Gene Targets and Approaches for Raising HDL

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Susceptibility to atherosclerosis in humans is inversely correlated to the concentration of plasma HDL. Over the past 10 years, the question of how HDL plays a direct role in the prevention of atherosclerosis has been the focus of intense research. The antiatherogenic effect of HDL may involve (1) promoting reverse cholesterol transport, during which excess cholesterol is routed from extrahepatic tissues back to the liver for elimination or reuse; (2) inhibition of lipoprotein oxidation; and (3) direct protection of the vessel wall from damages. These issues and other questions involving HDL metabolism and its role in atherosclerosis have been successfully addressed by use of mice in which genes believed to participate in HDL structure, metabolism, and its protective action on the vessel wall are overexpressed or inactivated by gene targeting. The ultimate goal is to apply this knowledge to a therapeutic purpose, eg, increasing HDL levels in patients suffering from atherosclerosis. A few years ago, a start was made to explore gene therapeutic approaches directed at manipulating the expression of genes that increase HDL or decrease LDL levels. Adenoviruses have been most commonly used for the delivery of genes to various target tissues in vivo. Still, considerable advances will have to be made until safe, stable, and prolonged expression of exogenous genes may someday be achieved in humans. Here, we briefly summarize the insights gained on HDL metabolism and its role in atherosclerosis.4 Overexpression or inactivation of genes, primarily in mice. We also summarize and discuss the progress that has been made toward increasing the expression of HDL-raising genes by use of adenovirus gene transfer technology and briefly outline the problems that remain to be overcome and discuss current technological strategies to achieve this goal.

The Table shows a list of HDL-modifying genes that have been overexpressed and/or inactivated in mice. They include structural genes like apolipoprotein (apo) A-I, A-II, and A-IV as well as genes that encode proteins that act on the HDL particle, such as cholesteryl ester transfer protein, hepatic lipase, lecithin-cholesterol acyl transferase (LCAT), and phospholipid transfer protein; a receptor for HDL, ie, scavenger receptor B-I (SR-BI); and serum paraoxonase (PON1), a protein that uses HDL as carrier and may have a protective effect on the vessel wall by inhibiting the oxidative modification of LDL. From the results of genetic studies with animals and humans, it becomes evident that beneficial effects, ie, an increase of circulating HDL concentrations and protection against atherosclerotic lesion development, might be expected from the overexpression of apoA-I, LCAT, SR-BI, and PON1 in vivo. These genes therefore are potentially useful targets for gene therapeutic approaches.

ApoA-I was one of the first genes targeted for overexpression in animals. Transgenic mice overexpressing human apoA-I have increased HDL cholesterol levels. The homogeneous HDL species that is normally present in mouse plasma was replaced by 2 populations of HDL particles resembling human HDL<sub>3a</sub> and HDL<sub>3b</sub>. In addition, these experiments provided the first direct experimental evidence that HDL may play an important role in preventing atherogenesis.4 Overexpression of human apoA-I significantly reduced the progression of atherosclerotic lesions both in C57BL/6 mice fed a high-fat/choleic acid–containing diet and also in chow-fed apoE-deficient mice. In contrast, in mice lacking apoA-I, HDL cholesterol was reduced to ≈20% of normal levels, and the plasma of these animals almost completely lacked α-migrating HDL particles.7 Despite this dramatic reduction of HDL cholesterol, no atherosclerotic lesions were observed in any of the homozygous apoA-I–deficient mice examined for up to 15 months, indicating that the absence of apoA-I by itself is not sufficient to cause atherosclerosis in mice.8

