Biochemical Pharmacology, Vol. 46, No. 10, pp. 1675–1680, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00
Pergamon Press Ltd

COMMENTARY

STRUCTURAL BASIS FOR THE PRESUMPTIVE ATHERO-THROMBOGENIC ACTION OF LIPOPROTEIN(a)

FACTS AND SPECULATIONS

ANGELO M. SCANU*

Departments of Medicine, Biochemistry and Molecular Biology and Committee on Genetics, University of Chicago, Chicago, IL 60637, U.S.A.

Background

All species of low density lipoproteins (LDL)†, the major carriers of cholesterol in the plasma, contain one copy of apoB100, an apolipoprotein with a mass of approximately 500 kDa containing 3–4% carbohydrates by weight [1]. It is now established that there are also in the circulation lipoproteins that contain apoB100 disulfide linked to a heavily glycosylated protein (30–35% carbohydrates by weight) called apolipoprotein(a) or apo(a). The link is presumed to occur between cys3734 of apoB100 and cys4057 of apo(a) [2]. There are important structural similarities between apo(a) and plasminogen in two main structural motifs: kringle and protease domains [3]. However, there are also several important differences between these two proteins: (1) plasminogen is present in all animal species; in turn lipoprotein(a) (Lp(a)) is confined to mammals high in the evolutionary scale, i.e. humans, apes and non-human primates except for the European hedgehog, an insectivor, (2) plasminogen is not associated with lipoproteins, whereas apo(a) floats with LDL due to its linkage to the highly lipophilic apoB100, (3) the plasma levels of plasminogen are relatively constant among individuals, whereas those of Lp(a) vary over 1000-fold, (4) there are important racial differences in the plasma levels of apo(a); this is not the case for plasminogen, (5) plasma Lp(a) but not plasminogen levels have been positively associated with an increased risk for atherosclerotic cardiovascular disease (ASCVD), (6) plasminogen has five kringles named 1 to 5; apo(a) is missing the first three, has 17 to 37 kringle 4-like repeats and one kringle 5-like domain, (7) the amino acid sequence of kringles 4 and 5 of plasminogen although similar differ significantly from those of apo(a), and (8) contrary to plasminogen, apo(a) cannot be converted into an active serine protease. Kringles consist of triple-looped structures containing approximately 80 amino acids exhibiting a three-dimensional organization maintained by three disulfide bonds linking highly conserved cysteine residues. Overall, it is apparent that in spite of the common ancestry of their genes both belonging to the same superfamily, apo(a) and plasminogen have evolved into two distinct proteins functioning under different metabolic control. This conclusion is in agreement with the observed lack of correlation between plasma levels of Lp(a) and plasminogen [4]. It is indeed puzzling that nature has helped in creating two protein molecules that during the course of millions of years have acquired distinct potential and become, under given sets of conditions, antagonistic to each other in key biological processes like that of fibrinolysis.

Athero-thrombogenic properties of apo(a)

The fact that Lp(a) has both LDL- and plasminogen-like properties has suggested that Lp(a) may represent a bridge between the areas of atherosclerosis and thrombosis [5]. However, the cardiovascular pathogenicity of this lipoprotein would only apply to plasma levels above arbitrary cut-off points between 20 and 30 mg/dL, in terms of overall Lp(a) mass, or 5 to 7 mg/dL in terms of protein, i.e. apoB100 plus apo(a) [4, 6]. The incidence of high plasma Lp(a) subjects varies from 15 to 20% depending particularly on race; for instance Afro-Americans have plasma levels that are 2- to 3-fold higher than whites for reasons that are not yet apparent. Plasma levels of Lp(a) are predominantly under genetic control and may be dependent on either the number of K-4 repeats [7] and/or on the activity of the promoter region of the apo(a) gene [8].

The notion associating high plasma levels of Lp(a) to cardiovascular pathogenicity has been mainly derived from retrospective epidemiological studies and has led to the speculation that under these conditions an increased number of Lp(a) particles traverse the arterial vessel endothelium and accumulate in the arterial intima [1, 9]. Here, these particles would undergo oxidation and be taken up by the resident macrophages which, as a consequence of the increased fat load, would be transformed into foam cells. In addition or alternatively, either native or modified Lp(a) particles, once in the intima,

* Tel. (312) 702-1775; FAX (312) 702-4534.

