sections (n=488) From Sudden Coronary Death Patients

Plaque Type	IEL Area (mm^2)	Stenosis (%)	Necrotic Core Area (%)	Macrophage (%CD68)
Pathologic intimal thickening (n=125)	6.5±4.0	43.0±16.1	0.1±0.4	0.1±0.2
Fibroatheroma (n=262)	9.2±4.9	64.5±17.8	11.2±13.2	1.1±1.5
Thin-cap fibroatheroma (n=46)	12.8±7.9	67.0±15.5	21.6±23.7	2.0±1.9
Plaque rupture (n=55)	13.2±6.4	79.8±14.4	29.0±19.0	5.3±5.4
P value	<0.0001**	<0.0001*	<0.0001***	<0.0001*

Values correspond to the means±SD. The parentheses show the number of sections. Significant differences between plaque types by Tukey-Kramer statistical analysis.

*Significant difference between all group comparisons with the exception of thin-cap fibroatheroma vs fibroatheroma.

**Significant difference between all group comparisons with the exception of thin-cap fibroatheroma vs rupture.

***Significant difference between all group comparisons.

TABLE 3. Morphometric Assessment of Vessel Area, Stenosis, Necrotic Core Size, and Macrophages Density in a Series of Human Coronary Sections (n=30) Embedded in OCT From 25 Sudden Coronary Death Patients Selected for Immunohistochemical Detection of Lipoprotein-Associated Phospholipase A_2 (Lp-PLA_2).

Plaque Type	IEL Area (mm^2)	Stenosis (%)	Necrotic Core Area (%)	Macrophage (%CD68)
pathologic intimal thickening (n=7)	10.4±2.5	45.8±18.8	0.00	3.1±3.2
fibroatheroma (n=8)	10.0±4.4	70.8±14.7	14.5±8.6	7.4±5.4
Thin-cap fibroatheroma (n=8)	9.0±1.7	82.4±8.8	32.1±18.3	8.2±4.1
Plaque rupture (n=7)	12.6±3.6	84.6±7.5	36.5±14.0	11.4±3.7
P value	ns	<0.0001*	<0.0001**	0.01***

Values correspond to the means±SD. The parentheses show the number of sections. Significant differences between plaque types by Tukey-Kramer statistical analysis. ns indicates no significance

*Significant difference between pathologic intimal thickening and all other plaque types.

**Significant difference between pathologic intimal thickening and fibroatheroma vs rupture and thin cap fibroatheroma.

***Significant difference between rupture and pathologic intimal thickening.

undergoing apoptosis revealed an increased susceptibility of macrophages to apoptosis, whereas detection of apoptotic nuclei in smooth muscle cells was generally low in all lesions types; occasional cells were negative for both markers. Apoptotic indices were significantly greater in plaque ruptures (30.6±15.7%) compared with lipid pool lesions referred to as pathologic intimal thickening (8.4±4.4, $P<0.02$). Further, double-labeling studies revealed colocalization of Lp-PLA_2 in cells undergoing apoptosis in regions showing a relatively high density of macrophages (Figure 2).

Discussion

The present findings indicate an association of Lp-PLA_2 expression in advanced ruptured and rupture-prone lesions we designate as thin-cap fibroatheromas. Immunostaining of lesions defined as pathologic intimal thickening or fibroatheromas showed only minimal reactivity to Lp-PLA_2 and when present, was mostly localized to the lipid pool or necrotic core, respectively. In contrast, thin-cap fibroatheromas and ruptured plaques showed extensive Lp-PLA_2 accumulation closely associated within the areas of the necrotic core and surrounding macrophages including those in the fibrous cap. Although Lp-PLA_2 was prominent in macrophages of advanced lesion, there was minimal expression in smooth muscle cells. Double staining experiments showed localization of Lp-PLA_2 within apoptotic macrophages suggesting that its products either represent a marker of apoptosis or they might play a causal role in the induction of cell death. Together, these findings suggest that Lp-PLA_2 may be closely linked with the progression and vulnerability of human coronary atheroma. Our findings are an important extension of an earlier study,[16] in which the expression of Lp-PLA_2 (mRNA and protein) was investigated in human aortic lesions. As in the present study, Lp-PLA_2 protein expression was greatest in advanced lesions, and simultaneous in situ hybridization and immunostaining identified macrophages as the primary source of Lp-PLA_2 secretion within these plaques.

