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Lipoprotein (a) downregulates lysosomal acid lipase and induces interleukin-6 in human blood monocytes

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Abstract

The association of elevated lipoprotein (a) (Lp(a)) with an increased risk for coronary events is clearly established. This increased risk may in part be due to the activation of monocytes as major cells involved in atherogenesis. High concentrations of plasma Lp(a) were shown to influence the gene expression of human blood monocytes and in the present study we demonstrate a reduced abundance of the lysosomal acid lipase (LAL) mRNA in monocytes of patients with coronary disease and selective Lp(a) hyperlipidemia. This is also supported by in vitro studies where purified Lp(a) but not low-density lipoprotein (LDL) was shown to downregulate mRNA levels of the LAL in control monocytes. A correlation of Lp(a) serum levels and the proinflammatory cytokine IL-6 was recently also described. Therefore, we investigated whether Lp(a) is capable to enhance the release of this acute phase cytokine from human blood monocytes. Purified Lp(a) led to an increased secretion of IL-6, but not TNF-α arguing against a general activation of these cells. The association of reduced LAL activity with the premature development of coronary artery disease has been demonstrated in patients with hypercholesterolemia, and in the present study we show for the first time that LAL expression is suppressed in monocytes from patients with Lp(a) hyperlipidemia and by purified Lp(a). In addition, increased levels of IL-6 also predict future cardiovascular events and IL-6 secretion was also induced by purified Lp(a).

Keywords: Lipoprotein (a); Lysosomal acid lipase; Interleukin-6; Blood monocyte; Atherogenesis

1. Introduction

Lipoprotein (a) (Lp(a)) is a low-density lipoprotein (LDL) particle where apoB100 is linked by a disulfide bond to apolipoprotein (a) (apo(a)). Lp(a) has been recognized as a risk factor for atherosclerosis [1,2] and the structural similarity of apo(a) with plasminogen may explain some of the atherogenic features of this lipoprotein [3]. Lp(a) exerts different effects on cells involved in the development of atherosclerotic lesions. First, Lp(a) increases the synthesis of plasminogen activator inhibitor-1 (PAI-1), the production of vascular adhesion molecule 1 (VCAM-1) and E-selectin in endothelial cells [4]. Second, the proliferation of vascular smooth muscle cells (VSMC) is enhanced by the reduced activation of plasminogen to plasmin, thus blocking the proteolytic activation of transforming growth factor-beta, an autocrine inhibitor of VSMC proliferation [5]. Third, in THP-1 macrophages, the secretion of interleukin-8 was found increased [6].

Whereas most of the published studies focus on the influence of elevated Lp(a) on the generation of the atherosclerotic plaque, the interaction of high Lp(a) with blood monocytes is poorly investigated. Blood monocytes are influenced by dyslipidemia, functionally abnormal monocytes have been reported in patients with hypercholesterolemia [7] and more mature monocytes are described in patients with xanthomatosis [8]. The monocyte chemoattractant protein-1 receptor CCR2 on circulating monocytes correlates with plasma LDL [9] and a significant increase of CD40 on monocytes of patients with moderate hypercholesterolemia has been published [10]. The expression of the variant activation antigen CD45RA by peripheral blood monocytes showed a positive correlation to plasma levels

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of the atherogenic lipoproteins LDL and Lp(a) [11]. Recently, we identified differentially expressed genes in monocytes from a patient with isolated Lp(a) hyperlipidemia compared to control monocytes using a microarray. Subsequent confirmation of these candidates in patients suffering from coronary heart disease (CHD) and high Lp(a), in healthy donors with high Lp(a) and controls with low Lp(a) revealed an induction of plasminogen activator inhibitor type 2 (PAI-2) in monocytes from male, but not from female individuals with high Lp(a). PAI-1 mRNA was found suppressed only in the patients' monocytes and not in healthy probands with high Lp(a) levels [12]. However, most of the identified candidate genes resulted from interindividual differences in the gene expression of the two donors and were not related to Lp(a) hyperlipidemia.