† Abbreviations: LDL, low density lipoproteins; VLDL, very low density lipoproteins; HDL, high density lipoproteins; Lp(a), lipoprotein(a), apo(a), apolipoprotein(a); TG, triglyceride(s); CE, cholesteryl ester(s); and ASCVD, atherosclerotic cardiovascular disease.

would become trapped through complexation with matrix components, i.e. proteoglycans, glycosaminoglycans, fibronectin, and collagen [10]. Immunodetectable apo(a) in the atherosclerotic plaques both in post-surgical and post-mortem specimens has been reported by several studies as well as its co-localization with fibrin [11]. However, the fact that apo(a) is present in the arterial wall does not necessarily imply that Lp(a) is causally related to the development of the atherosclerotic plaque since localization of apo(a) at the lesion sites may simply represent a secondary phenomenon. Promising clues in this direction come from recent studies showing that mice made transgenic for human apo(a), once fed an atherogenic diet, become more susceptible to the development of arterial lesions as compared with their control littermates [12]. The molecular mechanisms attending this interesting observation are yet to be elucidated, but one may speculate that apo(a) may facilitate the transfer of fats from the circulation to the arterial intima perhaps by modifying the endothelial permeability. It should be noted that whereas in the human plasma most of the apo(a) is associated with Lp(a), in those transgenic mice apo(a) circulated free due to its inability to form a linkage with mouse apoB100 [13]. Thus, the observations made in the mouse model may not necessarily apply to human biology.

A potential thrombogenic action of Lp(a) has been suggested by the striking homology of apo(a) with plasminogen on the assumption that the former may interfere with the physiological functions of the latter [1, 14, 15]. Some corroboration for this hypothesis has come from *in vitro* studies showing that Lp(a) can retard plasmin generation and also interferes with the binding of plasminogen to a specific binding site(s) on the cell membrane. However, in spite of these promising results, there are not yet convincing data documenting a thrombogenic action of Lp(a) *in vivo*. The reasons for the apparent discrepancy between *in vitro* and *in vivo* data are not apparent but invite consideration of several possibilities:

1. The action of Lp(a) at the cell surface may not be related to plasma levels, but rather to focal events.
2. *In vivo*, the interaction of Lp(a) with either the fibrinolytic system or the plasminogen binding sites in the cell membranes may be partially prevented by other blood components like tetranectin and fibronectin.
3. The action of Lp(a) may be dependent on the presence of certain classes of plasma lipoproteins, for instance triglyceride TG-rich particles originating either during the post-prandial state or in the fasting plasma of subjects with primary dyslipoproteinemias.
4. Except for subjects exhibiting very high plasma levels of Lp(a), and those are very few, the thrombogenic action of Lp(a) may only be manifested in the presence of thrombogenic diatheses attended for instance by an increased levels of plasma fibrinogen, factor VIIc, PAI-1 or α-2 antiplasmin. The thrombogenic action may also require pre-existing stenotic or injured arteries as recently shown experimentally in Cynamolgous monkeys [16].

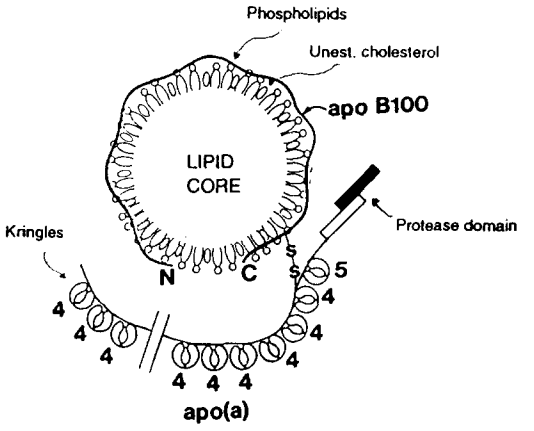

Fig. 1. Factors contributing to the heterogeneity of Lp(a). Each lipoprotein particle has a *lipid core* that contains the hydrophobic lipids triglycerides (TG) and cholesteryl esters (CE) and a *polar surface layer* comprised of phospholipids, unesterified cholesterol and protein. This protein is made of apoB100 disulfide linked to apo(a). Contributors to the Lp(a) size/density heterogeneity are the amount of lipids in the core and their relative abundance. On this basis, we can classify Lp(a) as TG- or CE-rich particles comparable to the apoB100-containing VLDL and LDL, respectively. Also contributing to the Lp(a) heterogeneity is the protein moiety. Since all Lp(a) particles contain only one mole of apoB100, the protein mass of Lp(a) will depend on the mass of the apo(a) polymorphs varying from 300 to 800 kDa. Thus, differences in either core, surface or both can generate several lipoprotein species. These differences are mainly under the control of the apo(a) gene but may be also influenced by either other genes or metabolic factors.

