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Inhibition and regression of atherosclerotic lesions⋆

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Abstract

Atherosclerosis, once believed to be a result of a slow, irreversible process resulting from lipid accumulation in arterial walls, is now recognized as a dynamic process with reversibility. Liver-directed gene therapy for dyslipidemia aims to treat patients who are not responsive to currently available primary and secondary prevention. Moreover, gene therapy strategies have also proved valuable in studying the dynamics of atherosclerotic lesion formation, progression, and remodeling in experimental animals. Recent results on the long-term effect of gene therapy suggest that hepatic expression of therapeutic genes suppresses inflammation and has profound effects on the nature of the atherogenic process.

Keywords

atherosclerosis; inhibition of lesion progression; lesion regression; inflammation; gene therapy; helper-dependent adenovirus

Abbreviations

AAV, adeno-associated virus; apo, apolipoprotein; CETP, cholesteryl ester transfer protein; EC, endothelial cell; FH, familial hypercholesterolemia; HDAd, helper-dependent adenovirus; HDL, high-density-lipoprotein; ICAM, intracellular adhesion molecule; INF-γ, interferon-γ; LDLR, low-density-lipoprotein receptor; PDGF, platelet-derived growth factor; SMC, smooth muscle cell; VCAM-1, vascular cell adhesion molecule-1; VLDLR, very-low-density lipoprotein receptor

The classical view of atherosclerosis was that it was a slow, irreversible process. However, mounting evidence now supports that it is a dynamic and reversible process. Animal studies in nonhuman primates and rabbits fed high cholesterol diets have revealed the possibility of lesion stabilization and even regression by an aggressive lipid lowering regimen (Armstrong & Megan, 1975; Aikawa *et al.*, 1998a; 1999; Kockx *et al.*, 1998). Furthermore, studies on thoracic aorta transplantation or transgenic mice carrying apolipoprotein E (apoE)-Mx1-*Cre* transgene have shown that long-term lipid normalization or stable expression of anti-atherogenic proteins induces regression of advanced lesions (Reis *et al.*, 2001; Raffai *et al.*, 2005). These studies also showed remodeling of arterial walls with decreased macrophage

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accumulation, reduced inflammation and increased collagen contents, providing a mechanistic explanation for the clinical benefit of lipid-lowering therapy (Libby & Aikawa, 2002).

Here we summarize current concepts of atherogenesis, recent progress made toward the development of liver-directed gene therapy for dyslipidemia, and how these approaches have contributed to our understanding of the dynamics of the atherosclerotic process. First, we will review the inflammatory response during progression of atherosclerosis, confer dynamics of atherosclerotic plaques, and discuss recent progress in vector biology. Finally, we will comment on our recent results on the inhibition, remodeling, and regression of atherosclerosis by liver-directed gene therapy in mouse models of dyslipidemia.

INFLAMMATION AND DEVELOPMENT OF ATHEROSCLEROSIS

Inflammation plays a central role in atherosclerosis (Glass & Witztum, 2001; Hansson, 2001; Libby, 2002; Nilsson *et al.*, 2005). The initial step of atherosclerosis is monocyte invasion into the intima (Fig. 1). Under normal conditions, monocytes and T cells in blood have random contact with, but do not adhere to, endothelial cells (ECs). However, the situation is changed with the onset of inflammation (Blankenberg *et al.*, 2003; Hoffman *et al.*, 2004).

Adhesion of leukocytes to endothelial cells (ECs)

When ECs undergo inflammatory activation in response to atherogenic stimuli such as low-density lipoprotein (LDL) accumulation in the arterial wall, they express various leukocyte adhesion molecules including P-selectin, E-selectin, intracellular adhesion molecules (ICAMs), vascular cell adhesion molecule-1 (VCAM-1), and integrins. Selectins are involved in the rolling and tethering of leukocytes. ICAMs, VCAM-1, and integrins induce firm adhesion of monocytes and T cells to ECs (Blankenberg *et al.*, 2003).

Transmigration

Once adherent to ECs, monocytes and T cells migrate into the intima (innermost layer of the arterial wall) in response to chemokines. Monocyte chemoattractant protein-1 (MCP-1) is one of such chemokines and is expressed by the ECs and smooth muscle cells (SMCs) (Boring *et al.*, 1998; Gu *et al.*, 1998).

