Lipoprotein(a) and Inflammation in Human Coronary Atheroma: Association With the Severity of Clinical Presentation

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Objectives. The purpose of this study was the investigation of the in vivo role of lipoprotein(a) [Lp(a)] and inflammatory infiltrates in the human coronary atherosclerotic plaque and their correlation with the clinical syndrome of presentation.

Background. Lipoprotein(a) is an atherogenic and thrombogenic lipoprotein, and has been implicated in the pathogenesis of acute coronary syndromes. Lipoprotein(a) induces monocyte chemoattraction and smooth muscle cell activation in vitro. Macrophage infiltration is considered one of the mechanisms of plaque rupture.

Methods. This study of atherectomy specimens investigated the in vivo role of Lp(a) at different stages of the atherogenic process, and its relationship with macrophage infiltration. We examined coronary atheroma removed from 72 patients with stable or unstable angina. Specimens were stained with antibodies specific for Lp(a), macrophages (KP-1), and smooth muscle cells (alpha-actin). Morphometric analysis was used to quantify the plaque areas occupied by each of the three antigens, and their colocalization.

Results. All specimens had localized Lp(a) staining; the mean fractional area was 58.2%. Ninety percent of the macrophage areas colocalized with Lp(a) positive areas, whereas 31.3% of the smooth muscle cell areas colocalized with Lp(a) positive areas. Patients with unstable angina (n = 46) had specimens with larger mean plaque Lp(a) areas than specimens from stable angina patients (n = 26): 64.4% versus 47.7% (p = 0.004). Unstable angina patients with rest pain (n = 28) had greater mean plaque Lp(a) area than unstable angina patients with crescendo exertional pain (n = 18): 71.1% versus 52.4% (p < 0.001). Mean KP-1 area was 31.2% in unstable rest angina versus 18.3% in stable angina (p = 0.05); alpha-actin area was greater in stable (48.5%) and crescendo exertional angina (48.8%) than in rest angina (30.4%). The strongest correlation between plaque KP-1 and Lp(a) area was in unstable rest angina (r = 0.88, p < 0.001), and between alpha-actin and Lp(a) areas in the crescendo exertional angina (r = 0.62, p < 0.01).

Conclusions. Lipoprotein(a) is ubiquitous in human coronary atheroma. It is detected in larger amounts in tissue from culprit lesions in patients with unstable compared to stable syndromes, and has significant colocalization with plaque macrophages. A correlation of plaque alpha-actin and Lp(a) area suggests a role of Lp(a) in plaque growth.

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Lipoprotein(a) [Lp(a)] is considered an independent risk factor for premature cardiovascular disease (1). Elevated serum lipoprotein Lp(a) levels have been associated with the development of myocardial infarction and the presence and extent of coronary (2–5), carotid (6), peripheral vascular (7)

and saphenous vein aortocoronary bypass graft (8,9) atherosclerosis, and with clinical restenosis after coronary intervention (10). Although the unique structural features of Lp(a) suggest both thrombogenic and atherogenic potential, the precise mechanism of Lp(a) action is still uncertain.

The Lp(a) molecule consists of a low density lipoprotein particle linked to apoprotein(a) [apo(a)]. Apoprotein(a) is a glycoprotein of variable size (11) that shares remarkable structural homology with plasminogen (12–14). Lipoprotein(a) has been implicated in the regulation of plasminogen activator inhibitor-1 expression in endothelial cells (15), and shown to inhibit endothelial cell surface fibrinolysis (16), to attenuate plasminogen binding to platelets (17) and to bind to plaque matrix components (18). Autopsy studies in humans have documented the presence of Lp(a) in aortic and coronary atherosclerotic plaques (19,20) and an apparent colocalization with fibrin(ogen) (21). Lipoprotein(a) has been localized at the site of mural thrombus (20), at the sites of fibrin deposition

> **Abbreviations and Acronyms**
>
> apo(a) = apoprotein(a)
> DCA = directional coronary atherectomy
> Lp(a) = lipoprotein(a)
> TGF-beta = transforming growth factor-beta
> VLDL = very low density lipoprotein

(19–21), and occasionally within tissue factor–positive macrophages in human thoracic aortic wall (19).