Therefore, the gene expression in monocytes of a second patient with high Lp(a) as the only obvious risk factor for premature atherosclerosis [13] was analyzed. By this approach, the probability to detect disease-related genes and to distinguish them from interindividual variances in gene expression is markedly increased. Besides the genes already described, the expression of the lysosomal acid lipase (LAL) was found suppressed in the patients' cells. Furthermore, purified Lp(a) also downregulates LAL mRNA in control monocytes and induces an enhanced release of IL-6, a chemokine with pro-inflammatory properties. Therefore, we conclude that these events may be relevant to the process of premature atherosclerosis in probands with high Lp(a).

2. Materials and methods

2.1. Patients and probands with elevated Lp(a)

Data of the patients with a history of myocardial infarction (P1, P2, P3, P4) [13] and healthy blood donors with elevated Lp(a) (L1 to L10) are listed in Table 1. Lp(a) was determined by a nephelometric test (Immuno AG, Vienna, Austria). Healthy blood donors with normal lipid status and Lp(a) plasma levels of less than 5 mg/dl were used as controls (C1 to C20). The isolation of blood monocytes was approved by the Medical Ethics Committee of the University Hospital of Regensburg, Germany.

The apo(a) isoforms were determined with sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by immunoblotting as previously described with the monoclonal antibody 1A2 [14]. The number of apoA-IV kringles is given in brackets and is as follows: P1 (15/20), P2 (14/32), P3 (19/30) and P4 (15/20). Lp(a) for the incubation of monocytes was isolated from healthy donors with high Lp(a). The number of apoA-IV kringles is: L1 (17/?), L2 (20/37), L3 (17/19) and L4 (18/28). All of the patients and probands had at least one small apo(a) isoform defined as 17 to 22 kringle IV repeats [15], which is in accordance with the higher Lp(a) concentrations.

2.2. Culture media and reagents

RPMI medium was from Gibco BRL (Karlsruhe, Germany). LPS, *E. coli* serotype O55:B5, and other laboratory reagents and chemicals were purchased from Sigma Chemical (Deisenhofen, Germany) unless noted otherwise. Nylon membranes (Genescreen) for Northern blotting were from NEN Life Science (Boston, MA) and [α^{32}P]dCTP was from Amersham Pharmacia Biotech (Braunschweig, Germany). Oligonucleotides were synthesized by MWG (Ebersberg, Germany). IL-6, IL-6 ELISA and TNF-α ELISA was from R&D Systems (Wiesbaden-Nordenstadt, Germany).

2.3. Isolation and culture of monocytes

Peripheral blood monocytes from healthy blood donors with elevated Lp(a), patients with Lp(a) hyperlipidemia and controls were isolated by leukapheresis followed by counterflow elutriation. Fractions containing >95% monocytes were pooled and cultured on plastic Petri dishes (1×10^6 cells/ml) in RPMI medium with 20% autologous serum for the indicated times.

2.4. Isolation of LDL and Lp(a)

LDL and Lp(a) lipoproteins were purified from citrated plasma of healthy donors as described by Kostner et al. [16]. Endotoxin in the isolated lipoproteins was determined by an assay from Sigma and was less than 0.01 ng/ml in the culture medium.

Table 1
Description of patients and probands with elevated Lp(a)

Probands	Number probands	Lp(a) (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	Age of the probands
Patients (P1 to P4)	4	113.2 ± 21.9	138.1 ± 10.7	39.6 ± 7.6	48.3 ± 6.4
Healthy probands with high Lp(a) (L1 to L10)	10	112.2 ± 27.3	139.4 ± 36.3	72.5 ± 21.2	34.6 ± 17.3
Controls (C1 to C20)	20	<5	103.4 ± 16.5	50.4 ± 18.4	35.2 ± 13.1

P1 to P4 are patients with Lp(a) hyperlipidemia and a history of myocardial infarction with the last event at least 7 years ago. L1 to L10 are healthy controls with high Lp(a) levels and C1 to C20 healthy controls with Lp(a) of less than 5 mg/dl.