In human LCAT-transgenic mice, a 20% to 60% increase in total cholesterol and cholesteryl esters, mainly in HDL, was observed.9,10 These studies using human A-I/LCAT–transgenic mice and human A-I/A-II/LCAT–transgenic mice demonstrated that small increases in LCAT activity are associated with large changes in lipoprotein cholesterol levels and that human LCAT has a preference for HDL containing human apoA-I. LCAT overexpression was found to regulate both LDL and HDL metabolism in cholesterol-fed rabbits and prevented diet-induced atherosclerosis.11 In contrast, HDLs in LCAT-transgenic mice display an abnormal composition and function and are ineffective in transporting HDL cholesterol to the liver, thus providing in vivo evidence for dysfunctional HDL as a potential mechanism leading to increased atherosclerosis in the presence of high plasma HDL levels.12 LCAT-deficient mice have reduced total cholesterol and HDL levels and provide an animal model for human LCAT-deficiency syndromes on which the role of LCAT in atherosclerosis and gene therapeutic approaches can be tested.13
SR-BI is a multifunctional receptor that also mediates HDL cholesterol transport into target tissues expressing SR-BI (mainly the adrenal gland and the liver). Overexpression of SR-BI by adenovirus gene transfer in mice resulted in the virtual disappearance of HDL from the plasma of the animals. Conversely, a knockout of SR-BI led to a doubling of plasma HDL cholesterol levels and an increase in the size of particles. The results from these studies reveal SR-BI to be a potential potent regulator of HDL levels in vivo.

PON1 is an esterase associated with HDLs in the plasma. It may confer protection against coronary artery disease by metabolizing proinflammatory oxidized lipids. HDLs isolated from PON1-deficient mice cannot protect LDLs from oxidation, and both LDL and HDL were shown to be more susceptible to oxidation than lipoproteins isolated from control mice in a tissue coculture model system. On an atherogenic diet, PON1-deficient mice developed somewhat larger atherosclerotic lesions than wild-type mice. These experiments suggest a moderately protective role for HDL-associated PON1 in vivo.

Conventional transgenic and knockout technologies have provided ample insight into the function of “HDL genes” and their direct or indirect roles in atherosclerosis. It is clear that augmentation of circulating HDL cholesterol exerts a protective effect against development of fatty streaks and may promote plaque regression. However, transgenic and knockout approaches are not applicable to the treatment of human patients. Viral gene transfer provides an attractive alternative for the delivery of the protective HDL-modulating genes, mainly to hepatocytes in vivo. A number of different adeno-viruses have been used to alter HDL levels in animals. These experiments have generally yielded results similar to those obtained by transgenic overexpression, thus validating this approach.

Studies on the use of adenoviral gene transfer for the prevention of atherosclerotic lesion development have aimed primarily at overexpressing apoA-I to raise HDL levels. In the studies by Kopfler et al and de Geest et al, overexpression of human apoA-I was achieved under the control of the powerful cytomegalovirus (CMV) promoter. Because of the short, transient nature of A-I protein expression, an antiantithrombotic effect was not observed. However, neointima formation after endothelial denudation was significantly reduced, indicating a direct protective effect of HDL on the vascular wall.

In the elegant study by Benoit et al in this issue of Circulation, long-term expression of human apoA-I by adenoviral gene transfer in mice has been achieved. In their experiments, the authors expressed the human A-I transgene under the control of the Rous sarcoma virus (RSV) promoter rather than the more commonly used CMV promoter. In wild-type mice, transgene expression from the RSV promoter persisted for ~3 weeks, only marginally longer than what is commonly observed for the CMV promoter. More importantly, however, when human apoA-I–transgenic mice were used as recipients, human apoA-I overexpression persisted for up to 10 weeks, suggesting that a response of the immune system against the heterologous protein was a major factor that prevented stable prolonged expression. This long-term overexpression of human apoA-I resulted in a clear antithrombotic effect. The study demonstrates that augmentation of expression of a normal endogenous gene, which does not cause an immunological response, by gene transfer is a potentially powerful therapeutic approach to slow or even reverse the progression of atherosclerotic disease. Still, several problems remain to be solved that affect gene expression after viral gene transfer before this approach may someday be successfully applied to human patients. They include, eg,
immune responses against viral coat proteins as well as size limitations imposed on the foreign DNA that can be incorporated into the virus. Adenoviral vectors in which all sequences encoding viral genes have been deleted offer the prospect of decreased host immune response to viral infection, decreased cellular toxicity, and increased capacity to accommodate large regulatory DNA regions. Initial results are encouraging and indicate significant advantages of regulated gene expression using genomic DNA for gene transfer and of adenoviral gene transfer vectors devoid of all viral coding sequences.20,21

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