Inflammation has been implicated in the pathogenesis of coronary disease. Recruitment of circulating monocytes is an early event in atherosclerosis (22), and enhanced macrophage infiltration in the atherosclerotic plaque has been correlated with acute clinical syndromes of rest angina and non–Q wave myocardial infarction (23). Macrophages have been specifically implicated in the induction of plaque rupture through digestion of fibrous caps (24), and they express tissue factor that is thought to promote local thrombogenicity (25). Plaque macrophages may represent a population of preexisting cells within the plaque in addition to newly attracted and differentiated circulating monocytes in response to a local stimulus (22,26,27). Our recent studies have implicated the apo(a) portion of unoxidized Lp(a) in the induction of monocyte chemotactic activity in human coronary and umbilical vein endothelial cells (28). Oxidized Lp(a) has also been shown to enhance in vitro adhesion of human monocytes to cultured endothelial cells (29). Furthermore, Lp(a) has also been localized in foam cells in human xanthomata (30). These experimental data indicate that Lp(a) may be an attractant of macrophages in the atheromatous plaque. Additionally, previous reports have associated Lp(a) with smooth muscle cell proliferation (31,32) an event that may lead to progressive luminal obstruction.

In this study, we have performed immunohistochemical staining on serial sections of atheroma removed from patients during percutaneous transluminal directional coronary atherectomy (DCA). We quantified and localized the tissue distribution of apo(a), of the KP-1 macrophage membrane antigen, and of alpha-actin, a smooth muscle cell marker. Using computerized morphometric analysis we determined the colocalization of apo(a) with macrophages and smooth muscle cells. We have also examined the relationship between plaque macrophage and Lp(a) areas, and the patient's clinical syndrome of presentation. Our findings indicate that Lp(a) is abundant in human coronary atheroma; it is detected in larger amount in patients with unstable angina as compared to stable angina, and colocalizes with plaque macrophages. A correlation of plaque alpha-actin and Lp(a) area suggests a role of Lp(a) in atherosclerotic plaque growth.

Methods

Patient population. Coronary specimens were evaluated from a total of 126 patients who underwent percutaneous transluminal DCA at Mount Sinai Hospital from June 1993 to December 1994. Inclusion criteria were successful intervention on a culprit de novo lesion, and clinical syndrome of stable angina (Canadian Cardiovascular Society classification [33]), crescendo exertional angina (Braunwald class I [34]) or rest angina within 48 h of the intervention (Braunwald class III [34]). In cases of mixed clinical picture, patients were classified according to the more severe and recent syndrome. Directional coronary atherectomy of vein graft lesions (n = 10), rescue DCA for failed angioplasty (n = 6) and unstable angina patients who had DCA of a nonculprit (n = 12) or restenotic (n = 10) lesion were excluded. Additionally, patients with too small or inappropriately stained specimens (see below) were excluded (n = 16). According to the standard practice at our Catheterization Laboratory, patients undergo angiography and intervention at the same setting, within 48 h of admission.

Atherectomy specimens. All tissue specimens from the culprit lesion were immediately immersed in 10% buffered formalin and routinely processed for paraffin embedding. Five-micrometer sections were serially cut, mounted on lysine-coated slides and stained with hematoxylin and eosin, a trichrome stain, and immunohistochemically as noted below.

Apoprotein(a) antibodies. Polyclonal rabbit antihuman apo(a) antibody was prepared as previously described (35). Rabbits were immunized with purified Lp(a) from a single donor. The immunoglobulin G fraction of the rabbit immune serum was isolated and sequentially absorbed with immobilized low density lipoprotein, lys-plasminogen and fibrinogen. The final antibody preparation had no reactivity against these antigens by Western immunoblotting analysis (36). The murine monoclonal antihuman antibodies, a-6 against kringle IV type 2 of apo(a) (amino-terminal specific) and a-40 (carboxy-terminal specific) were prepared as detailed previously (37).