2.5. Isolation of RNA and Northern blot analysis

Total cellular RNA from elutriation-purified monocytes was isolated by the guanidine isothiocyanate–cesium chloride technique [17]. Ten micrograms of total RNA was separated through a 1.2% agarose gel containing 6% formaldehyde and blotted onto nylon membranes. After cross-linking with UV-irradiation (Stratalinker model 1800, Stratagene, La Jolla, CA), the membranes were hybridized with a cDNA probe generated by RT-PCR. The LAL probe was amplified using the primers LAC1 (5′-CCCGGCAGGACAGCTCCAGA-3′) and LAC2 (5′-GTGTGACACAGCTCAAGTCCA GCTT-3′) and the IL-6 probe with IL6uni (5′-ATGAACTCCTTCTCCACAAGCGC-3′) and IL6rev (5′-GAAGAGCCCTCAGGC TGGACTG-3′). The membranes were stripped and subsequently hybridized with a human GAPDH probe (Clontech, Palo Alto, CA). The probes were radiolabeled with [α-^{32}P]dCTP using the Prime-It II Kit from Stratagene (Amsterdam Zuidoost, Netherlands). Hybridization and washing conditions were performed as recommended by the manufacturer of the membrane.

2.6. Microarray hybridization

mRNA was purified from total RNA by oligodT beads (Roche, Penzberg, Germany). Hybridization, signal detection and data analysis was performed by Incyte Genomics (for detailed information see http://www.incyte.com).

Two hundred nanograms of mRNA isolated from monocytes of patient 1 or patient 2 and control 1 were independently reverse-transcribed using Cy5- or Cy3-labeled nucleotides, respectively, mixed and simultaneously hybridized to the UniGEM™ V 2.0 microarray with 8556 human cDNAs spotted. The array was rinsed and scanned for the two fluorescent dyes independently. Data were analyzed using GemTools™ software and were calculated as expression ratios of patient to control mRNA.

2.7. Monitoring of gene expression by real-time RT-PCR

Two micrograms of total RNA were reverse-transcribed using the Promega Reverse Transcription System in a volume of 40 μl. Two microliters of cDNA were subsequently amplified in glass capillaries (LightCycler) using PCR primers specific for GAPDH and LAL. The reaction conditions were as follows: 2 μl cDNA, 2 μl 10× LightCycler-Fast Start DNA Master SYBR Green I (Roche Diagnostics GmbH, Mannheim, Germany), 2.4 μl $MgCl_2$ (25 mM), 1 μl of each primer (5 pmol/μl), in a total volume of 20 μl. The primers for GAPDH were: GAPDHuni: 5′-TTGGTATCGTGGAAGGACTCA, GAPDHrev 5′-TGTCATCATATTTGGCAGGTTT.

The primers for LAL were: LAC-385uni: 5′-GTGGATGGGCAACAGCAGAGG, LAC-803rev: 5′-CCTTCAGTATGACATGAGTGC.

Amplification in the LightCycler capillaries was for 45 (40) cycles for LAL (GAPDH) with initial incubation of 10 min at 95 °C for activation of TaqPolymerase. Cycling parameters were 10 s at 95 °C, 10 s 55 °C (60 °C for GAPDH) and 15 s at 72 °C (5 s for GAPDH). Fluorescence was monitored at 82 °C (85 °C for GAPDH). The second derivative method was used for quantification with the LightCycler software.

2.8. Generation of standard curves

For quantification of the results obtained by real-time PCR, we used the standard curve method. For this purpose, a stock of total RNA from control monocytes was serially diluted. A standard curve with 50, 25, 12.5, 6.25 ng total RNA was generated for LAL and GAPDH. The standard curves were used to determine the relative expression of LAL and GAPDH mRNA in each sample.

2.9. ELISA

Ten million cells were incubated for 24 h in RPMI, 20% autologous serum and LDL or Lp(a) as indicated. Ten microliters of the supernatant were used for IL-6 or TNF-α protein determinations. The ELISA was performed as recommended by the distributor (R&D Systems).

