Cell, Vol. 104, 503–516, February 23, 2001, Copyright ©2001 by Cell Press

Atherosclerosis: The Road Ahead

Review

Christopher K. Glass*†‡ and Joseph L. Witztum*
Specialized Center of Research on Molecular Medicine and Atherosclerosis
*Department of Medicine
Division of Endocrinology and Metabolism
†Department of Cellular and Molecular Medicine
University of California, San Diego
9500 Gilman Drive
La Jolla, California 92093

Complications of atherosclerosis are the most common causes of death in Western societies. In broad outline, atherosclerosis can be considered to be a form of chronic inflammation resulting from interaction between modified lipoproteins, monocyte-derived macrophages, T cells, and the normal cellular elements of the arterial wall. This inflammatory process can ultimately lead to the development of complex lesions, or plaques, that protrude into the arterial lumen. Plaque rupture and thrombosis results in the acute clinical complications of myocardial infarction and stroke (Navab et al., 1996; Ross, 1999; Steinberg and Witztum, 1999). Among the many genetic and environmental risk factors that have been identified by epidemiologic studies (Table 1), elevated levels of serum cholesterol are probably unique in being sufficient to drive the development of atherosclerosis in humans and experimental animals, even in the absence of other known risk factors. The elucidation of molecular mechanisms that control cholesterol biosynthesis and serum cholesterol levels (reviewed in Goldstein and Brown, 1977) led to the development of "statins," a potent class of cholesterol lowering drugs that have been proven to significantly reduce cardiovascular mortality in hypercholesterolemic patients (Gould et al., 1998). However, available statins are not sufficient to fully prevent the progression of atherosclerosis in many susceptible individuals. Indeed, even among individuals with the same cholesterol levels there is great disparity in the expression of clinical disease. This is most vividly illustrated by two subjects with the same founder gene defect leading to homozygous familial hypercholesterolemia, and similarly elevated plasma cholesterol levels. One, a male, died of coronary artery disease at age 3, while a female, despite developing symptoms of cardiovascular disease, died of unrelated causes at age 33 (Hobbs et al., 1990). Thus, an understanding of other factors contributing to atherogenesis is needed to develop new strategies for prevention and treatment. Here, we review recent studies of molecular mechanisms underlying the pathogenesis of atherosclerosis and provide examples of how emerging technologies may be used to accelerate the pace of discovery and facilitate development of novel therapeutic approaches.

‡To whom correspondence should be addressed (e-mail: cglass@ucsd.edu).

Lipoproteins and Atherosclerosis

Serum cholesterol is carried by several lipoprotein particles that perform the complex physiologic tasks of transporting dietary and endogenously produced lipids (reviewed in Witztum and Steinberg, 1995). Chylomicrons provide the primary means of transport of dietary lipids, while very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL) function to transport endogenous lipids. Triglyceride-rich VLDL particles containing apolipoprotein B-100 (apo B-100) and apolipoprotein E (apo E) are synthesized by the liver and function to transport fatty acids to adipose tissue and muscle. After triglyceride removal in peripheral tissues, a portion of the remaining VLDL remnants are metabolized to LDL particles by further removal of core triglycerides and dissociation of apolipoproteins other than apo B-100. In humans, the majority of serum cholesterol is carried by LDL particles.

While LDL has an essential physiological role as a vehicle for the delivery of cholesterol to peripheral tissues, increased LDL cholesterol levels are associated with increased risk of cardiovascular disease. LDL is taken up by cells via LDL receptors that recognize an N-terminal domain of apo B-100. The circulating level of LDL is determined in large part by its rate of uptake through the hepatic LDL receptor pathway, as evidenced by the fact that lack of functional LDL receptors is responsible for the massive accumulation of LDL in patients with homozygous familial hypercholesterolemia (Goldstein and Brown, 1977). The expression of LDL receptors is subject to feedback control by intracellular cholesterol levels. Low levels of intracellular cholesterol lead to activation of the SREBP transcription factors, which stimulate transcription of the LDL receptor gene and other genes involved in cholesterol biosynthesis (Brown and Goldstein, 1997). Statins lower circulating cholesterol levels indirectly by inhibiting HMG CoA-reductase, the rate limiting enzyme required for endogenous cholesterol biosynthesis. The resulting decrease in intracellular cholesterol leads to activation of SREBP, upregulation of LDL receptors, and enhanced clearance from plasma degradation of LDL, reducing its circulating levels.

Targeted disruption of the apo E or LDL receptor genes, or overexpression of the human apo B gene in mice, results in marked increases in VLDL and/or LDL cholesterol levels. When such animals are fed high cholesterol diets, plasma cholesterol levels are greatly exaggerated and reach values in excess of 1500–2000 mg/dl (Smithies and Maeda, 1995; Breslow, 1996). Although mice are normally very resistant to the development of atherosclerosis, the combination of these genetic and dietary manipulations results in massive hypercholesterolemia leading to extensive atherosclerotic disease that occurs throughout the aorta and has many features in common with human lesions. The development of murine models of atherosclerosis has revolutionized the approach to evaluating potential roles of specific pro-