Immunocytochemistry. Antibody staining was performed on deparaffinized and rehydrated sections. Lipoprotein(a) was detected with the absorbed polyclonal apo(a) antibody at a concentration of 8.1 μg/ml, with the a-6 monoclonal apo(a) antibody at a concentration of 1 μg/ml and the a-40 monoclonal apo(a) antibody at a concentration of 2 μg/ml. Only six randomly selected specimens were stained with the a-40 antibody. Macrophages were identified with a murine monoclonal antihuman CD-68 panmacrophage antibody (KP-1, M814, Dako) at a concentration of 7.6 μg/ml. Smooth muscle cells were identified with an antihuman smooth muscle cell alpha-actin antibody (M851, Dako) at a concentration of 0.1 μg/ml. Sections were washed in phosphate-buffered saline, blocked with H_2O_2 and the appropriate normal serum, incubated with primary antibody and reacted with the appropriate biotin-conjugated secondary antibody, and then with streptavidin conjugated with peroxidase (BioGenex, San Ramon, CA). Peroxidase activity was detected with 3-3′-diaminobenzidine. Sections were dehydrated, coverslipped and examined. Positive control, nonimmune negative and processing control slides were performed for each antigen stain. Each antibody stain was done as a batch using the same reagents, so as to obviate staining differences. Preabsorption of the apo(a) antibodies

yielded negative staining. According to our previous methodology (23), specimens with less than 1.5 mm^2 total tissue area (n = 42) were not stained immunohistochemically; additionally, specimens with poor immunohistochemical staining or missing an antigen stain were also excluded (n = 12). Serially cut tissue sections were immunostained for the assessment of specific colocalization.

Morphometric analysis. Microscopic analyses were conducted at ×100 magnification without knowledge of the clinical syndrome, and independently of the other stains. Total specimen plaque area, and segmental areas occupied by apo(a), KP-1 or alpha-actin positive staining were manually planimetered independently of each other using a microscope drawing tube. Images were digitized using a Sony DKC-5000 camera and an Epson ES-1200C scanner (EU-13, Seico-Epson Corp., Japan), processed and quantified with Adobe Photoshop 4.0 and NIH image 1.57 software, on a PowerMacintosh computer. Total plaque area and the total area stained by apo(a), KP-1 and alpha-actin were calculated. Images of KP-1 and alpha-actin staining were superimposed on Lp(a) images to determine the KP-1/Lp(a) and alpha-actin/Lp(a) overlap areas for each specimen (23).

Statistical analysis. Data were first compared for stable versus unstable angina. However, prior studies of atherectomy specimens in unstable angina had included only rest angina patients (23–27). To analyze our results in accordance with this literature and to clarify differences between subgroups of unstable angina, the data were further divided into three groups according to the patients' clinical syndrome: stable angina, crescendo exertional angina or rest angina. Results are expressed as mean ± SEM of the individual specimen measurements. Analysis of variance with Tukey–Kramer correction (for multiple comparisons) was used for comparisons between the three groups. The significance of the trend in Lp(a) area among the three groups was assessed with regression analysis. A two-tailed Student t test was used for comparison of continuous variables between two groups. Categorical variables were compared with Fisher's exact test. Linear regression analysis was performed for correlation between Lp(a) and KP-1 areas, and between Lp(a) and alpha-actin areas. Multivariate logistic regression analysis was performed with plaque KP-1 area as the independent variable; clinical (age, gender, clinical syndrome) and morphologic [Lp(a) area, alpha-actin area] parameters were included as dependent variables. The statistical software JMP 3.2 (SAS Institute, Cary, NC) was used in a PowerMacintosh computer, and a two-tailed probability p < 0.05 was considered significant.