3. Results

3.1. Identification of differentially expressed genes in monocytes from patients with isolated Lp(a) hyperlipidemia using a microarray

Recently, we have shown that by comparing the gene expression patterns of monocytes isolated from a patient with high Lp(a) (P1) and monocytes from a control individual with low Lp(a) (C1) using a microarray, most of the genes identified to be differentially expressed are the result of interindividual differences between unrelated donors and were not associated with high Lp(a) serum levels [12]. Therefore, an additional hybridization experiment with mRNA purified from monocytes of a second patient with high Lp(a) was performed. Genes that were similarly up- or downregulated in the two mRNA samples of the patients were PAI-2, PAI-1, defensins [12] and LAL. In the present study, we focus on the regulation of the LAL gene. LAL mRNA was suppressed 2.8-fold in monocytes isolated from patient 1 (P1) and 4.2-fold in monocytes from patient 2 (P2) in comparison to the control (C1). The mRNAs of additional lysosomal genes like acid sphingomyelinase and lysosomal cysteine proteases were not differentially expressed in patients versus control monocytes.

3.2. mRNA analysis of LAL in Lp(a) patients, probands with elevated Lp(a) and controls

Further analysis of LAL mRNA by Northern blot in monocytes from Lp(a) patients (P1, P2), healthy probands with high Lp(a) (L1, L2) and six controls with low Lp(a) confirmed that LAL mRNA is downregulated in Lp(a) patients (below the sensitivity of a Northern blot) (Fig. 1A). The expression of LAL mRNA in monocytes of the healthy probands L1 and L2 with serum concentrations of Lp(a) similar to that of the patients is like in the controls with low Lp(a) (C1 to C6). Because of the high variability of LAL mRNA expression in the control group, we determined LAL mRNA by real-time RT-PCR in the LightCycler. RNA isolated from the monocytes of 20 different control individuals, of 10 healthy individuals with high Lp(a) and of 4 patients (P1 to P4) was analyzed. LightCycler analysis was performed twice with a standard deviation of lower than 0.2 (not indicated here). The relative expression of LAL and GAPDH were determined using the standard curves and division of LAL expression by the GAPDH values was done for normalization. The relative normalized value for the LAL mRNA in the control group was 6.9 ± 8.7, for healthy probands with high Lp(a) 3.4 ± 1.6 and 1.4 ± 0.7 for the patients. This indicates a 2-fold reduction of LAL mRNA in healthy probands with high Lp(a) and a 4.9-fold reduction in patients with high Lp(a) when compared to the control group.

Due to the high variation of LAL mRNA levels in the control group, these data are not statistically significant. Nevertheless, the values indicate a downregulation of LAL mRNA in healthy probands with high Lp(a) and suppression of LAL mRNA in the patients.

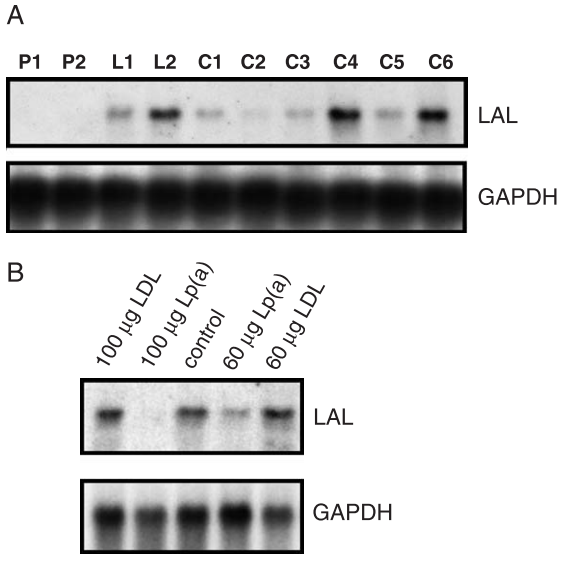

Fig. 1. Analysis of LAL expression in various individuals and in monocytes incubated with Lp(a) in vitro. (A) Elutriation-purified monocytes from patients with isolated Lp(a) hyperlipidemia (P1, P2), healthy controls with elevated Lp(a) (L1, L2) and six controls with low Lp(a) (C1 to C6) were incubated in 20% autologous serum for 24 h. RNA was isolated and 10 µg of total RNA was used in a Northern blot to analyze the expression of LAL. Equal RNA loading was confirmed by reprobing the blot with GAPDH. (B) Elutriation-purified monocytes from a donor with low Lp(a) were incubated with purified LDL (100 and 60 µg/ml), and with purified Lp(a) (100 and 60 µg/ml) in 20% autologous serum or in 20% serum alone (control) for 24 h. Total RNA was isolated and the expression of LAL and GAPDH was analyzed.

