Volume 325, number 3, 271–275 FEBS 12637 July 1993

Heterogeneous lipoprotein (a) size isoforms differ by their interaction with the low density lipoprotein receptor and the low density lipoprotein receptor-related protein/α_2-macroglobulin receptor

Winfried März[a], Angela Beckmann[b], Hubert Scharnagl[a], Rüdiger Siekmeier[b], Uli Mondorf[b], Irmtraud Held[c], Wolfgang Schneider[b], Klaus T. Preissner[d], Linda K. Curtiss[e], Werner Groß[a] and Manfred Hüttinger[3]

[a]*Gustav Embden-Center of Biological Chemistry and* [b]*Center of Internal Medicine, Johann Wolfgang Goethe-University, Frankfurt, Germany,* [c]*Department of Medical Chemistry, University of Vienna, Austria,* [d]*Haemostasis Research Unit, Kerckhoff-Klinik, Bad Nauheim, Germany* and [e]*The Scripps Research Institute, Department of Immunology, La Jolla, CA, USA*

Received 2 May 1993

Lipoprotein (a) (Lp(a)) is a complex of low density lipoprotein (LDL) with apolipoprotein (apo) (a). To examine the size distribution of Lp(a), plasma was separated by fast flow gel filtration and Lp(a):B complexes were determined in the eluate by enzyme immunoassays, in which detection was performed with monoclonal antibodies specific for apoB. Lp(a):B particles displayed apparent molecular masses (M_r) of 2×10^6 to at least 10×10^6. Lp(a) size isoforms differed by the expression of apoB epitopes and their interaction with cultured human skin fibroblasts. LDL was more effective in inhibiting binding, uptake, and degradation of low M_r Lp(a) than of high M_r Lp(a). In contrast, Glu-plasminogen, α_2-macroglobulin and tissue-type plasminogen activator were more effective in competing for the cellular degradation of high M_r Lp(a) than of low M_r Lp(a). Ligand blotting revealed that Lp(a) bound to the low density lipoprotein receptor, the low density lipoprotein receptor-related protein/α_2-macroglobulin receptor (LRP) and to two other endosomal membrane proteins. We propose that the LDL receptor preferentially internalizes low M_r Lp(a), whereas LRP may have a role in the clearance of high M_r Lp(a).

Lipoprotein (a); Apolipoprotein B; Low density lipoprotein receptor related protein; Plasminogen; α_2-Macroglobulin; Tissue type plasminogen activator; Human skin fibroblast

1. INTRODUCTION

The protein moiety of lipoprotein (a) (Lp(a)) is composed of apolipoprotein (apo) B-100 and apo(a) [1,2]. Lp(a) is a strong independent predictor for atherosclerosis [3]. Apo(a) is homologous to plasminogen [4]. This has put forth the hypothesis that Lp(a) exerts thrombogenic effects by inhibiting fibrinolysis [5].

Plasma levels of Lp(a) are largely determined by the rate of synthesis [6]. The plasma half life of Lp(a) is similar to that of LDL [6]. Reports coping with the role of the LDL receptor in Lp(a) clearance are conflicting. Evidence that Lp(a) can specifically bind to the LDL receptor comes from studies with cultured cells [2,7–10] and with partially purified receptors [11]. Most investigators, however, agree that the LDL receptor binds Lp(a) with significantly lower affinity than LDL, [8, 9], apo(a) presumably 'masking' the apoB-100 receptor binding domain [2,10]. Maartmann-Moe et al. claimed that the cellular uptake of Lp(a) is entirely LDL receptor-independent [12]. In humans, up-regulation of LDL receptors by cholestyramine [13] or HMG-CoA reductase inhibitors [14] does not reduce Lp(a).

