

## Exploring Genetically Modified Animals for Development of New Atherosclerosis Treatments in NHLBI

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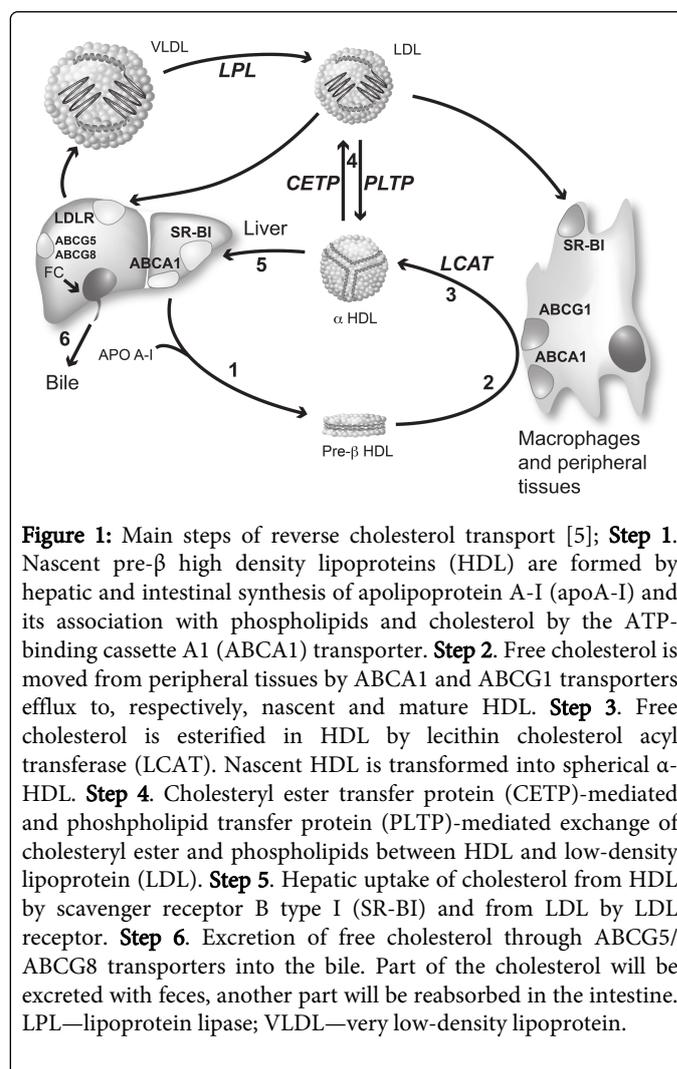
### Development of New Atherosclerosis

Atherosclerosis and complications associated with this pathology, such as coronary artery disease, remains a leading cause of morbidity and mortality in the world [1]. Dyslipidemia is one of the main risk factors leading to development of the atherosclerosis [2,3]. Peripheral (non-hepatic) cells, including arterial and aortic cells, obtain cholesterol from either de novo synthesis or from uptake of plasma lipoproteins. To prevent atherosclerosis, excess cholesterol must be removed from cells. This process takes place through the reverse cholesterol transport pathway [4,5], a process whereby cells efflux excess cholesterol to HDL, which subsequently delivers cholesterol to the liver for excretion into the bile (Figure 1).

Over the past 20 years, the Lipoprotein Metabolism Section (LMS) of NHLBI has created multiple lines of genetically modified animals to better understand the role of key genes involved in lipoprotein metabolism and lipid trafficking in the progression of atherosclerosis. Several transgenic and knockout animal models were created in LMS/MDB specifically for studies of the molecular mechanisms of RCT (Figure 1) [5]. Some of these models were also used to develop new treatments for atherosclerosis and other diseases associated with dyslipoproteinemia.

Among the first animal models created in our laboratory were mice and rabbits overexpressing the human lecithin cholesterol acyltransferase (LCAT) gene (Figure 1, step 3) under control of its endogenous promoter [6,7]. Both animal models clearly demonstrated the key role of LCAT in HDL biogenesis. Surprisingly, however, LCAT transgenic rabbits and mice responded quite differently to an atherogenic diet: in mice, LCAT overexpression enhanced the development of atherosclerotic lesions [8], whereas in rabbits, LCAT overexpression was highly protective against atherosclerosis [9].

In contrast to humans and rabbits, mice lack the cholesteryl ester transport protein (CETP) gene. CETP transfers cholesteryl ester from HDL to LDL, decreasing HDL and increasing LDL levels in plasma. Since rabbits and humans have CETP in plasma, their plasma cholesterol is mostly in LDL and so both rabbits and humans are more susceptible to atherosclerosis. As mice lack CETP, their plasma cholesterol is associated mostly with HDL, and so mice are highly resistant to atherosclerosis. After LCAT overexpression in mice, however, the lack of CETP caused HDL to become oversized and dysfunctional [8].



**Figure 1:** Main steps of reverse cholesterol transport [5]; **Step 1.** Nascent pre- $\beta$  high density lipoproteins (HDL) are formed by hepatic and intestinal synthesis of apolipoprotein A-I (apoA-I) and its association with phospholipids and cholesterol by the ATP-binding cassette A1 (ABCA1) transporter. **Step 2.** Free cholesterol is moved from peripheral tissues by ABCA1 and ABCG1 transporters efflux to, respectively, nascent and mature HDL. **Step 3.** Free cholesterol is esterified in HDL by lecithin cholesterol acyl transferase (LCAT). Nascent HDL is transformed into spherical  $\alpha$ -HDL. **Step 4.** Cholesteryl ester transfer protein (CETP)-mediated and phospholipid transfer protein (PLTP)-mediated exchange of cholesteryl ester and phospholipids between HDL and low-density lipoprotein (LDL). **Step 5.** Hepatic uptake of cholesterol from HDL by scavenger receptor B type I (SR-BI) and from LDL by LDL receptor. **Step 6.** Excretion of free cholesterol through ABCG5/ABCG8 transporters into the bile. Part of the cholesterol will be excreted with feces, another part will be reabsorbed in the intestine. LPL—lipoprotein lipase; VLDL—very low-density lipoprotein.