Monocyte maturation and T cell activation

In the intima, monocytes differentiate into macrophages upon induction by macrophage colony stimulating factor (M-CSF) (Ishibashi *et al.*, 1990; Clinton *et al.*, 1992). This causes the expression of scavenger receptors and the macrophages to engulf modified lipoproteins and fill with lipid droplets. Once in the intima, T cells encounter antigens such as modified LDL and heat-shock proteins. Upon activation by engagement of the receptor on T cells and antigens, the T cells produce cytokines such as interferon-γ (INF-γ) and further activate macrophages. The fat-laden macrophages so called foam cells and activated T cells are abundant in the fatty streak, which is the earliest stage of atherosclerosis.

Macrophages and smooth muscle cell (SMC) proliferation and plaque progression

Inflammatory molecules promote further growth of the atherosclerotic plaque. SMCs of the media migrate to the top of the intima to form a fibrous cap over the lipid core. The fibrous cap provides a shield between the thrombogenic material in the plaque's lipid core and the coagulation factors present in the intima. Foam cells secrete pro-inflammatory cytokines that amplify the local inflammatory response in the lesion and reactive oxygen species. The production of these mediators provides a signal amplification loop between acquired immunity (T cells) and innate immunity (monocyte/macrophages). Engagement of activated T cells and macrophages induces the secretion of tissue factors (TFs), pro-inflammatory cytokines, and

matrix metalloproteinases (MMPs) which degrade extracellular matrix necessary for strength of the plaque's fibrous cap.

Plaque rupture

When the plaque ruptures, it allows the circulating blood to contact the pro-coagulant protein, TF. Eventually macrophages die in a central core in the atherosclerotic plaque by apoptosis or necrosis, resulting in a necrotic core (Glass & Witztum, 2001; Libby, 2002).

IS ATHEROSCLEROSIS A DYNAMIC AND REVERSIBLE PROCESS?

The normal coronary artery has a trilaminar structure (Fig. 2). The ECs are in direct contact with the blood in the lumen over a basement membrane. The intima contains few SMCs within its extracellular matrix. The elastic lamina forms the barrier between the intima and media. SMCs synthesize and secrete elastin polymer that inhibits proliferation and regulates migration of SMCs to the intima (Karnik *et al.*, 2003). The media consists of multiple layers of SMCs tightly packed and embedded in an elastin-rich matrix and collagen. The adventitia is the outermost layer containing connective tissue.

Early atherosclerotic lesions

In early atherogenesis, migration of immune cells and the accumulation of lipids in macrophages lead to formation of fatty streaks (Libby, 2002; Libby & Aikawa, 2002). In humans, early atherosclerotic lesions are clinically silent, do not necessarily progress to advanced lesions (Stary *et al.*, 1994; 1995; Virmani *et al.*, 2000), and frequently regress spontaneously (Virmani *et al.*, 2000).

Vulnerable plaques

If proinflammatory conditions such as dyslipidemia persist, the lipid core grows. MMPs are secreted by the activated macrophages and degrade the extracellular matrix, while pro-inflammatory cytokines such as INF-γ inhibit collagen synthesis. These changes lead to a thin fibrous cap, large lipid core, and few SMCs, a phenotype of vulnerable plaques prone to rupture.

Plaque rupture

Disruption of the thin fibrous cap causes the direct contact of blood components to TF, initiating coagulation, recruitment of platelets, and ultimately formation of a thrombus. If the thrombus occludes the vessel, an acute myocardial infarction occurs.

Stenosis

Endogenous thrombolysis may eventually dissolve the thrombus. The wound healing response triggered by thrombin produced during blood coagulation stimulates SMC proliferation. Platelet-derived growth factor (PDGF) released from activated platelets stimulates SMC migration and collagen synthesis. This process results in a thick fibrous cap and expansion of the intima, offen in an inward direction. Stenotic lesions may impede blood flow under increased cardiac work demand, leading to ischemia and angina. Symptoms of advanced stenotic plaques are less susceptible to rupture due to the presence of thick fibrous caps.

Stabilization

A clinically important therapeutic goal is to reduce the incidence of acute coronary events. This aim can be accomplished by remodeling the vulnerable plaques qualitatively or functionally. For example, HMG-CoA reductase inhibitors (statins) have been shown to lower LDL cholesterol and stabilize lesions (Libby & Aikawa, 2001; Shah, 2002).