3.3. Regulation of LAL mRNA by purified Lp(a)

To further support the observation that Lp(a) regulates LAL expression, we incubated monocytes isolated from two healthy probands with purified Lp(a) or LDL for 24 h. Lp(a) and LDL were isolated from the plasma of healthy donors L1 to L4 and lipoproteins from the identical donor were used in one experiment. Northern blot analysis revealed a downregulation of LAL mRNA when the cells were incubated with 100 µg/ml Lp(a) for 24 h. LAL mRNA was suppressed 7.1 ± 2.1-fold as determined by LightCycler analysis of the corresponding mRNAs from two donors. Lp(a) (60 µg/ml) added for 24 h downregulate LAL mRNA 2.5 ± 1.4-fold (Fig. 1B). LDL did not influence LAL mRNA levels. A similar result was seen in the monocytic cell line THP-1 incubated with PMA to induce differentiation and 100 µg/ml Lp(a) or LDL for 24 h (not shown). LAL mRNA was downregulated in THP-1 cells treated with 100 µg/ml purified Lp(a) by a factor of 3.7 ± 1.3 whereas LDL did not modulate LAL expression in THP-1 cells.

The downregulation of LAL mRNA is only detected in monocytes cultivated in the presence of serum. Monocytes in serum-free medium supplemented with M-CSF and incubated with purified Lp(a) show no altered LAL mRNA expression, indicating that Lp(a) mediated regulation of LAL mRNA depends on a serum factor present in human serum.

3.4. Regulation of LAL mRNA by lipopolysaccharide (LPS)

Purified Lp(a) influences the gene expression of PAI-2 in a proinflammatory way, namely PAI-2 mRNA is induced by Lp(a) [12] and LPS [18]. However, to our knowledge, the regulation of LAL mRNA expression in human blood monocytes upon LPS treatment is not investigated. To see whether the suppression of LAL mRNA also resembles a proinflammatory response monocytes from two controls were incubated with different amounts of LPS. LPS, in 10 and 100 pg/ml concentrations, had no influence on LAL mRNA expression, but a significant downregulation is induced by 1, 10 and 100 ng/ml LPS in the presence of serum (Fig. 2). LightCycler expression analysis revealed a 5.3 ± 1.2-fold downregulation of LAL mRNA for donor 1 for 1, 10 and 100 ng/ml LPS and 12.8 ± 1.8-fold suppression for donor 2. Therefore, 1 ng/ml LPS is sufficient to downregulate LAL mRNA in human blood monocytes and higher amounts of LPS have no additional effect on the

mRNA levels of this gene. This indicates that LAL mRNA is suppressed in human monocytes by Lp(a) and LPS.

3.5. Lp(a) induces the secretion of IL-6

IL-6 was found to stimulate Lp(a) production in hepatocytes [19] and furthermore Lp(a) concentrations are related to serum IL-6 levels in some studies [20,21]. Therefore, it might well be that Lp(a) enhances the secretion of IL-6. We determined IL-6 and TNF-α in the supernatant of human monocytes incubated with serum alone, with 100 μg/ml LDL or 100 μg/ml Lp(a) for 24 h. Whereas TNF-α levels were unchanged (not shown), IL-6 secretion was significantly increased in cells treated with Lp(a) (Fig. 3A). IL-6 was 175.6 ± 35 pg/ml in the culture medium of monocytes cultivated in the presence of serum and 97.6 ± 83 pg/ml with LDL. Although LDL seems to downregulate IL-6 secretion, this difference was not significant using *t*-test. In the presence of Lp(a), monocytes released 687 ± 114 pg/ml IL-6, which is a nearly 4-fold and significant induction of IL-6 ($P < 0.001$).

In addition, IL-6 levels in the serum of patients with isolated Lp(a) hyperlipidemia (1.85 ± 0.2 pg/ml), in healthy probands with high Lp(a) (1.7 ± 0.38 pg/ml) and in controls with low Lp(a) (1.5 ± 0.36 pg/ml), were determined by ELISA. Although IL-6 levels seem to be slightly increased in Lp(a) patients and healthy probands with high Lp(a) compared to controls, this difference was not significant.