Consequently, LCAT overexpression in mice did not improve atherosclerosis but actually made it worse. In rabbits, overexpression of LCAT increased HDL levels and resulted in decreased atherosclerosis. Importantly, the atheroprotective ability of LCAT in rabbits was observed only when the animals had at least one copy of the LDL receptor (LDLR) gene [10]. Further experiments also showed significant LCAT and apoA-I interaction: when the apoA-I transgene was added to LCAT transgenic mice, total plasma and HDL cholesterol were increased to a much larger degree than either one of

these genes alone [11]. Finally, the key role of LCAT in HDL metabolism was confirmed in our lab by generation and study of LCAT knockout (KO) mice [12,13]. Comparing these model systems provided novel insights into the role of LCAT and CETP in lipoprotein metabolism and underscored the importance of choosing appropriate models for cardiovascular diseases.

Currently we are developing enzyme replacement therapy with injections of LCAT into LCAT-deficient patients. The feasibility of this approach was first tested in animal models. For example, the importance of the interaction between human LCAT with human apoA-I was revealed when we crossed LCAT-KO mice with human apoA-I transgenic mice and obtained animals that were especially responsive to infusion of human recombinant LCAT. LCAT-KO mice overexpressing human apoA-I, as well as the original LCAT-KO mice were very helpful in developing this new therapeutic approach [14]. Again, creating a new mouse model provided novel insights into lipoprotein metabolism – in this case with direct clinical impact.

Another interesting example of how genetically modified animal models led to important new insights was the work done in our laboratory on ATP-binding cassette (ABC) transporters, such as ABCA1 and ABCG1 (Figure 1, steps 1 and 2) and ABCG5/G8 (Figure 1, step 6). These transporters were found to play a key role in the trafficking of cholesterol and sterols [15-17]. Liver- and macrophage-specific overexpression of human ABCA1 in mice was associated with increased levels of HDL cholesterol, facilitating hepatic reverse cholesterol transport and biliary cholesterol excretion and protecting C57Bl/6N mice against diet-induced atherosclerosis [15,18]. Overexpression of ABCA1 decreased development of diet-induced atherosclerosis in mice, but only if the LDL receptor gene was functional [19], similar to the requirement for LDLr during overexpression of LCAT in rabbits [10]. Overexpression of the ABCG5/G8 transporter gene in the liver increased hepatobiliary sterol transport but did not alter aortic atherosclerosis in transgenic mice on C57Bl/6N, LDLr-KO or apoE-KO backgrounds [16]. Studies of ABCG1 transgenic mice with the transgene controlled by its natural promoter showed that enhanced expression of ABCG1 increased atherosclerosis in LDLr-KO mice, despite its role in promoting cholesterol efflux from cells [17]. These mouse models, in addition to delineating gene function, highlighted important interactions between genes involved in lipoprotein metabolism, which likely will be of even more interest as we enter the era of personal genome sequencing.

More recently, we have specifically designed genetically modified animals to investigate the role of endothelial cells in reverse cholesterol transport and development of cardiovascular diseases. For this purpose, we created mice overexpressing arginase 2, ABCA1, or SR-B1 specifically in endothelial cells [20-22]. Overexpression of human arginase 2 in endothelium was detrimental to the cardiovascular system, leading to endothelial dysfunction in transgenic mice, increased blood pressure and, when crossed with apoE-KO mice, increased aortic atherosclerotic lesions [20]. Analysis of mice overexpressing ABCA1 or SR-B1 in endothelial cells led us to suggest that the endothelium is a significant player in the removal of excess cholesterol from the periphery [21,22]. Overexpression of ABCA1 or SR-B1 in endothelium protected mice against diet-induced atherosclerosis. We also observed a profound anti-inflammatory effect from the expression of ABCA1 in endothelial cells in an ovalbumin-induced airway mouse model of asthma [23].

Our transgenic mice overexpressing human SR-B1 gene in liver helped to demonstrate that SR-B1 is a receptor for lipoprotein(a) [24].

Studies of genetically modified animals (mice and rabbits) created in the Lipoprotein Metabolism Section significantly contributed to our understanding of the mechanisms underlying dyslipidemia and atherosclerosis. These studies also led to development of novel treatments of atherosclerosis and other pathologies associated with abnormal lipid and lipoprotein metabolism (see reviews of animal models of atherosclerosis [25,26]). A detailed review of animal models designed for studies of the role of LCAT in biogenesis of HDL and RCT can be found in recent publications [5,27]

Animal models of atherosclerosis do have some limitations, including specificity of metabolic pathways, homogenous genetic background, price for large scale experiments, and so on (see for example review [28], where rabbits and mice were compared). However, even taking all these limitations into account, genetically modified animals are an extremely valuable source of new knowledge and efficient models for developing new treatments for atherosclerosis and other challenging human diseases.

We are now using some of the latest tools, such as zinc-finger nucleases, for creating new knockout mouse models and also inducible promoters for either selectively turning off or on genes of interest in select tissues.

Our journey is continuing....

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