Regression

"Vulnerable plaques" may potentially regress. This extraordinary possibility has been demonstrated in "thoracic aorta transplantation model" (Reis *et al.*, 2001).

EXPERIMENTAL GENE THERAPY FOR DYSLIPIDEMIA *VIA* LIVER-DIRECTED GENE TRANSFER HAS PROVIDED A MEANS TO STUDY THE DYNAMICS OF ATHEROSCLROTIC LESION DEVELOPMENT

The development of cholesterol-lowering drugs has greatly contributed to current primary and secondary preventions of cardiovascular disease. However, a substantial proportion of patients does not respond adequately to or tolerate these drugs, leaving therapeutic goals unattained. The need for novel therapies for these patients prompted the investigation of somatic gene therapy as an alternative treatment. In gene therapy, a gene delivery vector with high efficiency, specificity, and safety profile is required (Thomas *et al.*, 2003). The development of effective gene transfer vectors has further provided a useful tool to study the role of different circulating proteins and their receptors on the vascular biology of atherosclerosis in experimental animal models (Oka & Chan, 2002; 2004). The most popular vectors for liver-directed gene delivery are adeno-associated virus (AAV) and adenovirus (Ad).

AAV is a non pathogenic virus that encompasses a large number of serotypes. To date, studies on experimental gene therapy using AAV vectors have been based mainly on the use of eight different serotypes. The most commonly used serotype 2 AAV (AAV2) displays a relatively poor transduction efficiency, which severely limits its usefulness. Several AAV receptors and co-receptors have been identified, which include heparan sulphate proteoglycan, integrins, PDGF receptor, and sialic acid. The tissue distribution of these receptors or co-receptors determines cell type- or tissue-specific transduction (Grimm *et al.*, 2003; Burger *et al.*, 2004). A recently isolated AAV8 has been reported to be highly efficient in hepatic transduction (Gao *et al.*, 2002); it efficiently transduces hepatocytes following intravenous administration (Sarkar *et al.*, 2004; Nakai *et al.*, 2005). A drawback of AAV as a gene transfer vector is its relatively small cloning capacity of approx. 4.7 kb. This size limitation can be overcome by an intermolecular joining method using two AAV vectors (Duan *et al.*, 2000), but the value of such a procedure in practice remains to be determined. Nonetheless, AAV remains a popular viral gene delivery system. A major advantage of AAV is its ability to confer long-term transgene expression *in vivo*, by expressing transgene mainly as an extrachromosomal episome (McCarty *et al.*, 2004).

Helper-dependent adenoviral vector (HDAd) is the latest Ad vector which has an improved safety profile with sustained transgene expression *in vivo* in comparison with early generation Ads (Kochanek, 1999). It also has a large cloning capacity of up to 37 kb. Despite substantial efforts directed at improving the safety profile and prolonging transgene expression of early generation Ad vectors, the toxicity and immunogenecity of these vectors severely limited their usefulness for liver-directed gene therapy. Like early generation Ads, HDAds are associated with acute toxicity, which appears to be caused by the hosts' innate immune response (Schnell *et al.*, 2001) (Brunetti-Pierri *et al.*, 2004; Muruve *et al.*, 2004), resulting from the direct interaction of viral particles with innate immune effector cells upon systemic vector administration. Transient depletion of Kupffer cells has been found effective to reduce toxicity as well as increase hepatic transduction efficiency (Schiedner *et al.*, 2003). HDAd vector preferentially target the liver upon intravenous administration, and it is our preferred gene delivery vector in liver-directed experimental gene therapy in small animals.

DYSLIPIDEMIA AND THERAPEUTIC GENES

A number of potential therapeutic genes have been tested for their efficacy in experimental gene therapy for animal models of dyslipidemia. There are two broad mechanisms that affect atherosclerosis associated with dyslipidemia: 1) direct influence over lipoproteins, which includes lowering atherogenic lipoproteins, elevating anti-atherogenic lipoproteins, or facilitating reverse cholesterol transport; and 2) modulate the inflammatory reactions. In addition to the causative genes of dyslipidemia, genes that affect local inflammation in the arterial wall are now considered as potential therapeutic genes. Atherosclerosis is a slowly progressive disease that takes decades to develop in humans. Recent data from long-term experiments show that effects of long-term treatment almost always are different from those obtained from short-term experiments, underscoring the importance of such long-term observations (Oka & Chan, 2004).