The biologic role of Lp-PLA_2 in human atherosclerosis has not yet been definitively established, which is attributable, in part, to the proposed differences in atherogenic potential between the substrate and products of the enzymatic activity of this phospholipase. Initial reports ascribed atheroprotective characteristics to Lp-PLA_2, proposing that the enzyme is involved in hydrolyzing, and thereby inactivating, noxious polar phospholipids (ie, oxidized phosphatidylcholines in

TABLE 4. Lipoprotein-Associated Phospholipase A_2 (Lp-PLA_2), Apoptotic Cell Death by Cell Type, and Cell Density in Macrophage and Smooth Muscle Cell (SMC) Rich Regions

		Apoptosis (%)†		Cell Density ($cells/mm^2$)†	
Plaque Type	Lp-PLA_2 (%)	Macrophages	SMC's	Macrophages	SMC's
Pathologic intimal thickening (n=7)	0.4±0.5	8.4±4.4	1.0±1.3	1005±374	858±286
Fibroatheroma (n=8)	2.5±2.6	12.9±15.9	1.4±2.5	1247±493	1114±376
Thin-cap fibroatheroma (n=8)	11.8±5.4	22.7±8.4	1.5±1.3	1661±495	1183±257
Plaque rupture (n=7)	22.9±13.8	30.6±15.7	1.6±0.9	1796±430	1074±355
P value	0.0001*	0.02**	ns	0.03**	ns

Values correspond to the means±SD. The parentheses show the number of sections. Significant differences between plaque types by Tukey-Kramer statistical analysis. ns indicates no significance

*Significant difference between rupture vs thin cap fibroatheroma, fibroatheroma, and pathologic intimal thickening.

**Significant difference between rupture and pathologic intimal thickening.

†Apoptosis and cell density measurements were performed in the same regions.

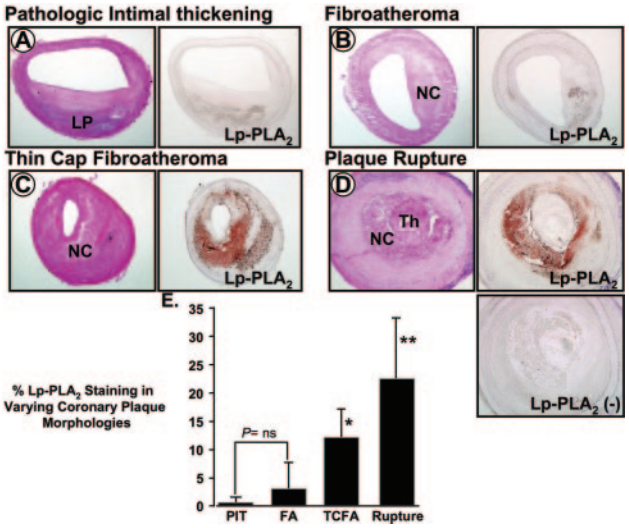

Figure 1. Serial cryostat sections showing lipoprotein-associated phospholipase A_2 (Lp-PLA_2) protein expression in varying human coronary plaques morphologies to include pathologic intimal thickening (PIT), fibroatheroma (FA), thin-cap fibroatheroma (TCFA), and rupture (panels A to D). The adjoining hematoxylin & eosin stained sections are also shown. The most intense Lp-PLA_2 staining was found within the necrotic core and adjoining macrophage rich areas of the plaque and was highly expressed in thin-cap fibroatheroma and ruptured lesions; Lp-PLA_2 (−)=negative control for Lp-PLA_2 staining after omission of the primary antibody. E, Bar graph of the % Lp-PLA_2 stained plaque area in various plaque morphologies; note highest intensity was observed in plaque ruptures. *$P<0.05$ vs FA or PIT; ** vs TCFA, FA, and PIT. LP indicates lipid pool; NC, necrotic core; Th, thrombus,

modified LDL) that may be responsible for promoting inflammation and atherosclerosis.[21–23] More recently, it has been proposed that the hydrolysis of polar phospholipids by Lp-PLA_2 generates large amounts of two downstream inflammatory mediators—lysophosphatidylcholine (lysoPC) and oxidized fatty acids—which elicit proinflammatory responses (ie, activation) from various cell types involved in atherosclerosis.[24] The specific mechanism that predominates in human atherosclerosis is not fully known; however, the findings from the present study add to accumulating epidemiology data supporting a proatherogenic role for Lp-PLA_2.