Results

The studied population (n = 72) included 26 patients with stable angina and 46 patients with unstable angina. Within the unstable angina population, 18 patients had primary crescendo exertional angina (Braunwald class I), and 28 patients had rest angina (Braunwald class III). The patients were 91% male, 18% diabetic, 38% hyperlipidemic and 55% hypertensive, and
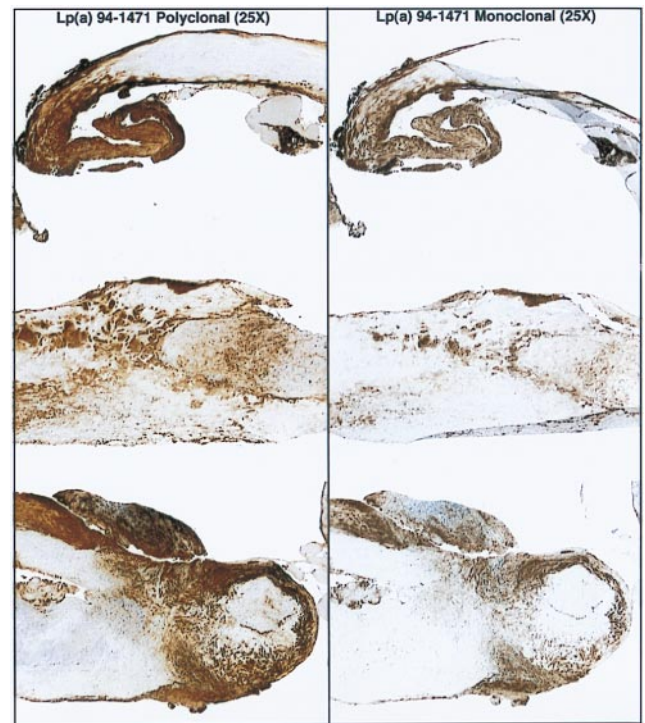

Figure 1. Demonstration of two Lp(a) stains, utilizing the polyclonal apoprotein(a) antibody (**left panel**) and the monoclonal apoprotein(a) antibody a-6 (**right panel**). The positive staining occupies the same plaque areas in both stains, but the polyclonal antibody stains with greater intensity. Lp(a) occupies the majority of the plaque. Original magnification ×40 (brown = peroxidase developed with 3-3′-diaminobenzidine).

had a mean age of 52 ± 1.1 years. The majority of lesions, 55%, were in the left anterior descending coronary artery. There were no differences among the groups with respect to the baseline clinical characteristics, coronary risk factors or the angiographic location of the treated lesions.

All patients had specimens with localized Lp(a) positive staining, 93% of patients had localized KP-1 positive staining and 94% had alpha-actin positive staining. There was no difference in the areas stained with the polyclonal apo(a) antibody or the a-6 monoclonal apo(a) antibody [specific for the amino-terminal of apo(a)] in all plaques examined (Fig. 1). Specimens stained with the a-40 antibody [specific for the carboxy-terminal of apo(a)] showed localized staining in areas that were also positive for the a-6 apo(a) antibody; however, there were certain areas in each specimen with a-6 positive staining that did not stain with the a-40 antibody.