These findings prompted us to test whether Lp(a) is taken up into cells by sites other than the LDL receptor. Previously, we have noticed that Lp(a) is heterogeneous with respect to particle size [15], and immunochemical data suggested that Lp(a) size isoforms differed by the expression of the receptor binding domain of apoB-100. Hence, we also addressed the question whether large and small Lp(a) molecules were targeted to different sites of endocytosis. In the course of our work we obtained evidence that a substantial proportion of large Lp(a) particles is internalized by LDL receptor-independent routes also involving the LDL receptor-related protein (LRP), a multifunctional cell surface receptor [16], which has been implicated in the clearance of chylomicron remnants [17] and protease-inhibitor complexes [18–22]. In contrast, small Lp(a) particles were

Correspondence: W. März, Gustav Embden-Center of Biological Chemistry, Johann Wolfgang Goethe-University, University Hospital, Theodor Stern-Kai 7, W-6000 Frankfurt/Main, Germany. Fax: (49) (69) 6301-5937 or 6301-6970.

Abbreviations: apo, apolipoprotein; α_2M, α_2-macroglobulin; EDTA, ethylenediaminetetraacetate; GPC, gel permeation chromatography; Lp(a), lipoprotein (a); M_r, relative molecular mass; HM_r-Lp(a), high molecular mass Lp(a); LM_r-Lp(a), low molecular mass Lp(a); LRP, low density lipoprotein receptor-related protein/α_2-macroglobulin receptor; tPA, tissue-type plasminogen activator; VLDL, LDL, HDL, very low, low and high density lipoproteins, respectively.

preferentially targeted to the classical LDL receptor pathway.

2. MATERIALS AND METHODS

2.1. *Gel permeation chromatography (GPC)*

40 μl fasting plasma (1.5–2 g/l EDTA K_2, 20.000 KIE/l aprotinin) were loaded onto a 300 mm column of Superose 6 (Pharmacia) and eluted with 200 mM NaCl, 100 mM Na_2HPO_4, pH 7.4, at a flow rate of 0.3 ml·min^{-1}.

2.2. *Enzyme immunoassay for Lp(a) B complexes*

Polyclonal anti-apo(a) (Immuno, Vienna) was diluted 1:500 and coated to microwell plates in 0.2 M sodium carbonate, pH 10.6. Wells were blocked with gelatin (10 g/l) in carbonate buffer for 1 h, and washed twice with 200 μl PBS (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4) containing 0.05% (v/v) Tween 20. GPC column fractions (100 μl) were applied to the wells and incubated for 2 h. The solid phase was washed three times with PBS/Tween 20. Lp(a):B complexes were detected with apoB specific monoclonal antibodies MB3, MB19, and MB47 [23–26], followed by biotinylated anti-mouse IgG (1:8000), an avidin:biotinylated peroxidase complex (1:2000) (Vector Laboratories, Burlingame, CA), and *o*-phenylenediamine as substrate. Neither plasminogen nor LDL reacted in this assay.

2.3. *Preparation of Lp(a) size isoforms*

Lp(a) was purified from the regenerate fluid of a dextran sulfate column based LDL-apheresis system (Kanegafuchi MA 01 – Liposorber LA 15). The regenerate fluid was concentrated by tangential flow filtration and ultracentrifuged, first at a density of 1.125 kg/l, then at 1 050 kg/l. The Lp(a) containing fraction was chromatographed on Biogel A-15m (200–400 mesh, equilibrated with 50 mM Tris-HCl, pH 7.4, 200 mM NaCl, 10 mg/l EDTA·Na_2, 20.000 KIU/l aprotinin, 1 mg/l NaN_3, column length 100 cm). The leading and the trailing part of the Lp(a) peak were considered high molecular mass (HM_r) and low molecular mass (LM_r) Lp(a), respectively. Both Lp(a) subfractions were free of LDL as determined by agarose gel electrophoresis [27] and intermediate gel immunoelectrophoresis [28]. HM_r-Lp(a) and LM_r-Lp(a) were iodinated as described [29].

2.4. *Binding, uptake and degradation of Lp(a)*

Normal human skin fibroblasts were grown in 24-well polystyrene plates in RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum and used at 75% confluence. Binding, uptake (binding *plus* internalization), and degradation were measured as described [30]. α_2-Macroglobulin (α_2M, Sigma) was activated with methylamine according to Ashcom et al. [31]. Recombinant tissue-type plasminogen activator (tPA, Thomae) was inactivated with 2 mM phenylmethylsulfonyl fluoride. LDL were prepared by preparative ultracentrifugation (1.019 to 1.050 kg/l).