Recently, there has been considerable interest in identifying new cardiovascular risk markers to help refine risk assessment and guide treatment decisions prior to those subjects experiencing ischemia or necrosis from acute coronary syndromes. An interesting question that requires additional research is whether differences exist between specific markers of plaque vulnerability and general markers of systemic inflammation. Although subtle, the differences may lie in the biologic plausibility (ie, causally involved) and specificity to the vascular inflammatory processes (versus systemic response). Examples of biomarkers with specificity toward vulnerable plaque include CRP, matrix metalloproteinases (MMPs), zinc peptidases (eg, pregnancy-associated plasma protein A [PAPP-A]), CD40 ligand, and myeloperoxidase. CD40 signaling, MMP-9, myeloperoxidase, and PAPP-A are plausible markers of plaque vulnerability because of their relationship with macrophages and fibrous cap integrity.[25–29] However, some (eg, myeloperoxidase, soluble CD40 ligand) are not specific to the vascular inflammatory processes. For other risk markers, such as CRP—arguably the most extensively studied of the inflammatory cardiovascular risk markers—its role in plaque vulnerability is less clear. Although numerous studies consistently indicate that adverse cardiovascular outcomes are associated with elevated plasma CRP levels, there is debate as to whether CRP is an active mediator of inflammation and atherosclerosis within the arterial vascular wall.[30] CRP has been detected in macrophages and within the lipid core in advanced coronary atheroma[31–33]; however, whether these findings reflect systemic (ie, liver) production and local uptake versus local production is not clear.[34]

Given the epidemiologic association between plasma Lp-PLA_2 and cardiovascular events in several studies and the findings from the present study, Lp-PLA_2 may be another important marker, and possibly a mediator, of plaque progression and vulnerability. The observation that Lp-PLA_2 staining in this study was most intense in regions that are abundant in lipids and oxidation products (ie, necrotic core) is consistent with the putative role of this enzyme. The expansion of the necrotic core is considered to be an important step in the progression toward plaque vulnerability and may be correlated with macrophage apoptosis. Oxidized LDL (ie, the substrate for Lp-PLA_2 hydrolytic activity) and lysoPC (ie, the product of Lp-PLA_2 hydrolytic activity) both have been associated with proapoptotic effects on macrophages.[35,36] Interestingly, in studies involving cultured human leukocytes, the addition of selective Lp-PLA_2 inhibitors to LDL before copper-induced oxidation decreased lysoPC production, reduced proinflammatory and cytotoxic effects elicited by oxidized LDL.[35,37] Although the precise signaling mechanism(s) capable of exerting proapoptotic effects of lysoPC are unclear, the naturally occurring Fas-FasL induction of caspase-3 cleavage may represent a viable pathway.[38]

In humans, Lp-PLA_2 in circulation is bound predominantly to low-density lipoprotein particles (≈80%), with the rest distributed among high-density lipoprotein and remnant-lipoproteins.[39,40] However, Lp-PLA_2 is also produced de novo within the atheroma by the same inflammatory infiltrate that is considered to be responsible for driving the inflammatory responses (ie, macrophages, T-lymphocytes, etc).[41,42] Regardless of how the enzyme reaches the intima (via LDL or de novo production), its substrates (ie, polar phospholipids) are abundant within the atheroma, either from modified LDL or from apoptotic/necrotic macrophages.[43] In fact, in advanced lesions, the toxic effects of lysoPC on macrophage apoptosis may ultimately result in a feedback cycle of increased lysoPC generation and apoptosis.[44] Another finding suggestive of a causal role for Lp-PLA_2 in atherosclerosis is that fibrous cap thickness is determined, in part, by the extent of macrophage infiltrate within the fibrous cap region. The products of Lp-PLA_2 activity (ie, lysoPC and oxidized fatty acids) are chemoattractants for circulating monocytes, and they are also involved in macrophage activation.[24,36,37] The potential role of macrophages in fibrous cap thickness and necrotic core expansion, together with relationship between macrophages and Lp-PLA_2 expression in the fibrous cap region may