Previous studies either *in vitro* or *in vivo* have not taken into consideration the issue of Lp(a) polymorphorphism both at the gene and at the protein levels, an issue that is discussed in the following section.

Lp(a) polymorphism

It is now recognized that like the other plasma lipoproteins, Lp(a) represents a class of particles that differ in size, density and composition pretty much as in the case of the apoB100-containing particles for which an established nomenclature exists, i.e. very low density lipoproteins (VLDL), LDL-1, LDL-2, and dense LDL [1]. However, Lp(a) heterogeneity represents a comparatively more complex issue (see Fig. 1) also attended by technical difficulties. Currently, the best recognized species are those that we may call "classic" Lp(a), i.e. cholesteryl esters (CE), LDL-like particles. Their density and size vary depending on the amount of total lipids that they carry and on the protein mass represented by one mole of apoB100 (around 500 kDa) and one or two moles of apo(a) varying between 300 and 800 kDa. The dense Lp(a) particles containing the high molecular weight apo(a) can be separated by density gradient ultracentrifugation

from the light Lp(a) particles having low molecular weight apo(a). This separation is optimal when dealing with heterozygous individuals where the two apo(a)s exhibit significant differences in molecular weight and also in homozygous subjects Lp(a) band. Thus, from the knowledge of the apo(a) phenotype one can reasonably predict Lp(a) particle heterogeneity.

An unanswered issue is whether the association of apoB100 with apo(a) occurs either in the liver cell, in the circulation or both. In terms of TG-rich particles containing apoB100-apo(a) that we call TG-Lp(a), they have been shown to occur both in the medium of long-term primary hepatocyte cultures [17] and in the late post-prandial state of a meal enriched in saturated fatty acids [18]. These particles may be the equivalents of the apoB100-containing VLDLs and as such undergo progressive lipolysis by the action of the enzymes lipoprotein lipase and hepatic lipase to generate denser CE-rich Lp(a) species. Documentation for the *in vivo* occurrence of this lipolytic process is yet to be obtained. However, its occurrence can be documented *in vitro* by subjecting isolated post-prandial TG-Lp(a) to lipolysis by the enzyme lipoprotein lipase.*

The two likely possibilities for the origin of CE-Lp(a) particles are either intracellular production followed by a direct secretion by the liver or formation in the circulating plasma as a result of the linking of apo(a) to apoB100-containing particles. These two possibilities are not mutually exclusive. The evidence supporting the latter possibility rests on experiments in mice made transgenic for human apo(a). When those animals were injected intravenously with human LDL, these lipoproteins associated with the circulating free recombinant apo(a) for form Lp(a) particles, which by the criteria used were considered indistinguishable from those of naturally occurring Lp(a) [13]. It has also been shown that a recombinant preparation of apo(a) containing 17 kringles can associate with human LDL *in vitro* [13]. Based on these data one may conclude that at least some of the species of Lp(a) are generated in the plasma, suggesting that the rate-limiting step for their formation is the amount of free reactive apo(a) and the type of LDL species in the circulation.

If the linking between apoB100 and apo(a) can, in fact, occur in the plasma, it should be possible to establish a correlation between LDL and Lp(a) heterogeneity and also assess whether apo(a) preferentially associates with specific classes of LDL. It must be noted that no correlation between plasma levels of LDL and Lp(a) has been described and that agents that lower the plasma levels of LDL even markedly have no effect on Lp(a). What we know is that by linking to apo(a), Lp(a) changes dramatically its metabolic fate. In this regard it is interesting to note that TG-Lp(a) contrary to CE-Lp(a) particles contain apoE, an apolipoprotein that may direct them to receptors that recognize apoE as a ligand [18]. Thus, apolipoproteins extrinsic to the Lp(a) structure may determine the catabolism of these particles. In terms of the CE-Lp(a) particles, the knowledge of their catabolic fate is not yet firmly established although a significant participation of the LDL receptor appears unlikely [1].