2.5. *Ligand blotting*

Endosomal fractions enriched for LRP were prepared as described [32]. 100 μg of total protein were separated on 5–10% polyacrylamide gradient gels and blotted according to Daniel et al. [33]. Blots were incubated with Lp(a) subfractions and immunostained with monoclonal anti-apo(a) (cl356, Pharmacia) and anti-mouse antibodies conjugated with alkaline phosphatase.

3. RESULTS

To analyse the size distribution of apo(a) containing particles, plasma was fractionated by size exclusion chromatography. Lp(a) was determined in the eluate by enzyme immunoassays, in which detection was carried out with monoclonal antibodies for apoB. Fig. 1 shows a typical elution profile. Lp(a):B complexes eluted over a broad M_r range: the largest Lp(a):B particles appeared in the void volume, the smallest ones co-eluted with LDL. The apparent M_r of Lp(a):B complexes ranged from 2×10^6 (small LDL) to at least 10×10^6 (VLDL). The profiles measured with MB3 and MB47 were virtually identical. However, when Lp(a):B complexes were detected with MB19, maximum immunoreactivity was observed at higher M_r than with MB3 or MB47. For instance, the highest concentration of Lp(a):B complexes detected with MB19 eluted after 28 min; the highest concentration determined with MB3 or MB47 eluted after 36 min. Thus, the ratio MB47/MB19 increased as the size of the Lp(a):B complexes decreased.

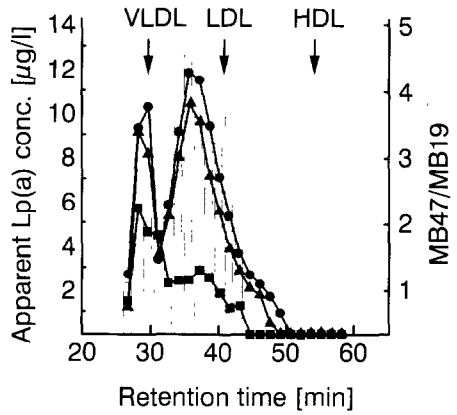

Fig. 1 Size distribution of Lp(a):B complexes in plasma. A plasma samples from a female donor (Lp(a) 236 mg/l; apo(a) phenotype: B) was separated by gel permeation chromatography. Lp(a):B complexes were measured in the eluate using enzyme immunoassays with apoB specific monoclonal antibodies: MB3 (circles), MB19 (squares), and MB47 (triangles). The shaded bars represent the ratios of MB47 to MB19 immunoreactivity. Retention times of VLDL, LDL, and HDL are indicated by vertical arrows.

To elucidate the functional significance of Lp(a) size heterogeneity, we studied the interaction of HM_r-Lp(a) and LM_r-Lp(a) with human skin fibroblasts. As shown in Fig. 2, radiolabelled HM_r-Lp(a) and LM_r-Lp(a) did not differ as to total binding, uptake, and degradation. However, when a 20-fold excess of unlabelled LDL was added, uptake of LM_r-Lp(a), but not of HM_r-Lp(a) was abolished. In keeping with this, LDL competed for the degradation of LM_r-Lp(a) to a greater extent than for the degradation of HM_r-Lp(a). Unlike uptake and degradation, binding of Lp(a) to fibroblasts at 4°C was only slightly competed for by LDL, but the inhibitory effect of LDL on LM_r-Lp(a) binding was still greater than on HM_r-Lp(a) binding.

We further analyzed the abilities of HM_r-Lp(a) and LM_r-Lp(a) to compete with each other, with LDL, with Glu-plasminogen, α_2M and tPA for degradation by human skin fibroblasts (Fig. 3). Unlabelled HM_r-Lp(a) and Glu-plasminogen competed with labelled HM_r-Lp(a) for cellular degradation. LM_r-Lp(a) and LDL