INHIBITION OF ATHEROSCLEROSIS PROGRESSION

LDLR- and apoE-deficient mice are popular models of atherosclerosis. Familial hypercholesterolemia (FH) is an autosomal recessive genetic disorder caused by defective LDLR. Despite the development of improved primary and secondary interventions, treatment for homozygous FH is limited and often does not achieve the therapeutic goal (Hopkins, 2003; Naoumova *et al.*, 2004). Therefore, FH is a good target for gene therapy. In contrast to FH, apoE deficiency in humans is a rare genetic disease with a mild phenotype compared with that in mice. This is considered to be due to differences in lipoprotein physiology between two species, notably the lack of cholesteryl ester transfer protein activity and the presence of hepatic apoB mRNA editing activity in mice. ApoE-deficient mice develop atherosclerosis spontaneously on a regular chow diet. Furthermore, it has been shown that in older apoE-deficient mice, atherosclerotic plaques in the brachiocephalic artery show considerable similarity to the vulnerable human plaques (Rosenfeld *et al.*, 2000). Therefore, this model has been used in many studies of atherosclerosis (Meir & Leitersdorf, 2004).

Irrespective of the vector used, many gene therapy studies for dyslipidemia have found retardation of atherosclerotic lesion progression after delivery of appropriate therapeutic genes (Chen *et al.*, 2000; Kawashiri *et al.*, 2001; 2002; Oka *et al.*, 2001; Belalcazar *et al.*, 2003; Mertens *et al.*, 2003; Nomura *et al.*, 2004; Pastore *et al.*, 2004). The most remarkable example showing the efficacy of a HDAd vector was reported in apoE deficient mice (Kim *et al.*, 2001). A single intravenous administration of HDAd containing mouse apoE gene into apoE deficient mice resulted in lifetime correction of hypercholesterolemia. Plasma apoE levels were near normal 2.3 years after treatment. Aorta in control mouse was covered 100% by atherosclerotic lesions, whereas aortas in vector treated mice were essentially lesion-free. Furthermore, this study demonstrated the feasibility of re-administration of HDAd vector to re-stimulate transgene expression. Re-administration of the same HDAd vector is inhibited by generation of neutralizing antibodies against vector capsid proteins. However, these antibodies are serotype-specific, which allows re-administration of the same vector of a different serotype.

First generation Ad vector-mediated transfer of the LDLR gene to the liver of LDLR deficient mice (Ishibashi *et al.*, 1993) or Watanabe heritable hyperlipidemic rabbits (Kozarsky *et al.*, 1994; Li *et al.*, 1995) resulted in significant but transient plasma cholesterol lowering. The transient nature of the lipid lowering after LDLR gene therapy is related in part to an immune response against the induced LDLR expression in mice lacking LDLR (Kozarsky *et al.*, 1996; Chen *et al.*, 2000). Lebherz and coworkers have used AAV vectors with serotype 2 (AAV2), 7 (AAV2/7) and 8 (AAV2/8) cap proteins. They have employed a liver-specific promoter to express the LDLR and have administered these viral constructs into LDLR deficient mice fed a high-fat western diet (Lebherz *et al.*, 2003). They found that serum

cholesterol levels were significantly lower in mice treated with AAV2/7 and AAV2/8 twenty weeks after treatment. Furthermore, mice treated with AAV2/7 and AAV 2/8 had 44% and 62% less lesions, respectively, than control mice. AAV2 LDLR treated mice had a 12% inhibition of lesion progression, which was not statistically significant. The success of the modified AAV2/8 is explained in part by the fact that nearly 85% of hepatocytes were transduced by AAV2/8 compared with less than 5% transduction by AAV2.