The microarray data revealed no significant differences in the mRNA levels of IL-6 and TNF-α in monocytes isolated from P1, P2 and C1. Northern blot analysis with a IL-6-specific probe was performed. IL-6 mRNA is easily detectable by this method and the variance in IL-6 mRNA in monocytes from different donors is low (Fig. 3B). The IL-6 mRNA expression was also not induced in monocytes treated with purified Lp(a) in vitro (Fig. 3C), indicating a posttranscriptional regulation. We further investigated whether IL-6 may mediate LAL suppression in monocytes.

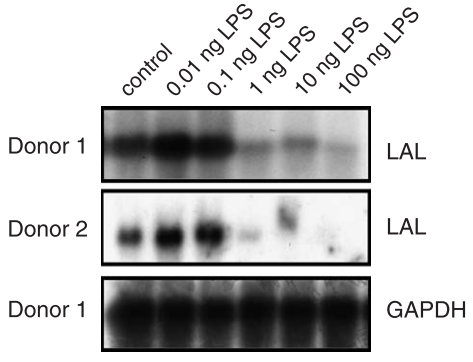

Fig. 2. Northern blot analysis of LAL mRNA in monocytes incubated with LPS. Elutriation-purified monocytes from two donors were incubated with LPS (10 pg/ml, 100 pg/ml, 1 ng/ml, 10 ng/ml and 100 ng/ml) or in 10% autologous serum alone for 24 h. Expression of LAL mRNA and GAPDH mRNA (donor 1) was analyzed.

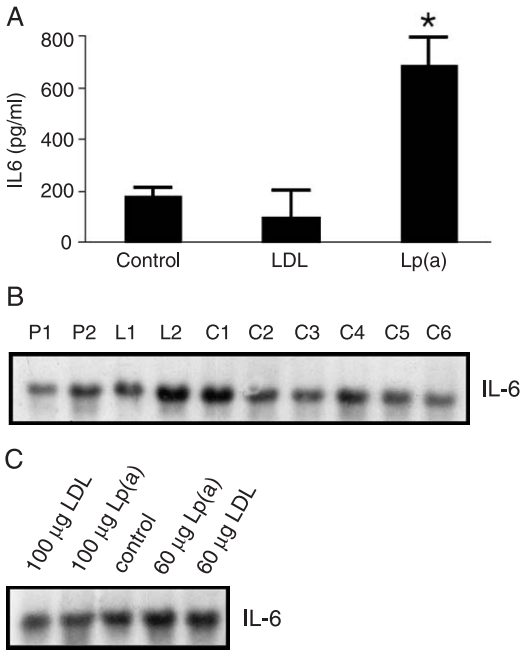

Fig. 3. Analysis of IL-6 expression in various individuals and in monocytes incubated with Lp(a) in vitro. (A) Elutriation-purified monocytes were incubated in 20% autologous serum for 24 h. Secreted IL-6 was determined in the supernatant of monocytes from four probands with low Lp(a) incubated with serum alone, with 100 μg/ml LDL or with 100 μg/ml purified Lp(a). IL-6 is given as pg/ml. Mean values obtained from four independent determinations ± S.D. are shown. Significance was determined using *t*-test (*$P < 0.001$). (B) Elutriation-purified monocytes from patients with isolated Lp(a) hyperlipidemia (P1, P2), healthy controls with elevated Lp(a) (L1, L2) and six controls with low Lp(a) (C1 to C6) were incubated in 20% autologous serum for 24 h. RNA was isolated and 10 μg of total RNA was used in Northern blots to analyze the expression of IL-6. (C) Elutriation-purified monocytes from a donor with low Lp(a) were incubated with purified LDL (100 and 60 μg/ml) or with purified Lp(a) (100 and 60 μg/ml) in 20% autologous serum or in 20% serum alone (control) for 24 h. Total RNA was isolated and the expression of IL-6 was analyzed.

Monocytes were incubated with 10 ng/ml IL-6 for 24 h or LPS as control. Whereas LPS downregulates LAL mRNA, IL-6 did not change LAL mRNA levels (not shown), indicating that induced IL-6 secretion does not mediate the suppression of LAL mRNA.

