Enhanced Atherosclerosis in Lp(a) WHHL Transgenic Rabbits

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ABSTRACT: High lipoprotein(a) [Lp(a)] levels form a major risk factor for the development of atherosclerosis. The risk of elevated Lp(a) concentrations is significantly increased in patients who also have high levels of LDL cholesterol. Although the relation between Lp(a) and atherosclerosis has been reported in numerous studies, little is known about whether Lp(a) would exacerbate the complicated lesion formation *in vivo*. To test the hypothesis that increased plasma levels of Lp(a) may enhance the development of atherosclerosis in the setting of hypercholesterolemia, we generated WHHL transgenic rabbits expressing human apolipoprotein (a) and compared the atherosclerotic lesions with those of nontransgenic WHHL rabbits.

KEYWORDS: Lp(a); atherosclerosis; transgenic rabbit; WHHL; hypercholesterolemia

INTRODUCTION

Lipoprotein(a) [Lp(a)] is an atherogenic lipoprotein of both lipid composition and the presence of apolipoprotein (apo) B-100 [apoB-100]. Lp(a) is distinguished from LDL by an additional protein component designated as apolipoprotein (a) [apo(a)], which is complexed to apoB-100 by disulfide linkage.[1,2] Since the discovery of Lp(a) by Berg in 1963, numerous cross-sectional and prospective studies have revealed that high plasma levels of Lp(a) are associated with human cardiovascular disease such as coronary heart disease, stroke, and restenosis.[1,3–5]

To define the metabolic and pathologic consequences of Lp(a), our laboratory generated transgenic rabbits expressing human apo(a) and showed that human apo(a) is efficiently assembled into Lp(a) in the plasma.[6] Recently, we reported that Lp(a) substantially increases the development of atherosclerosis in transgenic rabbits fed a cholesterol-rich diet.[7] To further elucidate whether Lp(a) may enhance the development of atherosclerosis in the setting of hypercholesterolemia, we generated WHHL transgenic rabbits with LDL receptor deficiency and studied their atherosclerotic lesions.

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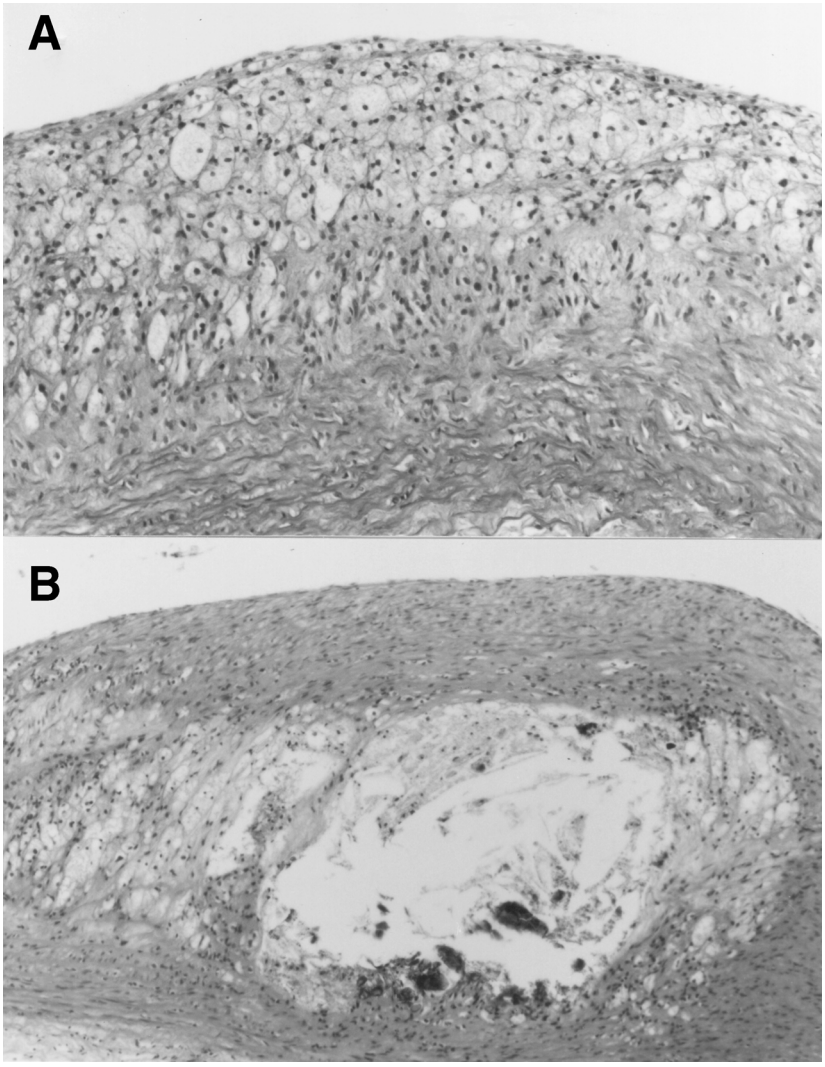