In the entire study population (n = 72), the mean total plaque area was 5.6 ± 0.9 mm^2, the % Lp(a) area 58.2 ± 4.2%, the % KP-1 area 25.1 ± 4.2% and the % alpha-actin area 41.2 ± 5.9%. KP-1 positive areas significantly overlapped with Lp(a) positive areas; there was 90.1 ± 4.2% KP-1/Lp(a) colocalization. On the other hand, alpha-actin positive areas were predominantly detected on Lp(a) negative areas; there was 31.3 ± 6.1% alpha-actin/Lp(a) colocalization. An example is shown in Figure 2.
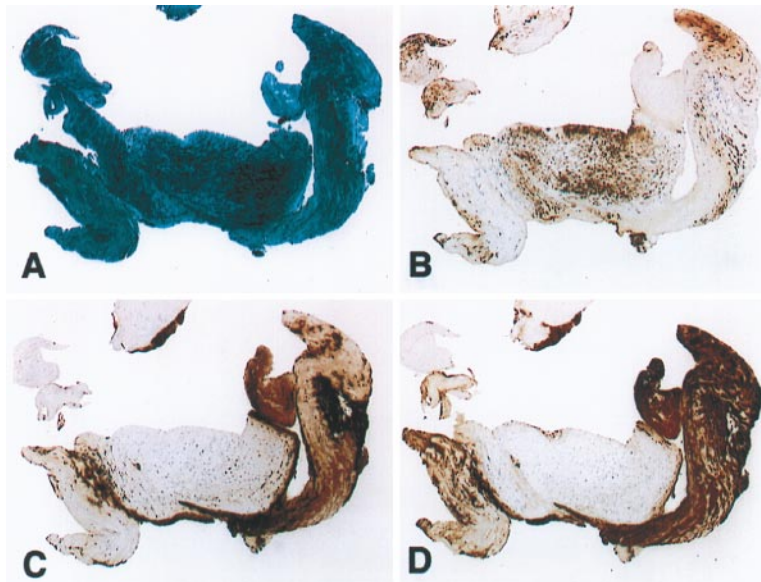
Figure 2. Comparative staining of an atherosclerotic plaque of a rest angina patient: eosin (**A**), alpha-actin (**B**), KP-1 (**C**) and polyclonal apoprotein(a) antibody (**D**). The distribution of Lp(a) and macrophages is nearly identical (**C, D**). Smooth muscle cells occupy a certain portion of the Lp(a) area, but they also localize in Lp(a) negative area.

Plaque Lp(a) area was 64.4 ± 3.5% in unstable angina (n = 46) versus 47.7 ± 4.9% in stable angina (n = 26) patients, p = 0.004. There were trends toward greater macrophage area (28.9 ± 3.9% vs. 18.3 ± 4.5%, p = 0.09) and lower smooth muscle cell area (37.2 ± 4.4% vs. 45.8 ± 6.8%, p = 0.1) in unstable compared to stable angina patients.

The distribution among the three groups (stable angina, crescendo exertional angina, rest angina) of total plaque area and of fractional specimen areas occupied by Lp(a), KP-1, and alpha-actin and their overlap are shown in Table 1. All groups had similar total plaque areas from the retrieved specimens. Lipoprotein(a) positive area was significantly greater with more unstable syndromes (rest angina > crescendo exertional angina > stable angina, p for trend <0.001). Macrophage area was significantly greater in unstable rest angina compared to stable angina, whereas alpha-actin areas were similar among the groups. Additionally, there were no differences among the groups with respect to the Lp(a)/macrophage or Lp(a)/alpha-actin colocalizations.

Irrespective of localization of the staining, there were significant linear correlations (Figure 3) between the plaque areas of Lp(a) and macrophages in all groups: r = 0.88 in unstable rest angina (p < 0.0001) (Fig. 3), r = 0.60 in crescendo exertional angina (p = 0.01) and r = 0.73 in stable angina (p < 0.001). On the other hand, plaque alpha-actin and Lp(a) amounts correlated significantly in the crescendo exer-

tional angina group (r = 0.62, p < 0.001) (Fig. 4), but less so in rest angina (r = 0.37, p = 0.05) or in stable angina (r = 0.24, p = 0.25).

In the multivariate analysis controlling for the clinical syndrome, age, gender, total plaque area and plaque alpha-actin area, the plaque Lp(a) area was the single most powerful correlate of plaque macrophage area (p = 0.01), whereas the clinical syndrome was no longer a significant correlate of the fractional macrophage area.

Discussion

This is the first report to examine human coronary atherectomy specimens for the presence and extent of Lp(a) by morphometric analysis of immunohistologic tissue sections. Morphometric analysis has also been used to quantify the area of the plaque containing macrophage membrane antigen (KP-1) and alpha-actin, a marker of smooth muscle cells. We have quantified the colocalization of these antigens with apo(a), and have correlated these findings with the clinical syndrome observed in the patients from whom the atherectomy specimens were obtained. While Lp(a) has been previously demonstrated in atherosclerotic plaques, this report shows the extent of plaque Lp(a) positive area, its colocalization with macrophages and its relationship with unstable angina. Thus, our results support an important in vivo role of