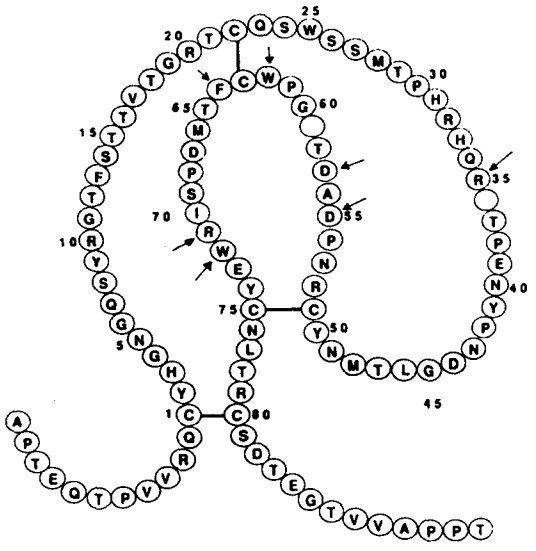

Fig. 2. Human kringle 4-37 with its multiple loop structure held together by three disulfide bonds. The position of the 7 amino acids comprising the lysine binding pocket is shown by arrows. The same amino acids are present in human plasminogen kringle 4 except that arg35 is replaced by lys.

Functional heterogeneity

The information associating changes in Lp(a) structure to changes in function is currently limited. Only recent studies have shown that Lp(a) of different density exhibit differences in fibrin binding attributable to apo(a) size [19]. The molecular basis for this observation is unclear except that it may relate to differences in the number of apo(a) K4 repeating units characterizing the two Lp(a) species used. It is also possible that the degree of particle lipidation may have an effect on the binding of Lp(a) to lysine/fibrin, but this is an area where information is lacking. In this respect it would be important to gather more experimental data on the TG-Lp(a) particles.

Substantial new knowledge on Lp(a) functional polymorphism has been gathered recently at the level of apo(a) sequence polymorphism. These studies have shown that in apo(a) the lysine/fibrin binding pocket that comprises seven amino acid residues (two anionic, asp55/asp57; two cationic, arg35/arg71 and three nonpolar, trp62/trp72/phe64) is located in kringle 4-37 (see Fig. 2) and that the substitution of trp72 by arg causes a defective binding of Lp(a) for lysine/fibrin [20]. This original observation was made in the rhesus monkey, but more recent studies have shown that mutations in kringle 4-37 can also occur in humans.†

The above findings are of interest in that they document that apo(a) can undergo mutations that are capable of affecting its thrombogenic potential.

* Edelstein C and Scanu AM, unpublished observations.
† Scanu AM, unpublished observation.

Based on the *in vitro* results, the apo(a) mutant with a trp72 → arg substitution should be considerably less thrombogenic than its wild-type counterpart and thus relatively more "benign" from the cardiovascular standpoint. In this context it would be of interest to explore whether people in the age group above 70 with no history of ASCVD and high plasma levels of Lp(a) may have mutated "benign" forms of apo(a).

Considerations in terms of preventive cardiology

The notion that Lp(a) is a quantitative trait expressed early in life and transmitted in an autosomal co-dominant mode [6, 7] along with the postulated cardiovascular pathogenicity of this lipoprotein particle invite the conclusion that it would be important to know the plasma Lp(a) levels in all members of families with history of premature ASCVD. Those with high plasma Lp(a) would know that they have inherited a cardiovascular risk factor and would be strongly motivated to avoid additional risks. On the other hand, those subjects with a "benign" form of Lp(a) would be at a comparatively lesser risk even in the presence of high plasma levels of Lp(a). Based on current knowledge it would not be necessary to determine plasma Lp(a) levels in the whole population but rather target the analyses at a high risk particularly when the other lipids and lipoprotein parameters cannot readily explain the history of ASCVD [1]. The search for potentially benign Lp(a) mutants is currently confined to very few specialized laboratories but once the techniques are refined and standardized they should enter the general clinical chemistry laboratory to complement the data on Lp(a) quantification. It should be kept in mind that quantification of Lp(a) requires direct analyses contrary to LDL whose cholesterol values can be derived from the knowledge of total plasma cholesterol and high density lipoprotein (HDL) cholesterol [21].