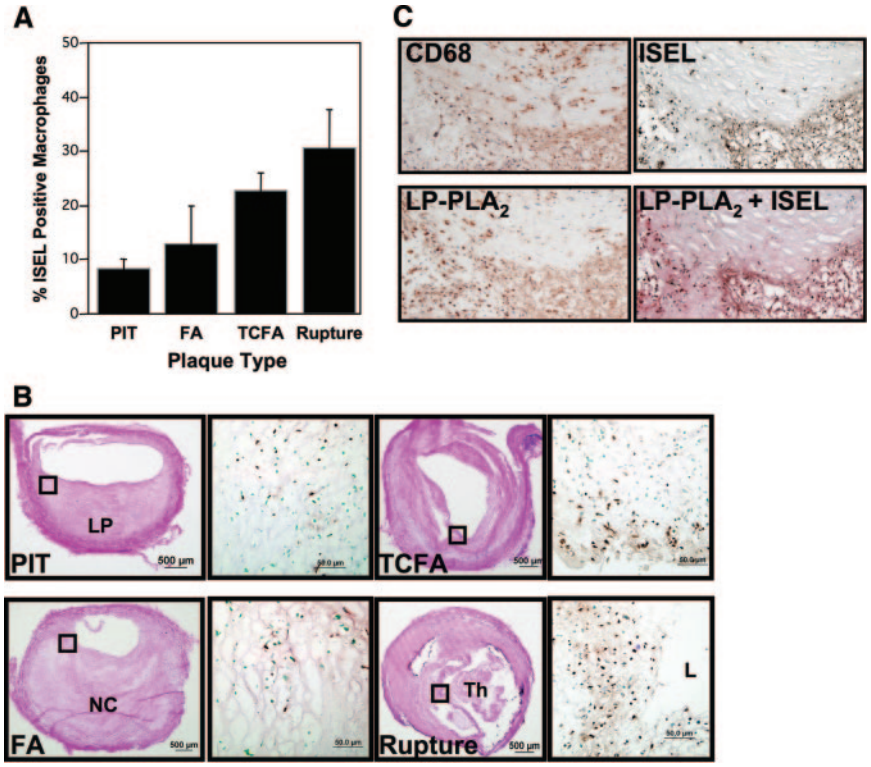

Figure 2. Macrophage apoptosis and colocalization of lipoprotein-associated phospholipase A_2 (Lp-PLA_2) in various human coronary plaque morphologies. A, Macrophage apoptosis based on the percentage of nuclei positive by in situ end labeled (ISEL) was maximal in ruptures followed by thin-cap fibroatheromas (TCFAs) and least in pathologic intimal thickening (PIT). B, Representative serial cryostat sections from various plaque types demonstrating DNA fragmentation staining (brown nuclear reaction); the counterstain is methylgreen. The adjoining hematoxylin & eosin stained sections are also shown. Numerous ISEL-positive nuclei were found in ruptures and TCFAs relative to less advanced plaque types. C, High-resolution images of serial sections stained for macrophages (CD68), ISEL, and Lp-PLA_2. Note: Double labeled sections for Lp-PLA_2 and ISEL shows colocalization in similar areas strongly positive for macrophages. L indictes lumen; LP, lipid pool; NC, necrotic core; Th, thrombus.

indicate that Lp-PLA_2 is involved in plaque vulnerability, particularly in the progression from TCFA to plaque rupture. As such, Lp-PLA_2 hydrolytic activity may represent an important biologic pathway confirming that TCFAs are indeed the precursor lesion to ruptured plaques. Although the findings from the present study are intriguing and suggest biologic plausibility and specificity to vascular inflammation, definitively demonstrating a causal role of Lp-PLA_2 for the progression atherosclerosis and plaque vulnerability will likely require additional research beyond necropsy studies.

In summary, this is the first study to characterize the expression of Lp-PLA_2 protein within human coronary atheroma of various morphologies or phenotypes. Lp-PLA_2 was expressed by macrophages within the fibrous cap region of rupture-prone and ruptured lesions, and Lp-PLA_2 staining colocalized with apoptotic macrophages. In advanced lesions, Lp-PLA_2 staining also was intense in regions abundant in lipids and oxidation products (eg, necrotic core). Based on these findings, Lp-PLA_2 and the products of its enzymatic activity may play an expanded role in promoting plaque instability, and additional studies are warranted to explore the potential causal role for Lp-PLA_2 in plaque progression and vulnerability.

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