Table 1. Histopathologic Data

	Total Plaque Area (mm²)	% Lp(a) Positive Area	% KP-1 Positive Area	% Alpha-Actin Positive Area	% KP-1 Lp(a) Overlap	% Alpha-Actin Lp(a) Overlap
Stable angina (n = 26)	5.9 ± 0.8	47.7 ± 4.9*	18.3 ± 4.5†	48.5 ± 6.8‡	87.5 ± 6.0	30.0 ± 6.0
Crescendo exertional angina (n = 18)	5.8 ± 1.1	52.4 ± 5.4*	24.9 ± 7.2	48.8 ± 5.4	87.9 ± 4.4	30.6 ± 5.9
Rest angina (n = 28)	5.1 ± 0.8	71.1 ± 3.4*	31.2 ± 4.7†	30.4 ± 5.8‡	92.2 ± 3.1	34.9 ± 6.6

*p < 0.001 by analysis of variance among the three groups with Tukey–Kramer correction for multiple comparisons; p for trend <0.001. †Greater KP-1 positive area in rest versus table angina (p = 0.05). ‡Lower alpha-actin positive area in rest versus stable angina (p = 0.02). Variables are expressed as mean ± SEM.
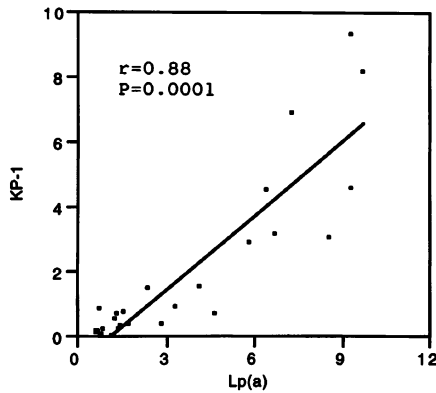
Figure 3. Correlation between Lp(a) and KP-1 in rest angina ($r^2 = 0.77$, p < 0.0001).

Lp(a) in the pathogenesis of atherosclerosis and the acute coronary syndromes, and expand on prior in vitro findings on the potential mechanisms of action of Lp(a) in the atherosclerotic plaque.

In this study, apo(a) antigen was present in atheroma from stable and unstable angina patients. Furthermore, of all the specimens studied, 58.2 ± 4.2% of total plaque area was apo(a) positive, documenting that Lp(a) was a major component of the atherosclerotic plaque in the studied population. A murine monoclonal antibody directed against kringle IV type 2 repeats (a-6) located at the amino-terminal end of apo(a) was used to confirm the specificity of the immunopurified rabbit apo(a) antibody used to document localization of apo(a). Both the monoclonal and the polyclonal apo(a) antibody stained identical areas of the tissue sections (Fig. 1), indicating that the polyclonal anti-apo(a) antibody reacted only with the apo(a) antigen. Recent studies have shown that a variety of inflammatory proteolytic enzymes cleave the apo(a) portion of Lp(a) into carboxy-terminal and amino-terminal domains (38,39). Peripheral blood neutrophils have also been shown to cleave Lp(a) into similar domains (39,40). Fragments from the amino-terminal of apo(a) have been documented in both human urine (41) and plasma (42), indicating that the frag-

Figure 4. Correlation between Lp(a) and alpha-actin in crescendo exertional angina ($r^2 = 0.38$, p < 0.001).
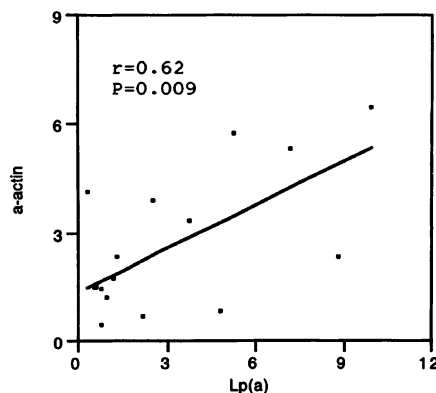
mentation of apo(a) occurs in vivo. Our studies have shown that human neutrophils and peripheral blood monocytes cleave fibrin-bound Lp(a), releasing the amino-terminal portion of apo(a) from the fibrin surface, whereas the carboxy-terminal of apo(a) remained bound to fibrin (40). These observations suggest that proteolysis by inflammatory cells may be responsible for the fragmentation of apo(a) observed in vivo.