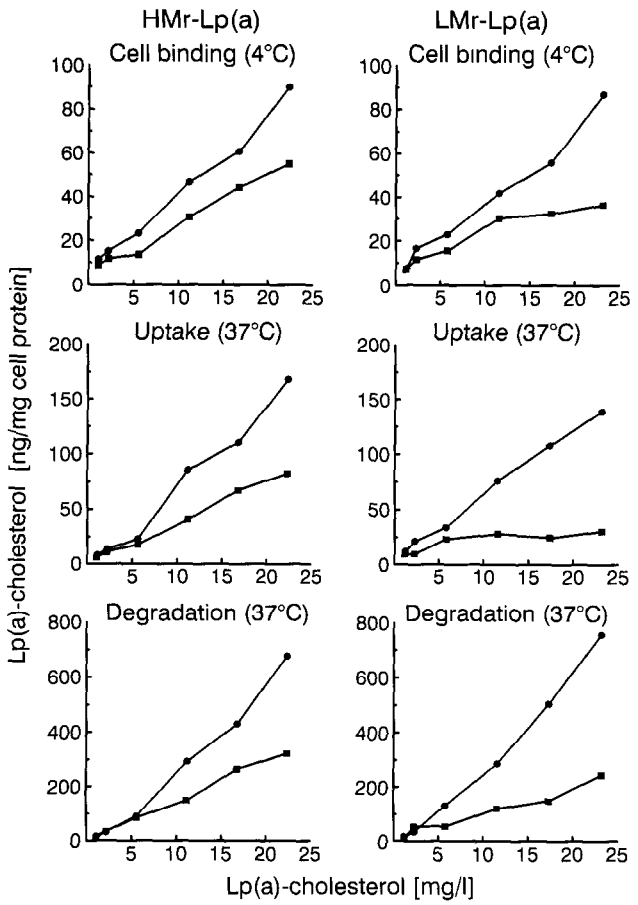

Fig. 2. Binding, uptake, and degradation of high molecular mass (HM_r) Lp(a) and low molecular mass (LM_r) Lp(a). Normal human fibroblasts were grown as described in section 2. To up-regulate LDL receptors, cells were switched to 10% (v/v) human lipoprotein deficient serum 40 h before the experiment. Binding (4°C), uptake (37°C), and degradation (37°C) of [^{125}I]HM_r-Lp(a) and [^{125}I]LM_r-Lp(a) were determined as described [30]. Lp(a)-concentrations are expressed in terms of cholesterol. Circles: labelled Lp(a) in the absence of LDL. Squares: labelled Lp(a) in the presence of excess unlabelled LDL. Each data point is the mean of triplicate determinations.

were ineffective as competitors for HM_r-Lp(a). Unlabelled LM_r-Lp(a) competed with labelled LM_r-Lp(a), whereas unlabelled HM_r-Lp(a) did not. LDL effectively displaced LM_r-Lp(a), and Glu-plasminogen had no effect on LM_r-Lp(a) degradation. Activated α_2M and inactivated tPA inhibited the cellular degradation of Lp(a) (Fig. 3). Inhibition was greater with HM_r-Lp(a) than with LM_r-Lp(a). Combinations of α_2M and tPA had no additional effects, compared to the two competitors alone.

On ligand blots with LRP enriched endosomal membranes Lp(a) bound to the LDL receptor, to LRP-515, the 515 kDa subunit [34] of LRP, and to two other proteins of unknown molecular identity (Fig. 4). Staining of LRP-515 with HM_r-Lp(a) appeared more intense than with LM_r-Lp(a).

4. DISCUSSION

In this study, we have worked out three novel aspects concerning the metabolism of Lp(a): we demonstrate that circulating Lp(a) is heterogeneous with respect to size, we suggest that this heterogeneity is of physiological relevance, and we postulate that LRP, apart from its role in the clearance of chylomicron remnants [17] and protease–inhibitor complexes [18–22], is involved in the metabolism of Lp(a).