Therapeutic considerations

On the premise that synthesis of apo(a) occurs in the liver and that this process plays an important role in the regulation of the plasma levels of Lp(a), it is apparent that the most fruitful efforts at lowering plasma Lp(a) levels should be at the hepatocyte level. However, our current knowledge of the factors that regulate the expression of the apo(a) gene is rather limited and, in consequence, none of the approaches to treat high plasma Lp(a) levels has a solid rationale. The unresponsiveness of plasma Lp(a) to diets is well established although no rigorous long-term studies have been carried out to-date. If in fact the TG-Lp(a) particles prove to be important contributors to the plasma Lp(a) levels, then special diets designed to reduce hepatic TG synthesis may prove to be useful. The same consideration may apply to pharmaceutical agents among which niacin in doses of 3–4 g daily has been shown to lower plasma Lp(a) levels significantly [22]. However, this positive experience has not been universal. In general, we must wait for further developments in the area of Lp(a) pharmacology; while waiting, it is advisable to target the efforts towards the correctable risk factors for ASCVD [23].

Considerations about "normal" plasma levels of Lp(a)

Plasma levels of Lp(a) are considered as normal when they are below cut-off points above which Lp(a) has been associated with an increase in the risk for ASCVD. However, the reported cut-off points have varied from 20 to 30 mg/dL in terms of total Lp(a) mass and are in need of standardization. Irrespective of these limitations, it is clear that about 20% of the population enjoys apparently "safe" levels [1, 7], raising questions about their potential physiological role. Unfortunately, this is an area where factual knowledge is lacking and only speculations are permissible. One possibility is that the Lp(a) cholesterol that escapes the LDL receptor route is used for cell membrane formation in rapidly regenerating tissues as in wound healing [9]. Another highly speculative possibility is that, at physiological levels, Lp(a) may contribute to the preservation of the anticoagulant state at the endothelial surface and also favor the generation of plasmin, the serine protease involved in clot lysis.

Lp(a) physiology may also relate to its heterogeneity. In this context preliminary data suggest that the light forms of Lp(a) are potentially less thrombogenic than the dense forms, i.e. the ones containing high molecular weight species of apo(a) irrespective of plasma levels [17]. It is also possible that Lp(a) mutants with a lysine/fibrin binding defect may serve beneficial functions along the lines discussed above. The presence of Lp(a) only in animals high in the evolutionary scale (except for the European hedgehog) also suggests that having Lp(a) may be of advantage from the evolutionary standpoint. In this respect we need to know whether animals classified as Lp(a) based on protein analyses remain negative on both the RNA and DNA levels.

Summary and perspectives

During the past 6 years, Lp(a) has emerged from relative obscurity to a position of prominence in the area of cardiovascular research. Those interested in lipids and atherosclerosis have had and continue to have an active interest in this lipoprotein particle because of its LDL characteristics and its potential for atherogenesis. At the same time those interested in coagulation and fibrinolysis have welcomed the investigation of a new factor that appears to play a role in their experimental systems. As a result, two groups of researchers traditionally following independent investigational paths have found something in common to explore and in some instances joined forces in this exploration. In general, Lp(a) has facilitated the bridging of two important research areas and promoted joint productivity and new knowledge on many aspects of the biology of Lp(a), on the mechanism(s) underlying the observation derived mainly from epidemiological data that plasma Lp(a) levels are associated with an increased risk for ASCVD, and on the possible physiological significance of this class of lipoprotein particles.

In terms of Lp(a) biology the recent elucidation of the structure of the apo(a) gene and its promoter region [24, 25] has opened up a challenging area of

research concerning the factors controlling gene expression and regulation. Advances have also been made in the understanding of the mechanisms of Lp(a) assembly utilizing both cell culture systems [26] and transgenic mice [12, 13]. The information that has emerged thus far, although preliminary, holds promise for exciting new developments. The same applies to the area of metabolism where much remains to be done particularly in the area of the structure/function relationships among various Lp(a) species.