Our group has shown that HDAd directed anti-atherogenic gene transfer is a powerful way of inhibiting atherosclerosis development/progression both short- and long-term. A 24 week treatment with HDAd-mouse VLDLR inhibited the lesion progression by 87% in LDLR deficient mice fed a high cholesterol diet (Oka *et al.*, 2001). A humoral anti-LDLR developed in a minority of mice treated with the vector. In mice that did not display such a response, LDLR gene therapy was even more effective in cholesterol lowering and protection against atherosclerosis (Nomura *et al.*, 2004). Long-term efficacy of HDAd-LDLR gene therapy is also excellent. When LDLR deficient mice were treated at 12 weeks of age with a low dose of HDAd-LDLR, they responded with lowering of their plasma cholesterol that lasted at least 108 weeks. By that time, all of the control mice had died, and the aorta was heavily covered with thick lesions compared to the scattered lesions found in treated mice. In addition to inhibition of lesion progression, HDAd-LDLR treatment caused lesion remodeling from a vulnerable-looking to a more stable-appearing phenotype with a marked reduction in VCAM-1 expression, well-preserved collagen-rich extracellular matrix, and increased α-actin staining. These results are consistent with the studies that have shown that long-term lipid lowering contributes to plaque stabilization (Aikawa *et al.*, 1998a; 1998b; Verhamme *et al.*, 2002).

REGRESSION OF ADVANCED ATHEROSCLEROTIC LESIONS

Promoting the regression of advanced atherosclerotic lesions or remodeling “vulnerable plaques” to a more stable phenotype undoubtedly is an important therapeutic goal. However, in contrast to inhibition of lesion progression, induction of lesion regression is more difficult to prove experimentally. Tangirara and coworkers have reported that the treatment of LDLR deficient mice with a second generation Ad vector expressing apoA-I resulted in a 70% reduction of pre-existing fatty streaks in *en face* lesion analysis after only 4 weeks treatment (Tangirala *et al.*, 1999). This is in contrast to our long-term studies. After 24 weeks treatment with HDAd expressing human apoA-I (HDAd-hgAI), LDLR deficient mice having pre-existing advanced lesions showed 50% reduction of lesion progression. The plasma apoA-I levels were maintained at or above normal human levels for the duration of the study. Nevertheless, we found dramatic remodeling of the plaque to a more stable-looking phenotype (Belalcazar *et al.*, 2003).

In addition to apoA-I, there is also considerable interest in apoE as an anti-atherogenic protein (Davignon, 2005). Two papers reported that second generation Ad vector expressing human apoE induced regression of early fatty streaks and advanced complex lesions only after 4–6 weeks treatment in apoE deficient mice (Tsukamoto *et al.*, 1999; Harris *et al.*, 2002). In another study, apoE deficient nude mice were treated with a first generation Ad expressing apoE at 17 weeks old. The authors found regression of fatty streaks 6 months after the treatment (Desurmont *et al.*, 2000).

Given our success in using apoE3, we tested the efficacy of long-term hepatic apoE3 expression on advanced lesions in apoE deficient mice. Mice were fed a high cholesterol diet for 30 weeks to induce atherosclerosis and then treated with HDAd containing human apoE3 gene. After 36 weeks, lesion areas in treated mice had not significantly regressed compared to the baseline group that were sacrificed at vector administration, whereas those in control group had progressed over 340%. However, in spite of similarities in lesion area, the lesion thickness in

treated mice was significantly smaller (a 28% reduction) than in baseline mice, suggesting that the induction of lesion regression occurs even in advanced lesions (Paul *et al.*, manuscript in preparation). It should be noted that apoE3 is derived from the liver in this model. In contrast, apoE was derived from both the liver and macrophages in the thoracic aorta transplantation model (Reis *et al.*, 2001).

ApoE could potentially be anti-atherogenic *via* mechanisms other than cholesterol lowering. This possibility has been tested in LDLR deficient mice. Hepatic apoE3 expression not only reduced lesions (Tsukamoto *et al.*, 2000), but also stimulated a 61% regression after 6-week treatment without affecting plasma cholesterol levels. This lesion regression was accompanied by the reduction of urinary, LDL-associated and arterial wall isoprostane, an index of *in vivo* oxidant stress (Tangirala *et al.*, 2001).

COMBINATION TREATMENT

Anti-atherogenic proteins and lipid lowering proteins work *via* different mechanisms. ApoA-I elevates high-density lipoprotein (HDL), which has anti-inflammatory and anti-oxidative effects, and also promotes reverse cholesterol transfer. ApoE may be anti-atherogenic by its diverse functions (e.g., anti-oxidant effect, inhibition of T cell proliferation, inhibition of SMC proliferation and migration, inhibition of platelet aggregation, promotion of reverse cholesterol transport through apoA-I or apoA-I-devoid HDL, etc.) (Davignon, 2005). LDLR works mainly by lowering atherogenic non-HDL lipoproteins. Therefore, there is considerable interest in the outcome of combined therapy. Although hepatic apoE3 expression alone inhibited atherosclerosis progression in LDLR deficient mice, the addition of LDLR with apoE3 did not show an additive or synergistic effect on either cholesterol lowering or atherosclerosis after 6-weeks treatment (Kawashiri *et al.*, 2001). Similarly, the atherosclerotic lesion progression was reduced by LDLR or apoA-I single treatment, but the effects of combined treatment was not greater than those of a single gene treatment (Kawashiri *et al.*, 2002). It should be noted that the expression of therapeutic genes was transient and the treatment lasted only for 6 weeks in both studies.