The present study documents that the amino-terminal portion of apo(a), as recognized by the monoclonal antibody a-6, colocalized with the plaque areas stained by the polyclonal anti-apo(a), indicating that this portion of the apo(a) remained bound to the plaque, even if cleavage had occurred. The staining of serial tissue sections with the a-40 murine monoclonal antibody, directed against the carboxy-terminal portion of apo(a), colocalized with the a-6 positive staining, suggesting that the apo(a) in the plaque was intact. The intensity of the a-40 staining was weaker than that of the a-6 staining, and there were areas that stained positive with a-6 and negative with a-40. It is not clear whether this was due to hidden epitopes in the carboxy-terminal portion of apo(a) unable to react with the a-40 monoclonal antibody, or whether the apo(a) was cleaved with loss of the carboxy-terminal portion of the molecule.

Lesion histopathology and the clinical syndrome of presentation. We also report for the first time a relationship between the amount of Lp(a) in the atheromatous plaque and the cardiac clinical syndrome. The fractional plaque Lp(a) area was increased in severe unstable (rest) angina compared to crescendo exertional angina and to stable angina (71.1 ± 3.4% > 52.4 ± 5.4% > 47.7 ± 4.9%, p < 0.001). Fractional plaque apo(a) positive area was 58.2 ± 4.2%, greater than the fractional KP-1 positive area (25.1 ± 4.2%). However, 90.1 ± 4.2% of the KP-1 positive staining was localized on apo(a) positive areas, which was significantly different than random colocalization (p < 0.001). The KP-1/apo(a) overlap did not differ significantly with the clinical syndromes. Additionally, apo(a) area, not the clinical syndrome, was the most powerful correlate of the plaque KP-1 area in our multivariate analysis.

The major etiology of acute coronary syndromes is localized thrombus formation upon a disrupted or eroded plaque; however, it is likely that most ruptured plaques heal endogenously, without clinical manifestation, and unstable angina may also develop without plaque disruption or even thrombus formation (27,43). We have previously reported that unstable angina Braunwald class III (i.e., with rest pain within 48 h) is associated with a higher incidence of angiographically complex and thrombotic lesions compared to less severe unstable angina syndromes (44). Within plaques from rest angina patients, macrophages have been shown to accumulate close to the fibrous caps and are thought to promote plaque disruption (24). The mechanism by which macrophages accumulate in the atherosclerotic plaque remains unknown.

According to the "response-to-injury" hypothesis, growth factors and cytokines are secreted from dysfunctional vascular endothelial cells, promoting increased adherence of circulating

monocytes and T lymphocytes to the vessel wall (22). Lipoprotein(a) may induce the accumulation of monocytes/macrophages in the plaque and thereby increase the propensity for plaque rupture. The significant Lp(a)/macrophage colocalization, as well as the strong correlation between plaque Lp(a) and macrophage area, provide support to prior in vitro data that implicated the apo(a) moiety of Lp(a) in monocyte chemoattraction (28). In addition, after plaque rupture has occurred in these patients, the lipid core of the plaque is exposed to flowing blood, and thrombus formation occurs (43). Lipoprotein(a) may enhance local thrombogenicity by the competitive antagonism of apo(a) with plasminogen in cell membrane receptors, thereby inhibiting quiescent endogenous fibrinolysis, and increasing the likelihood for development of a severe acute clinical syndrome (12–17).

Prior in vitro reports have provided potential mechanisms for the Lp(a)–macrophage interaction. Cultured macrophages have been reported to acquire the ability to internalize Lp(a) via an apo(a) receptor (45,46) different from the part of apo(a) that antagonizes plasminogen (46); thus, association with macrophage cell membrane may not preclude plasminogen antagonism. Additionally, we have shown that the very low density lipoprotein (VLDL) receptor can initiate the internalization and degradation of Lp(a) in murine embryonic fibroblasts infected with human VLDL receptor complementary DNA (36). It has also been demonstrated that the VLDL receptor is found on vascular endothelium (47) and colocalizes with foam cells in human atherosclerotic plaque tissue (36). These studies suggest that cellular uptake of Lp(a), mediated by the VLDL receptor, may contribute to the macrophage–foam cell transformation.