Lp(a) has been shown to be heterogeneous by rate zonal ultracentrifugation [35], lysine affinity chroma-

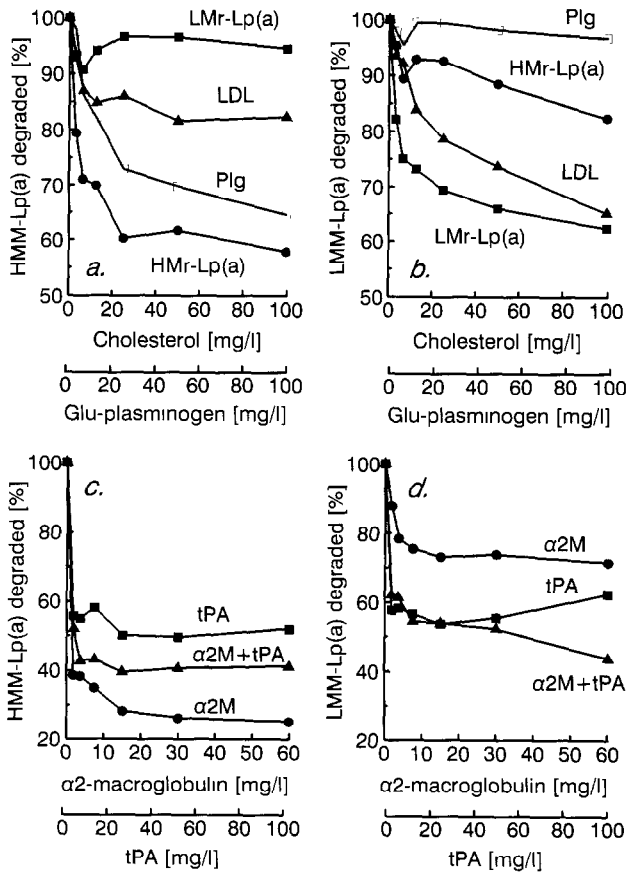

Fig. 3. Inhibition of degradation of radioactively labelled high molecular mass (HM_r) Lp(a) and low molecular mass (LM_r) Lp(a) in fibroblasts by unlabelled HM_r-Lp(a), LM_r-Lp(a), LDL, plasminogen, α_2-macroglobulin and tissue-type plasminogen activator. Top panels (a and b). Cells were maintained in 10% (v/v) lipoprotein deficient serum for 40 h, and then received labelled HM_r-Lp(a) and LM_r-Lp(a) (5 μg/ml Lp(a)-cholesterol). Unlabelled HM_r-Lp(a) (circles), LM_r-Lp(a) (squares), LDL (triangles) and Glu-plasminogen (open squares) were used as competitors at concentrations indicated on the abscissas. Degradation of ^{125}I-labelled HM_r-Lp(a) (a) and ^{125}I-labelled LM_r-Lp(a) (b) were assayed by measuring the amount of trichloroacetic acid-soluble radioactivity in the medium [30]. Bottom panels (c and d): Identical conditions, except that the cells were not pre-incubated with lipoprotein deficient serum. Activated α_2-macroglobulin (circles), tissue-type plasminogen activator (squares) and mixtures of both (triangles) were added as unlabelled competitors at the indicated concentrations. c: degradation of ^{125}I-labelled HM_r-Lp(a); d: degradation of ^{125}I-labelled LM_r-Lp(a).

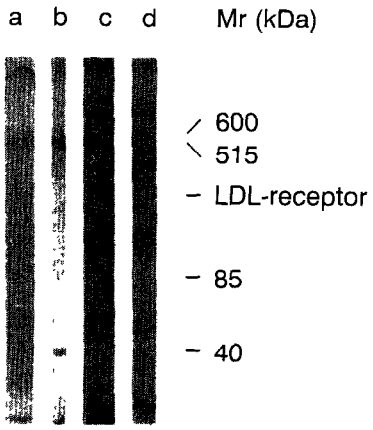

Fig. 4. Binding of Lp(a) to endosomal membrane proteins by ligand blotting. Endosomal membrane proteins were separated by SDS-polyacrylamide gel electrophoresis and blotted to nitrocellulose. Strips were probed as follows: (a) total protein stain; (b) antibodies for LRP-515 and the 39 kDa LRP associated protein [47]. (c) HM_r-Lp(a); (d) LM_r-Lp(a). In lanes c and d, Lp(a) was detected with the apo(a) specific monoclonal antibody cl356; no staining was obtained when Lp(a) was omitted.

