Editorial

Lipoprotein(a) and Atherosclerosis

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Lipoprotein(a) [Lp(a)] is composed of 1 particle of LDL attached to apo(a), a glycoprotein that has striking homology to plasminogen and tremendous size heterogeneity owing to the presence of multiple repeats of plasminogen-like kringle 4 modules (see References 1 and 2 for a review). Since Lp(a) was discovered by Berg, numerous cross-sectional and prospective studies have revealed associations between high plasma levels of Lp(a) and atherosclerotic vascular diseases, such as coronary heart disease and stroke (see References 1 and 3 for a review). Although several pathogenic roles for Lp(a) have been proposed, it has not been completely elucidated how high Lp(a) levels are associated with vascular diseases. Transgenic animal models give invaluable means to provide answers to the intractable question of whether high Lp(a) causes atherosclerosis and not vice versa. Lawn and colleagues[4] first reported the generation of apo(a) transgenic mice in 1992. Fatty streak lesion formation in the aortic sinus of these mice was substantially increased compared with that of the nontransgenic controls when fed an atherogenic diet. However, another laboratory failed to reproduce the atherogenic effects of the overexpressed apo(a) with or without the overexpressed human apoB-100, even though the same transgene was used.[5,6] It may be possible that a certain strain of mouse is resistant to the atherogenic effects of apo(a). In this context, apo(a) transgenic rabbits have emerged as an alternative model in which the atherogenicity of apo(a) as well as that of Lp(a) can be reasonably reevaluated. Recently, 2 lines of apo(a) transgenic rabbits have been reported independently.[7,8] In the current issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Fan and colleagues[9] have demonstrated that transgenic expression of apo(a) in the liver aggravates atherosclerosis in rabbits fed a high-cholesterol diet.

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In contrast to mice whose atherosclerosis is normally limited to the area of the aortic sinus, the sites of lesions in the cholesterol-fed rabbits were more widely distributed in the apo(a) transgenic line.[9] Lesion formation was increased in all of the sites examined: the aorta, carotid, iliac, and coronary arteries. This diffuse spatial distribution may be related to the increased incidence of coronary, peripheral vascular, and cerebrovascular diseases in patients with increased Lp(a) levels. It is of particular interest that atherosclerotic lesions were mostly composed of vimentin-positive immature or dedifferentiated smooth muscle cells (SMCs), not of macrophages. Together with the observation that apo(a) was deposited almost exclusively in the extracellular matrixes, the authors speculated that apo(a) stimulates the phenotypic transformation of vascular SMCs. In this regard, impaired activation of transforming growth factor-β (TGF-β) was proposed to explain the mitogenic effects of apo(a) on SMCs.[10] Because of its structural resemblance to plasminogen, apo(a) has inhibitory effects on the production of plasmin by competing with plasminogen. Because latent TGF-β is converted to its active form by means of plasmin, the activation of TGF-β is suppressed in the presence of increased concentrations of apo(a). Active TGF-β has inhibitory effects on the proliferation and migration of SMCs. Therefore, apo(a) may stimulate the proliferation and migration of SMCs through inhibiting TGF-β activation as a whole, thus contributing to the development of atherosclerosis. Neither phenotypic transformation nor proliferation of SMCs has, however, been directly demonstrated in the apo(a) transgenic mice.

In addition to the mitogenic effects of apo(a) on SMCs, apo(a) is believed to possess thrombogenic properties. For the reasons mentioned above, apo(a) is considered to inhibit fibrinolysis by competing with plasminogen. Given that this presumption is correct, mural thrombi, once generated, are expected to be resistant to fibrinolytic degradation, resulting in exaggerated responses of wound repair. However, compelling in vivo evidence for the thrombogenic properties of apo(a) is lacking. It will be of great interest to test these attractive, but still elusive, hypotheses in the apo(a) transgenic rabbits.

This animal model is also useful for investigating the property of association between apo(a) and rabbit apoB-100. In humans, apo(a) is covalently associated with Cys-4326 of apoB-100. In mice, the association of apo(a) with apoB-100 is not covalent because mouse apoB-100 lacks Cys-4326.[11] In rabbits, apo(a) is associated with rabbit apoB-100 more tightly than with mouse apoB-100 because nondenaturing polyacrylamide gel electrophoresis (PAGE) showed that most of apo(a) was in Lp(a).[7–9] However, the association might not be covalent because apo(a) and apoB-100 migrated differently under nonreducing conditions of SDS-PAGE. This finding is consistent with the fact that rabbit apoB-100 also lacks Cys-4326. In apo(a) yeast artificial chromosome transgenic rabbits, as reported by Rouy et al,[7] $\approx$20% of plasma apo(a) is covalently associated with rabbit apoB-100 via a cysteine residue other than Cys-4326. It would also be interesting to know which is more atherogenic, free apo(a) or Lp(a). Introduction of the apo(a) transgene into human apoB-100 transgenic mice did not significantly potentiate the development of aortic fatty lesions in the absence or presence of the LDL receptor.[5,6,12] Probably the atherogenic effects of

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apoB-100 may have overwhelmed the subtle effects of apo(a) and masked the difference.