The fact that the clinical classification was no longer a significant correlate of the plaque macrophage infiltration when plaque Lp(a) area was entered in the multivariable statistical model may reflect the relative discrepancy between pathologic events within the plaque and specific ischemic symptomatology, that is, that the majority but not all patients with acute coronary syndromes have a disrupted plaque (37,48), and that a minority of stable angina patients may also have a complex or disrupted lesion (49). The fact that Lp(a) was the single statistically significant correlate of plaque macrophage area suggests that plaques with high Lp(a) area had the highest macrophage area, and these plaques may have been the ones that produced plaque disruption with superimposed thrombosis, regardless of the severity of the clinical presentation.

In the present study, Lp(a) area was associated with smooth muscle cell actin stained area in plaque tissue from patients with crescendo exertional angina. In this population there is relatively low incidence of angiographically complex lesions and intracoronary thrombus (44). In this subset of patients, Lp(a) may have a role in the growth of atherosclerotic plaque and the transformation from stable to unstable angina. The association between the extent of Lp(a) and vascular smooth muscle cell areas in crescendo exertional angina (Fig. 3), and a 31% colocalization is of interest since the VLDL receptor is expressed on smooth muscle cells, as well as on endothelial cells and macrophages (47,50). The colocalization of Lp(a) with smooth muscle cells extends previous experimental observations concerning the modulation of transforming growth factor-beta (TGF-beta). Lipoprotein(a) has been shown to inhibit the formation of TGF-beta in a coculture of bovine endothelial and smooth muscle cells by preventing the formation of cell surface–bound plasmin, an activator of latent TGF-beta. Since TGF-beta deficiency promotes smooth muscle cell migration, it was postulated that this mechanism might occur in the vessel wall in vivo (31). Grainger et al, extended these observations by demonstrating that Lp(a) stimulated vascular smooth muscle cell proliferation in tissue culture (32). Confirming evidence for the role of Lp(a) in vivo has been provided by the finding that TGF-beta formation is inhibited by apo(a) in the aorta of the human apo(a) transgenic mouse by inhibiting activation of plasminogen to plasmin (32,51).

The possible effects of Lp(a) on vascular smooth muscle cell proliferation and migration may be critical in coronary atherosclerotic plaque growth (52), progressive luminal narrowing and the development of accelerated ischemic symptoms even in the absence of plaque disruption or thrombus formation (53).

Conclusions. In summary, we report that Lp(a) is an extensive component of the human coronary atherosclerotic plaque of living patients. This is the first description of an association of plaque Lp(a) area with the clinical syndrome of unstable angina. We also found that plaque Lp(a) correlates with the amount and location of macrophage infiltration, and that plaque Lp(a) area is associated with the extent of smooth muscle cell area in crescendo exertional angina patients. Our findings provide support of an in vivo role of Lp(a) in the atherosclerotic plaque and the development of acute coronary syndromes.

Limitations. Antithrombotic therapy prior to DCA may have altered plaque composition; however, the routine practice in our hospital of performing early intervention within 48 h of admission provides a rather uniform time frame for DCA specimen collection in all patients. With respect to unstable angina, only specimens from culprit lesions, as determined by the operator and based on established criteria (44,49), were included in this study. Analysis of nonculprit lesions might have offered an additional control group. Studies of artherectomy specimens are always subject to sampling error of the removed tissue. In the present study, blood Lp(a) levels were not provided for a correlation of plaque Lp(a) area with the blood Lp(a) level. With respect to smooth muscle cells, alpha-actin staining may underestimate their presence in the atherosclerotic plaque, since when these cells proliferate they change from the contractile to the proliferative phenotype.

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