tography [10], and chromatofocusing [36]. Whereas the heterogeneous density distribution was attributed to the known apo(a) polymorphism [37], no relationship could be delineated between the apo(a) polymorphism and Lp(a) heterogeneity revealed by lysine binding [10] or chromatofocusing [36]. We found that Lp(a) is heterogeneous with respect to particle size, even in individuals expressing only one apo(a) isoform (data not shown). Hence, Lp(a) size heterogeneity is also independent of the apo(a) polymorphism. The M_r of the genetically determined apo(a) isoforms ranges between 400,000 and 700,000. Evidently, this difference is too small to account for the 2×10^6 to at least 10×10^6 size variation found by gel filtration. Therefore, other causes including the presence of more than one apo(a) molecule per Lp(a) particle or the formation Lp(a)–LDL complexes [38] may underlie Lp(a) size heterogeneity.

The reason for the heterogeneity notwithstanding, Lp(a) particles of different size differed in the expression of apoB-100 epitopes. Compared to the epitope for MB19 which is located at or near the amino-terminal residue 71, the apparent number of MB47 epitopes was high on LM_r-Lp(a). The MB47 epitope consists of two non-linear domains including amino acid residues 3429–3453 and 3507–3523; it is located in the vicinity of the apoB-100 receptor binding domain [26]. Thus, we hypothesized that the apoB-100 receptor binding domain was more exposed on LM_r-Lp(a) than on HM_r-Lp(a). Consequently, LM_r-Lp(a) should be taken up by the LDL receptor at a higher rate than HM_r-Lp(a).

As expected, LDL was more effective in inhibiting cellular uptake of LM_r-Lp(a) than of HM_r-Lp(a). HM_r-Lp(a) and LM_r-Lp(a) poorly competed with each other for degradation, indicating that the two Lp(a) subclasses used different routes of internalization. Plasminogen competed for the degradation of HM_r-Lp(a), suggesting that plasminogen binding sites [39,40] may be involved in Lp(a) internalization. The cellular uptake of HM_r-Lp(a) was inhibited by methylamine-activated α_2M and inactivated tPA. Because both are ligands for LRP [18–20], this strongly suggests that LRP functions as a receptor for Lp(a). Consistently, Lp(a) bound to LRP-515, the large subunit of LRP [34], in vitro.

In addition to the LDL receptor and LRP, two other endosomal proteins seem to interact with Lp(a). So far, nothing is known on their identity. One speculative possibility is that they are related to plasminogen receptors which have been found to bind Lp(a) with high affinity [39,40]. However, whether or not these receptors undergo endocytosis is presently unknown.

The precise mechanism by which Lp(a) interacts with LRP is not clear. Some homology exists between kringles 1 and 2 in tPA and the kringles in apo(a) [41]. It is, therefore, tempting to speculate that binding of Lp(a) to LRP depends on apo(a). Beyond this, endocytosis of Lp(a) may require the formation of multimeric complexes consisting of Lp(a), LRP and other Lp(a) binding proteins (e.g. plasminogen receptors or LDL receptors), similar to the model proposed by Herz et al. [22] for the uptake of urokinase-type plasminogen activator–plasminogen activator inhibitor 1 complexes by LRP.

Although these issues have to be worked out in future studies our data are clear with respect to the fact that some of the uptake and degradation of Lp(a) in cells does not rely on the LDL receptor and that LRP contributes to the LDL receptor-independent component. There are multiple lines of evidence suggesting that the greater part of the catabolism of Lp(a) does not proceed via the LDL receptor pathway: Turnover studies in rats failed to show a relationship between LDL receptor activity and Lp(a) clearance [42,43]. In a heterozygous patient with familial hypercholesterolemia Krempler et al. found that Lp(a) was metabolized faster than LDL [6], and Knight et al. reported that the catabolic rate of Lp(a) was normal in familial hypercholesterolemia [44]. Drugs known to stimulate LDL receptors did not lower Lp(a) [13,14]. In individuals heterozygous for familial defective apoB-100, Lp(a) contained approximately 50% of the binding-defective apoB-100, compared to approximately 75% in the LDL fraction from these patients, suggesting that the LDL receptor plays a minor role in Lp(a) clearance [45]. Finally, our multiple-pathway model for Lp(a) catabolism is easily reconciled with the observation that several-fold over-expression of LDL receptors in transgenic mice increases Lp(a) elimination [46]: Normally most Lp(a) is metabolized via LDL receptor independent routes, and the LDL receptor pathway becomes relevant only, when LDL receptor expression is extremely enhanced.