We hypothesized that long-term stable gene expression could allow more significant effects. LDLR deficient mice were fed a high cholesterol diet for 36 weeks and then treated with HDAd-hgAI or HDAd-LDLR vector alone or in combination. Atherosclerotic lesion area was measured by quantitative morphometry 28 weeks after the treatment. HDAd-hgAI treatment inhibited the lesion progression, but mice treated with HDAd-LDLR alone or combination induced lesion regression (Chao *et al*., manuscript in preparation). These results indicate that HDAd-mediated LDLR gene therapy is highly effective in inhibition of lesion progression and in inducing atherosclerotic lesion regression.

The progressive accumulation of macrophages and other immune cells in the atherosclerotic plaques is one of the hallmarks in atherosclerosis. Recruitment of monocytes into the intima and their retention there contributes to the progression of plaques (Llodra *et al.*, 2004). Many studies including our results found that regression and remodeling of atherosclerotic lesions are associated with reduced macrophages in the lesions. The mechanism of macrophage retention and potential reverse transmigration into blood flow has not been well understood. Polansky has recently proposed a mathematical model which might explain cell motility in atherosclerotic lesion (Polansky, 2003).

CONCLUSIONS

Gene therapy was introduced with great expectations as the latest frontier in gene medicine. Despite the initial optimism, the field has experienced some setbacks. In the areas of dyslipidemia, the progress in liver-directed gene delivery has proved invaluable in studying

vascular biology and the pathogenesis of atherosclerosis. Data on the long-term effects of gene delivery support the feasibility of achieving a clinical goal in stabilization or regression of vulnerable atherosclerotic plaques by a gene therapy approach. Although additional work is needed, the field is slowly but relentlessly moving toward fulfilling its promise made decades ago.

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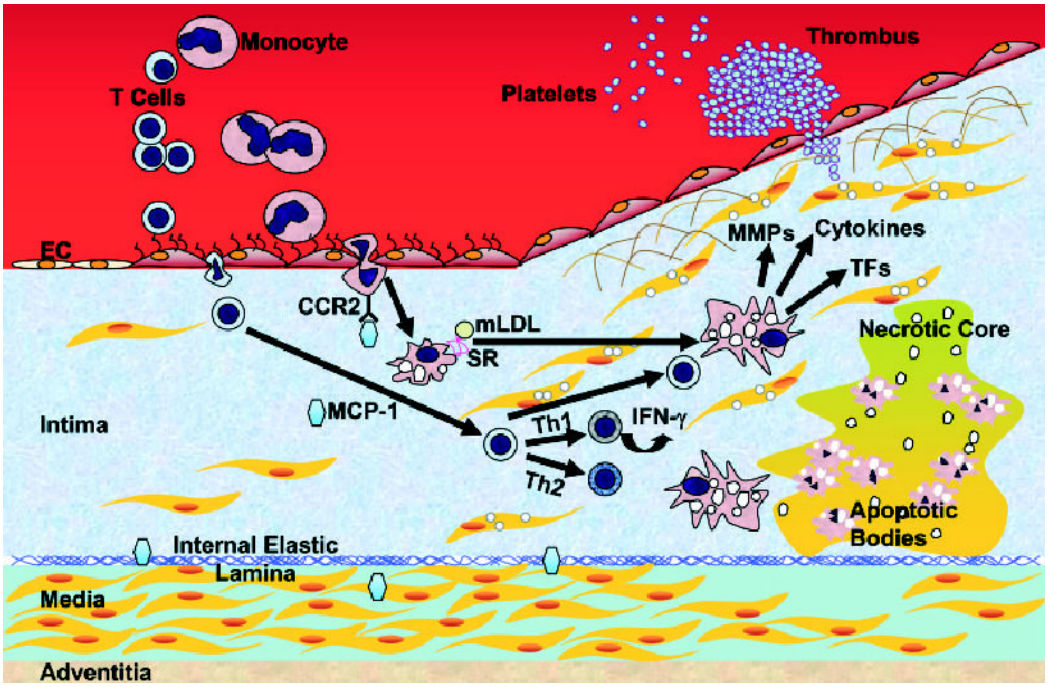

Figure 1. Development of atherosclerosis.