4. Discussion

Elevated plasma Lp(a) is a risk factor for premature cardiovascular disease [1,2]. Lp(a) modulates the gene expression of cells involved in the formation of atherosclerotic lesions namely endothelial cells [4], smooth muscle cells [5] and macrophages [6]. We recently described that Lp(a) also alters the gene expression of human blood monocytes with PAI-2 being induced and PAI-1 secretion being diminished [12]. In this study, we focus on the reduced expression of LAL mRNA identified in the monocytes of two patients with high Lp(a) and cardiovascular disease by microarray hybridization. This was also con-

firmed by Northern blot analysis. Using real-time quantitative PCR, the expression of LAL mRNA was studied in the monocytes of 4 patients with high Lp(a), 10 healthy probands with high Lp(a) and 20 controls. We found a 2-fold reduction of LAL mRNA in monocytes from healthy probands with high Lp(a) and a 4.9-fold reduction in patients with high Lp(a) when compared to the control group. LAL mRNA expression is highly variable within the controls and to further support our observation in vitro studies were performed. Monocytes were incubated with purified Lp(a) and LAL mRNA was found suppressed in these cells whereas the incubation with LDL as a control had no influence on LAL mRNA levels. This indicates that the LDL moiety of Lp(a) does not suppress LAL expression and even oxidized LDL was demonstrated not to impair lysosomal hydrolysis of cholesteryl esters in macrophages [22].

LAL contributes to the prevention of lipid overload in the liver, spleen and macrophages [23], and LAL activity is reduced in cholesteryl ester storage disease (CESD) patients [24,25]. This disorder is characterized by high LDL cholesterol and an accumulation of cholesteryl esters and triglycerides in most tissues and early atherosclerosis [26]. The severity of the disease correlates with the residual LAL activity [25,27]. Plasma cholesterol and triglyceride levels are not elevated in patients with high Lp(a) [28] but they are in patients with reduced LAL activity [26]. Therefore, we suggest that LAL activity in the liver as a main organ determining plasma LDL and HDL levels is not significantly altered. Lipid overload of macrophages is unlikely to change plasma LDL but may promote the generation of atherosclerotic lesions [29].

Atherosclerosis is an inflammatory disease [30] and the monocyte response to atherogenic stimuli and to proinflammatory mediators like LPS is often similar. Indeed, LPS, the most potent inflammatory stimulus, also downregulated LAL mRNA expression in human blood monocytes.

The proinflammatory cytokine IL-6 stimulates the secretion of apo(a) from hepatocytes [19] and a correlation of Lp(a) levels and IL-6 in patients with high apo(a) isoforms and monoclonal gammopathy was recently reported [21]. Therefore, we investigated whether Lp(a) may induce IL-6 secretion in human blood monocytes. IL-6 release was significantly enhanced in the cells incubated with purified Lp(a) in vitro whereas TNF-α release was not induced by this atherogenic lipoprotein. IL-6 levels in the serum of patients with isolated Lp(a) hyperlipidemia, in healthy probands with high Lp(a) and in controls with low Lp(a) were determined. Although IL-6 levels slightly declined from Lp(a) patients to healthy Lp(a) probands to controls, the difference was not significant. Baggio et al. [20] published similar data. In their study, IL-6 levels did not correlate to high Lp(a) with the exception of the centenarians. IL-6 concentrations were significantly higher in centenarians with high Lp(a) when compared to centenarians with low Lp(a). Similarly, PAI-1 secretion is reduced in monocytes from patients with high Lp(a) and also in in vitro studies [12]. An inverse relation of Lp(a) and PAI-1 was demonstrated in the serum of type 2 diabetic patients [31], whereas PAI-1 was not reduced in the serum of patients with high Lp(a) not suffering from type 2 diabetes.

Lp(a) stimulates the secretion of IL-6, an acute phase cytokine inducing apo(a) production in hepatocytes and furthermore downregulates LAL in monocytes from patients with isolated Lp(a) hyperlipidemia and in monocytes incubated with Lp(a) in vitro. Therefore, high Lp(a) may contribute to the premature development of severe atherosclerosis by impairing lysosomal cholesterylester hydrolysis and enhancing the secretion of the multifunctional proinflammatory cytokine IL-6.

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