Acknowledgements: We thank Mrs. Sabine Black, Mrs. Bettina Donnerhak and Mrs. Ulrike Stein for technical assistance and Pharmacia Diagnostics AB for providing monoclonal antibody cl356. Part of this study was supported by the Austrian Science Foundation (S4605).

REFERENCES

[1] Utermann, G. (1989) Science 246, 904–910.
[2] Armstrong, V.W., Walli, A.K. and Seidel, D. (1985) J. Lipid. Res. 26, 1314–1323.
[3] Kostner, G.M., Avogaro, P., Cazzolato, G., Marth, E. and Bittolo-Bon, G. (1981) Atherosclerosis 38, 51–61.
[4] McLean, J.W., Tomlinson, J.E., Kuang, W.-J., Eaton, D.L., Chen, E.Y., Fless, G.M., Scanu, A.M. and Lawn, R.M. (1987) Nature 330, 132–137.
[5] Miles, L.A. and Plow, E.F. (1990) Thromb. Haemostas. 63, 331–335.
[6] Krempler, F., Kostner, G.M. and Bolzano, K. (1980) J. Clin. Invest. 65, 1483–1490.
[7] Havekes, L., Vermeer, B.J., Brugman, T. and Emeis, J. (1981) FEBS Lett. 132, 169–173.
[8] Floren, C.-H., Albers, J.J. and Biermann, E.L. (1981) Biochem. Biophys. Res. Commun. 102, 636–639.
[9] Krempler, F., Kostner, G.M., Roscher, A., Haslauer, F., Bolzano, K. and Sandhofer, F. (1983) J. Clin. Invest. 71, 1431–1441.
[10] Armstrong, V.W., Harrach, B., Robenek, H., Helmhold, M., Walli, A.K. and Seidel, D. (1990) J. Lipid Res. 31, 429–441.
[11] Steyrer, E. and Kostner, G.M. (1990) J. Lipid Res. 31, 1247–1253.
[12] Maartmann-Moe, K. and Berg, K. (1981) Clin. Genet. 20, 352–362.
[13] Vessby, B., Kostner, G., Lithell, H. and Thomis, J. (1982) Atherosclerosis 44, 61–71.
[14] Kostner, G.M., Gavish, D., Leopold, B., Bolzano, K., Weintraub, M.S. and Breslow, J.L. (1989) Circulation 80, 1313–1319.
[15] März, W., Siekmeier, R. and Groß, W. (1991) Thromb. Haemostas. 65, 714.
[16] Herz, J., Hamann, U., Rogne, S., Myklebost, O., Gausepohl, H. and Stanley, K.K. (1988) EMBO J. 7, 4119–4127.
[17] Kowal, R.C., Herz, J., Weisgraber, K.H., Mahley, R.W., Brown, M.S. and Goldstein, J.L. (1990) J. Biol. Chem. 265, 10771–10779.
[18] Strickland, D.K., Ashcom, J.D., Williams, S., Burgess, W.H., Migliorini, M. and Argraves, W.S. (1990) J. Biol. Chem. 265, 17401–17404.
[19] Kristensen, T., Moestrup, S.K., Glieman, J., Bendtsen, L., Sand, O. and Sottrup-Jensen, L. (1990) FEBS Lett. 276, 151–155.
[20] Bu, G., Williams, S., Strickland, D.K. and Schwartz, A.L. (1992) Proc. Natl. Acad. Sci. USA 89, 7426–7431.
[21] Orth, K., Madison, E.L., Gething, M.-J., Sambrook, J.F. and Herz, J. (1992) Proc. Natl. Acad. Sci. USA 89, 7422–7426.
[22] Herz, J., Clouthier, D.E. and Hammer, R.E. (1992) Cell 71, 411–421.
[23] Young, S.G., Bertics, S.J., Curtiss, L.K., Casal, D.C. and Witztum, J.L. (1986) Proc. Natl. Acad. Sci. USA 83, 1101–1105.
[24] Young, S.G. and Hubl, S.T. (1989) J. Lipid Res. 30, 443–449.