New biological functions have been increasingly assigned to Lp(a) and/or apo(a): chemotactic activity on monocytes, angiogenic effects because of resemblance to angiostatin, presence of platelet-activating factor acetylhydrolase activity, enhancement of the expression of intercellular adhesion molecule-1 on endothelial cells, and so forth. Their physiological relevance can be tested in vivo by using apo(a) transgenic rabbits.

Besides the mystery of bona fide functions of Lp(a), extreme polymorphism of apo(a) and huge variation of the plasma levels of Lp(a) (up to 1000-fold) have fascinated many clinical geneticists. The most polymorphic region of apo(a) is the kringle 4–encoding region. The first (type 1) and last 8 (types 3 through 10) repeats of the kringle 4 tandem array are present in only 1 copy. The number of type 2 kringle 4 sequences varies from 3 to 43 and is responsible for the size heterogeneity.[13] Ninety percent of the variation in plasma levels is accounted for by the apo(a) gene. Forty percent to 70% of the variation is explained by the size of the apo(a) isoform, which is inversely related to the plasma levels of Lp(a), which are largely determined by the difference in the production rate in the liver. Moreover, there is a great difference in the distribution of plasma levels of Lp(a) among different ethnic groups. For example, African-Americans have higher plasma levels of Lp(a) than do whites. The isoform-concentration relationship can be easily tested by generating transgenic animals with the use of constructs that are designed to express different isoforms.

More important, it remains unknown whether pharmacological intervention to lower Lp(a) levels inhibits the progression of atherosclerosis. Currently, sex steroid hormones and nicotinic acids are known to significantly decrease plasma levels of Lp(a). Given the difficulties encountered in clinical studies, the apo(a) transgenic rabbit should be an excellent animal model in which the antiatherogenic efficacy of these compounds can be tested preclinically.

References

1. Utermann G. Lipoprotein(a). In: Scriver CR, Beaudet AL, Sly WS, eds. *The Metabolic and Molecular Bases of Inherited Disease*. New York, NY: McGraw-Hill; 1995:1887–1912.
2. Hobbs H, White AL. Lipoprotein(a): intrigues and insights. *Curr Opin Lipidol*. 1999;10:225–236.
3. Djurovic S, Berg K. Epidemiology of Lp(a) lipoprotein: its role in atherosclerotic/thrombotic disease. *Clin Genet*. 1997;52:281–292.
4. Lawn R, Wade DP, Hammer RE, Chiesa G, Verstuyft JG, Rubin EM. Atherosclerosis in transgenic mice expressing human apolipoprotein(a). *Nature*. 1992;360:670–672.
5. Mancini F, Newland DL, Mooser V, Murata J, Marcovina S, Young SG, Hammer RE, Sanan DA, Hobbs HH. Relative contributions of apolipoprotein(a) and apolipoprotein-B to the development of fatty lesions in the proximal aorta of mice. *Arterioscler Thromb Vasc Biol*. 1995;15:1911–1916.
6. Sanan D, Newland DL, Tao R, Marcovina S, Mooser V, Hammer RE, Hobbs HH. Low density lipoprotein receptor-negative mice expressing human apolipoprotein B-100 develop complex atherosclerotic lesions on a chow diet: no accentuation by apolipoprotein(a). *Proc Natl Acad Sci U S A*. 1998;95:4544–4549.
7. Rouy D, Duverger N, Lin SD, Emmanuel F, Houdebine LM, Denefle P, Viglietta C, Gong E, Rubin EM, Hughes SD. Apolipoprotein(a) yeast artificial chromosome transgenic rabbits: lipoprotein(a) assembly with human and rabbit apolipoprotein B. *J Biol Chem*. 1998;273:1247–1251.
8. Fan J, Challah M, Shimoyamada H, Shiomi M, Marcovina S, Watanabe T. Defects of the LDL receptor in WHHL transgenic rabbits lead to a marked accumulation of plasma lipoprotein(a). *J Lipid Res*. 2000;41:1004–1012.
9. Fan J, Shimoyamada H, Challah M, Sun H, Marcovina S, Honda K, Watanabe T. Transgenic rabbits expressing human apolipoprotein(a) develop more extensive atherosclerotic lesions in response to a cholesterol-rich diet. *Arterioscler Thromb Vasc Biol*. 2000;21:88–94.
10. Grainger D, Kemp PR, Liu AC, Lawn RM, Metcalfe JC. Activation of transforming growth factor-β is inhibited in transgenic apolipoprotein(a) mice. *Nature*. 1994;370:460–462.
11. Chiesa G, Hobbs HH, Koschinsky ML, Lawn RM, Maika SD, Hammer RE. Reconstitution of lipoprotein(a) by infusion of human low density lipoprotein into transgenic mice expressing human apolipoprotein(a). *J Biol Chem*. 1992;267:24369–24374.
12. Callow M, Verstuyft J, Tangirala R, Palinski W, Rubin EM. Atherosclerosis in transgenic mice with human apolipoprotein B and lipoprotein (a). *J Clin Invest*. 1995;96:1639–1646.
13. Lackner C, Cohen JC, Hobbs HH. Molecular definition of the extreme size polymorphism in apolipoprotein(a). *Hum Mol Genet*. 1993;2:933–940.

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