From the pathological standpoint we still know little about the mechanisms underlying the epidemiological observation that Lp(a) represents an independent risk factor for ASCVD [4, 27]. This correlation is not an undisputed one in that there are some discordant data and views on the subject. The current uncertainties may be explained by the following: (1) poor definition of the cut-off points of normal Lp(a) levels, (2) lack of standardization of the Lp(a) assay, (3) studies conducted without taking into account Lp(a) polymorphism and thus population heterogeneity, and (4) too much reliance on retrospective studies without a sufficiently critical appraisal of potentially confounding coexisting cardiovascular risk factors. Moreover, from the mechanistic standpoint it is yet unclear whether the cardiovascular pathogenicity of Lp(a) should be viewed in terms of atherogenesis, thrombogenesis or both. It is reasonably well established that apo(a) is present in the atherosclerotic plaque but its causative role in the formation of the lesion has not been established. Among issues to be resolved are: (1) the contribution of Lp(a) cholesterol vs LDL cholesterol to foam cell formation, (2) the importance of apo(a)-fibrin(ogen) complexation in the arterial intima, and (3) the role of apo(a) oxidation as well as its interaction with extracellular matrices. It would also be important to define how much of the atherothrombotic action is dependent on just plasma Lp(a) levels or on the actual amount of Lp(a) or modified Lp(a) trapped in the arterial intima, considering that the two events may not be necessarily related.

Major advances in the near future are expected to derive from the studies on gene expression and on the identification of the regulatory elements acting at the level of the promoter region of the apo(a) gene. Although an inverse correlation between plasma levels of Lp(a) and apo(a) size due to the number of kringle 4 repeats has been recognized, it has now become apparent that there are cases where this correlation does not apply and that heterogeneity in Lp(a) plasma levels may be present in subjects with a single apo(a) isoform [28]. In those cases, studies at the level of the promoter region, correlating for instance sequence data with plasma Lp(a) levels, may prove useful, and preliminary data to this effect have appeared.

As to the physiological role of Lp(a) the paucity of knowledge in this area should encourage concerted efforts to fill this gap. It is clear that Lp(a) is not essential for life in that many animal species have no detectable Lp(a) in their plasma although in these $Lp(a)^-$ animals no systematic search for RNA message has ever been carried out. In this regard, transgenic animals as well as animals transfected with the apo(a) gene by homologous recombination may provide helpful clues. Moreover, the study of human mutants should prove highly valuable in this regard.

Acknowledgements—The original studies by the author cited in this review were supported by Program Project Grant NHLBI 18577. The author wishes to thank Celina Edelstein for constructive suggestions and Jill Voss for help in the editing of the manuscript.

REFERENCES

1. Scanu AM and Fless GM, Lipoprotein (a): Heterogeneity and biological relevance. *J Clin Invest* **85**: 1709–1715, 1990.
2. Guevara J Jr, Spurlino J, Jan AY, Yang C-Y, Tulinsky A, Venkataram Prasad BV, Gaubatz JW and Morrisett JD, Proposed mechanisms for binding of apo(a) kringle type 9 to apoB100 in human lipoprotein(a). *Biophys J* **64**: 686–700, 1993.
3. McLean JW, Tomlinson JE, Kuang WJ, Eaton DL, Chen EY, Fless GM, Scanu AM and Lawn RM, cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. *Nature* **330**: 132–137, 1987.
4. Scanu AM, Lawn R and Berg K, Lipoprotein(a) and atherosclerosis. *Ann Intern Med* **115**: 209–218, 1991.
5. Scanu AM, Lipoprotein(a): A potential bridge between the fields of atherosclerosis and thrombosis. *Arch Pathol Lab Med* **112**: 1045–1047, 1988.
6. Berg K, Lp(a) lipoprotein: An overview. In: *Lipoprotein (a)* (Ed. Scanu AM), pp. 1–20. Academic Press, New York, 1990.
7. Utermann G, The mysteries of lipoprotein(a). *Science* **246**: 904–910, 1989.
8. Wade DP, Clarke JG, Lindahl GE, Liu AC, Zysow BR, Meer K, Schwartz K and Lawn RM, 5′ control regions of the apolipoprotein(a) gene and members of the related plasminogen gene family. *Proc Natl Acad Sci USA* **90**: 1369–1373, 1993.
9. Lawn RM, Lipoprotein(a) in heart disease. *Sci Am* **6**: 54–60, 1992.
10. Bihari-Varga M, Gruber E, Rotheneder M, Zechner R and Kostner GM, Interaction of lipoprotein(a) and low density lipoprotein with glycosaminoglycans from human aorta. *Arteriosclerosis* **8**: 851–857, 1988.
11. Beisiegel V, Niendorf A, Wolf D, Reblin T and Rath M, Lipoprotein(a) in the arterial wall. *Eur Heart J* **11** (Suppl E): 174–183, 1990.
12. Lawn RM, Wade DP, Hammer RE, Chiesa G, Verstuyft JG and Rubin EM, Atherogenesis in transgenic mice expressing human apolipoprotein(a). *Nature* **360**: 670–672, 1992.
13. Chiesa G, Hobbs HH, Koschinsky ML, Lawn RM, Maika SD and Hammer RE, Reconstitution of lipoprotein(a) by infusion of human low density lipoprotein into transgenic mice expressing human apolipoprotein(a). *J Biol Chem* **267**: 24369–24374, 1992.
14. Loscalzo J, Lipoprotein (a). A unique risk factor for atherothrombotic disease. *Arteriosclerosis* **10**: 671–679, 1990.
15. Miles LA and Plow EF, Lp(a): An interloper into the fibrinolytic system? *Thromb Haemost* **63**: 331–335, 1990.
16. Koudy Williams J, Bellinger DA, Nichols TC, Griggs TR, Bumol TF, Fouts RL and Clarkson TB, Occlusive arterial thrombosis in cynomolgus monkeys with varying plasma concentrations of lipoprotein(a). *Arteriosclerosis Thromb* **13**: 548–554, 1993.
17. Edelstein C, Davidson NO and Scanu AM, Oleate stimulates the formation of triglyceride-rich particles containing apoB100-apo(a) in long-term primary