[25] Curtiss, L.K. and Edgington, T.S. (1982) J. Biol. Chem. 257, 15213–15221.
[26] Young, S.G., Witztum, J.L., Casal, D.C., Curtiss, L.K. and Bernstein, S. (1986) Arteriosclerosis 6, 178–188.
[27] Neubeck, W., Wieland, H., Habenicht, A., Müller, P., Baggio, G. and Seidel, D. (1977) Clin. Chem. 23, 1296–1300.
[28] Gaubatz, J.W., Cushing, G.L. and Morrisett, J.D. (1986) in: Plasma Lipoproteins. Part B. Characterization, Cell Biology, and Metabolism (Albers, J.J. and Segrest, J.J. Eds.) vol. 129, pp. 167–186, Academic Press, Orlando.
[29] Sinn, H.J., Schrenk, H.H., Friedrich, E.A., Via, D.P. and Dresel, H.A. (1988) Anal. Biochem. 170, 186–192.
[30] Goldstein, J.L., Basu, S.K. and Brown, M.S. (1983) in: Biomembranes. Part L. Membrane Biogenesis (Fleischer, S. and Fleischer, B. Eds.) vol. 98, pp. 241–260. Academic Press, Orlando.
[31] Ashcom, J.D., Tiller, S.E., Dickerson, K., Cravens, J.L., Argraves, W.S. and Strickland, D.K. (1990) J. Cell. Biol. 110, 1041–1048.
[32] Huettinger, M., Retzek, H., Hermann, M. and Goldenberg, H. (1992) J. Biol. Chem. 267, 18551–18557.
[33] Daniel, T.O., Schneider, W.J., Goldstein, J.L. and Brown, M.S. (1983) J. Biol. Chem. 258, 4606–4611.
[34] Herz, J., Kowal, R.C., Goldstein, J.L. and Brown, M.S. (1990) EMBO J. 9, 1769–1776.
[35] Fless, G.M., Rolih, C.H. and Scanu, A.M (1984) J. Biol. Chem. 259, 11470 11478.
[36] Grinstead, G.F. and Ellefson, R.D. (1988) Clin. Chem. 34, 1036–1040
[37] Utermann, G., Kraft, H.G., Menzel, H.J., Hopferwieser, T. and Seitz, C. (1988) Hum. Genet. 78, 41–46.
[38] Trieu, V.N. and McConathy, W.J. (1990) Biochemistry 29, 5919–5924.
[39] Miles, L.A., Fless, G.M., Levin, E.G., Scanu, A.M. and Plow, E.F. (1989) Nature 339, 301–303.
[40] Hajjar, K.A., Gavish, D., Breslow, J.L. and Nachman, R.L. (1989) Nature 339, 303–305.
[41] Castellino, F.J., Urano, T., deSerrano, V.S. and Beals, J.M. (1990) in: Lipoprotein (a) (Scanu, A.M. Ed.) pp. 87–101. Academic Press, San Diego.
[42] Harkes, L., Jürgens, G., Holasek, A. and Berkel, T.J.C. (1988) FEBS Lett. 227, 27–31.
[43] Ye, S.Q., Keeling, J., Stein, O., Stein, Y. and McConathy, W.J. (1988) Biochim. Biophys. Acta 963, 534–540.
[44] Knight, B.L., Perombelon, Y.F.N., Soutar, A.K., Wade, D.P. and Seed, M. (1991) Atherosclersis 87, 227.
[45] Perombelon, Y.F.N., Gallagher, J.J., Myant, N.B., Soutar, A.K. and Knight, B.L. (1992) Atherosclerosis 92, 203–212.
[46] Hofmann, S.L., Eaton, D.L., Brown, M.S., McConathy, W.J., Goldstein, J.L. and Hammer, R.E. (1990) J. Clin. Invest. 85, 1542–1547.
[47] Strickland, D.K., Ashcom, J.D., Williams, S., Battey, F., Behre, E., McTigue, K., Battey, J.F. and Argraves, W.S. (1991) J. Biol. Chem. 266, 13364–13369.