FIGURE 1. Aortic atherosclerosis from (**A**) non-Trg WHHL and (**B,C**) Trg WHHL rabbits. (**A**) fatty streak; (**B**) fibrous plaque; (**C**) complicated plaque associated with calcification. Magnification 100×. [*Figure 1C is on the following page.*]

METHODS

Transgenic (Trg) rabbits expressing human apo(a) were crossbred with homozygous WHHL rabbits, as described previously.[8] By selectively breeding, we obtained two groups of WHHL rabbits: nontransgenic (nonTrg) homozygous WHHL rabbits [apo(a)$^{0/0}$/LDLr$^{-/-}$] and homozygous Trg WHHL rabbits expressing human

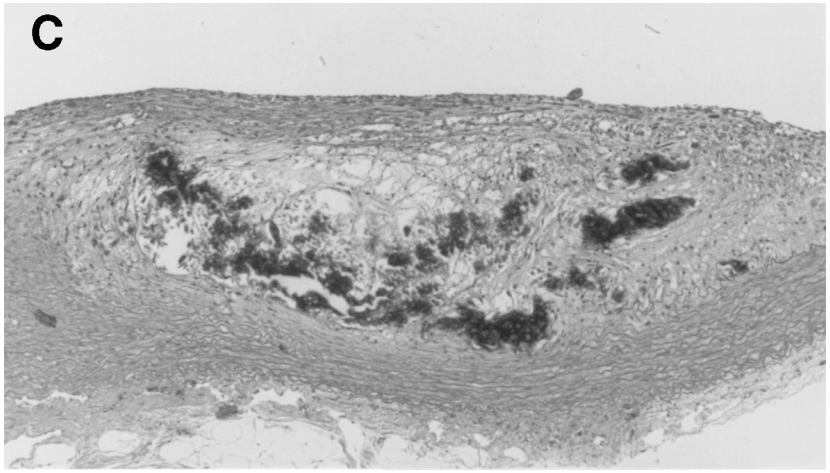

FIGURE 1C. [*Legend is on previous page*.]

apo(a) [apo(a)$^{+/0}$/LDLr$^{-/-}$]. These rabbits were fed a chow diet and were studied at the age of 7 months. Plasma lipids, lipoproteins, and aortic atherosclerosis were examined by the methods described recently.[7]

RESULTS AND DISCUSSION

Trg WHHL rabbits had similar levels of plasma total cholesterol, triglycerides, and HDL-cholesterol (data not shown). Also, their lipoprotein profiles were essentially identical (data not shown). Trg WHHL rabbits had as high as ~15 mg/dl of Lp(a) in plasma. Microscopically, compared to the lesions of non-Trg WHHL, which were mainly composed of foam cells (FIG. 1A), the atherosclerotic lesions in Trg rabbits were characterized by increased formation of fibrous plaques and atheroma (FIG. 1B and C). In particular, the lesions in Trg rabbits were often associated with calcification which was barely evident in non-Trg rabbits (FIG. 1C). The complicated lesions in Trg WHHL rabbits were uniformly found in aorta, carotid, iliac, and coronary arteries. Immunohistochemical study revealed that the lesions of transgenic WHHL rabbits were predominated by fibrous plaque, whereas in non-Trg rabbits, the lesions were largely those of fatty streaks, which were composed of macrophage-derived foam cells. When stained with antibodies against apo(a) and apoB, we could see that apo(a) was colocalized with apoB and closely associated with the lesions, suggesting that Lp(a) may be involved in lesion formation. By an image analysis system, we quantitated the lesions in all parts of aorta. Trg WHHL rabbits contained more advanced lesions in the aortic arch than did non-Trg WHHL littermates. In the thoracic and abdominal aortas, nonTrg WHHL had only fatty streak, whereas Trg WHHL had more than 60% advanced atherosclerosis.

We conclude that Lp(a) accelerates fibrous plaque formation and calcification in WHHL rabbits and that this transgenic WHHL rabbit model may provide a valuable model to study advanced atherosclerotic lesions.

The mechanism(s) for the complicated lesions found in Trg WHHL rabbits are currently unknown. We speculate that Lp(a) or apo(a) may enhance the expression of some genes that are related to calcification. This hypothesis is currently under investigation.

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