Monocytes and T-leukocytes do not adhere to ECs under normal conditions. **1. Adhesion**. When ECs undergo inflammatory activation they express adhesion molecules, and leukocytes are trapped by ECs. **2. Transmigration**. Once adherent, leukocytes migrate into the intima. **3. Foam cell formation.** In the intima, monocytes differentiate into macrophages and take up lipids. **4. Progression.** T cells are activated and further stimulate macrophages. SMCs of the media migrate to the top of the intima to form a fibrous cap over the lipid core. Further activation of macrophages produces MMPs that degrade the extracellular matrix and weaken the fibrous cap. **5. Plaque rupture.** When the plaque ruptures, it allows the blood to contact to the procoagulant protein, tissue factor, and activate the coagulant cascade. Macrophages eventually die in a central core by apoptosis or necrosis, which forms a necrotic core in the lesion. IFN-γ, interferon-γ; MCP-1, monocyte chemoattractant protein-1; mLDL, modified LDL; MMP, matrix metalloproteinase; SR, scavenger receptors; TF, tissue factor. **Note:** Mast cells play an important role in atherogenesis. The interaction of the chemokine receptor CCR3 on the surface of mast cells and eotaxin, a chemoattractant, may facilitate the trans-migration of these cells. In the intima, they undergo degranulation and release factors that contribute to atherogenesis. Mast cells are not indicated.

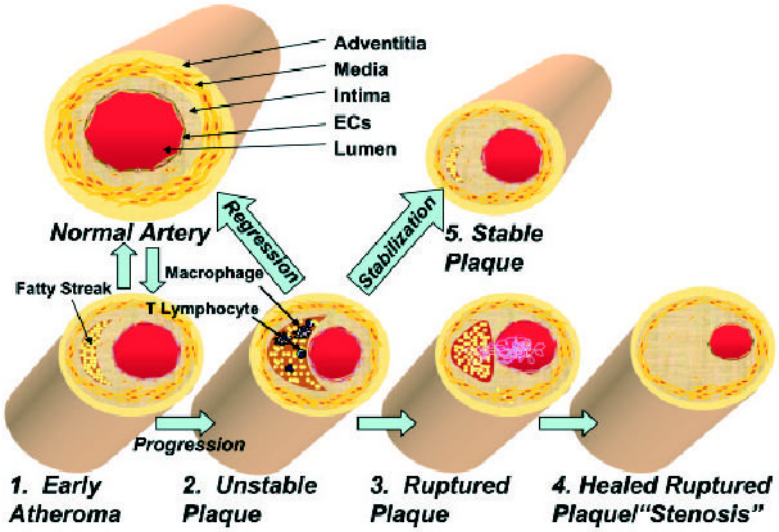

Figure 2. Evolution of atheroma.

The normal human coronary artery has a trilaminar structure. **1. Early atherosclerotic lesions**. In early atherogenesis, migration of immune cells and the accumulation of lipids in macrophages lead to the formation of fatty streaks. These early lesions frequently regress spontaneously. **2. Vulnerable plaque.** If inflammatory conditions persist, the lipid core grows. Further activation of macrophages secretes matrix degrading enzymes and weakens the fibrous cap, which leads to vulnerable plaques prone to rupture. **3. Plaque rupture.** Disruption of the fibrous cap causes the direct contact of blood components to tissue factor and initiates coagulation. This leads to the formation of the thrombus. **4. Stenosis.** A wound healing response stimulates smooth muscle cell migration and collagen synthesis. This process results in a thick fibrous cap and expansion of the intima, which constricts the lumen. **5. Stabilization.** Drug treatment or gene therapy for dyslipidemia remodels the nature of the vulnerable plaques, which reduces the incidence of acute coronary events. **6. Regression.** Advanced atherosclerotic lesions could be regressed under aggressive lipid lowering or drug treatments.