cultures of human hepatocytes. *Chem Phys Lipids*, in press.
18. Scanu AM, Pfaffinger D and Edelstein C, Postprandial Lp(a): Identification of a triglyceride-rich particle containing apo E. *Chem Phys Lipid*, in press.
19. Fless GM and Snyder ML, Polymorphic forms of Lp(a) with different structural and functional properties: Cold induced self-association and binding to fibrin and lysine-Sepharose. *Chem Phys Lipids*, in press.
20. Scanu AM, Miles LA, Fless GM, Pfaffinger D, Eisenbart J, Jackson E, Hoover-Plow JL, Brunck T and Plow EF, Rhesus monkey lipoprotein(a) binds to lysine Sepharose and U937 monocytoid cells less efficiently than human lipoprotein(a). Evidence for the dominant role of kringle 4_{37}. *J Clin Invest* **91**: 283–291, 1993.
21. Kurschinski DT, Dennen DA, Garcia M and Scanu AM, Letter to the Editor: "Plasma lipoprotein(a) and the Friedewald formula". *Clin Chem* **35**: 2157, 1989.
22. Brewer HB, Effectiveness of diet and drugs in the treatment of patients with elevated Lp(a) levels. In: *Lipoprotein(a)* (Ed. Scanu AM), pp. 211–218. Academic Press, New York, 1990.
23. Scanu AM, Lipoprotein(a): A genetic risk factor for premature coronary heart disease. *JAMA* **267**: 3326–3329, 1993.
24. Malgaretti N, Acquati F, Magnaghi P, Bruno L, Pontoglio M, Rocchi M, Saccone S, Della Valle G, D'Urso M, LePaslier D, Ottolenghi S and Taramelli R, Characterization by yeast artificial chromosome cloning of the linked apolipoprotein(a) and plasminogen genes and identification of the apolipoprotein(a) 5′ flanking region. *Proc Natl Acad Sci USA* **89**: 11584–11588, 1992.
25. Ichinose A, Multiple members of the plasminogen-apolipoprotein(a) gene family associated with thrombosis. *Biochemistry* **31**: 3114–3118, 1992.
26. White AL, Rainwater DL and Lanford RE, Intracellular maturation of apolipoprotein(a) and assembly of lipoprotein(a) in primary baboon hepatocytes. *J Lipid Res* **34**: 509–517, 1993.
27. Berg K, Inherited lipoprotein variation and atherosclerotic disease. In: *The Biochemistry of Atherosclerosis* (Ed. Scanu AM), pp. 419–480. Marcel Dekker, New York, 1979.
28. Cohen JC, Chiesa G and Hobbs HH, Sequence polymorphisms in the apo(a) gene: Evidence for dissociation between apo(a) size and plasma Lp(a) levels. *J Clin Invest* **91**: 1630–1636, 1993.