Table 1. Risk Factors for Development of Atherosclerosis

Factors with a Significant Genetic Component
Elevated levels of LDL and VLDL
Low levels of HDL
Elevated lipoprotein (a)
Hypertension
Diabetes Mellitus
Male gender
Elevated levels of homocysteine
Elevated levels of hemostatic factors, e.g., fibrinogen
Metabolic syndrome
Insulin resistance
Obesity
Family history
Environmental Factors
Smoking
Lack of exercise
High fat diet
Infectious agents

teins in lesion development. The crossing of these animals with mice that have been engineered to overexpress or lack genes of interest has led to a growing list of proteins that accelerate or retard the rate at which lesions develop, and/or alter lesion composition, examples of which are provided in Table 2 and described throughout this review. The products encoded by these genes thus represent potential targets for therapeutic intervention in humans. It is important to note that the development of atherosclerosis in these murine models, as in virtually all animal models of atherosclerosis, is driven by extreme elevations in circulating cholesterol levels that result in the formation of extensive lesions over a time scale of weeks to months. In contrast, the development of atherosclerosis in humans evolves over decades and advanced lesions are typically less cellular than those observed in animal models. Furthermore, thrombotic events that account for myocardial infarction in humans have not been consistently observed in mice. In spite of these caveats, genetic studies in mice provide the most powerful approach available to validate roles of candidate genes in vivo.

Initiating Events; LDL Modification

Atherosclerotic lesions begin as fatty streaks underlying the endothelium of large arteries (Figures 1A and 2). Recruitment of macrophages and their subsequent uptake of LDL-derived cholesterol are the major cellular events contributing to fatty streak formation. Many lines of evidence suggest that oxidative modifications in the lipid and apolipoprotein B (apo B) components of LDL drive the initial formation of fatty streaks (Navab et al., 1996; Steinberg and Witztum, 1999). Indeed immunologic evidence of LDL oxidation is observed even in human fetal arteries prior to the presence of macrophages (Napoli et al., 1997). The specific properties of oxidized LDL (oxLDL), usually studied following oxidation of native LDL in vitro, depend on the extent of modification. This can range from "minimal" modification, (mmLDL) in which the LDL particle can still be recognized by LDL receptors (Navab et al., 1996), to extensive oxidation, in which the apo B component is fragmented and lysine residues are covalently modified with reactive breakdown products of oxidized lipids (Steinberg and Witztum, 1999). Such particles are not bound by the LDL receptor, but rather by several so-

Table 2. Examples of Genes that Influence Development of Atherosclerosis in Hypercholesterolemia Mice

Gene	Experiment	Genetic Background	Effects on Lesion Area	Proposed Mechanism	Reference
Atherogenic Genes					
12/15-LO	Knockout	apoE$^{-/-}$	⇓	Decreased LDL oxidation	Cyprus et al., 1999;
	Overexpression	LDL R$^{-/-}$	↑	Increased LDL oxidation	Harats et al., 2000 Detmer et al., 2000;
iNOS	Knockout	apoE$^{-/-}$	↓	Decreased LDL oxidation	Behr-Roussel et al., 2000
M-CSF	Knockout	apoE$^{-/-}$	⇓	Decreased macrophage infiltration	Smith et al., 1995; Qiao et al., 1997
MCP-1	Knockout	LDL R$^{-/-}$ apoE$^{-/-}$	⇓	Decreased macrophage infiltration	Gu et al., 1999; Gosling et al., 1999
CCR2	Knockout	apoE$^{-/-}$	⇓	Decreased macrophage infiltration	Boring et al., 1998
P- and E-selectin	Combined knockout	LDL R$^{-/-}$	↓	Decreased monocyte adherence	Dong et al., 1998
CXCR-2	Knockout	LDL R$^{-/-}$	↓	Decreased macrophage residence	Boisvert et al., 1998
SR-A	Knockout	apoE$^{-/-}$	↓	Decreased uptake of oxLDL	Suzuki et al., 1997
CD36	Knockout	apoE$^{-/-}$	⇓	Decreased uptake of oxLDL	Febraio et al., 2000
IFNγ receptor (R0)	Knockout	apoE$^{-/-}$	↓	Decreased inflammatory responses, increased apoAIV	Gupta et al., 1997
CD154	Knockout	apoE$^{-/-}$	↓	Decreased CD40 signaling	Lutgens et al., 1999
IL-10	Knockout	C57 BL/6J	↑	Increased inflammatory responses	Pinderski et al., 1999; Mallat et al., 1999
Antiatherogenic Genes					
Paraoxinase	Knockout	apoE$^{-/-}$	↑	Reduced clearance of oxidized lipids	Shih et al., 2000
apo A-I	Knockout	h apoB transgene	↑	Decreased reverse cholesterol transport	Voyaziakis et al., 1998
	Overexpression	apo E$^{-/-}$ LDL R$^{-/-}$	↓	Increased reverse cholesterol transport	Benoit et al., 1999; Tangirala et al., 1999
PPARγ	Knockout	LDL R$^{-/-}$	↑	Altered macrophage function	Chawla et al., 2001;
	Agonist	LDL R$^{-/-}$	↓		Lie et al., 2000
SR-B1	Knockout	LDL R$^{-/-}$	↑	Decreased reverse cholesterol transport	Huszar et al., 2000
	Overexpression	LDL R$^{-/-}$	↓	Increased reverse cholesterol transport	Kozavsky et al., 2000

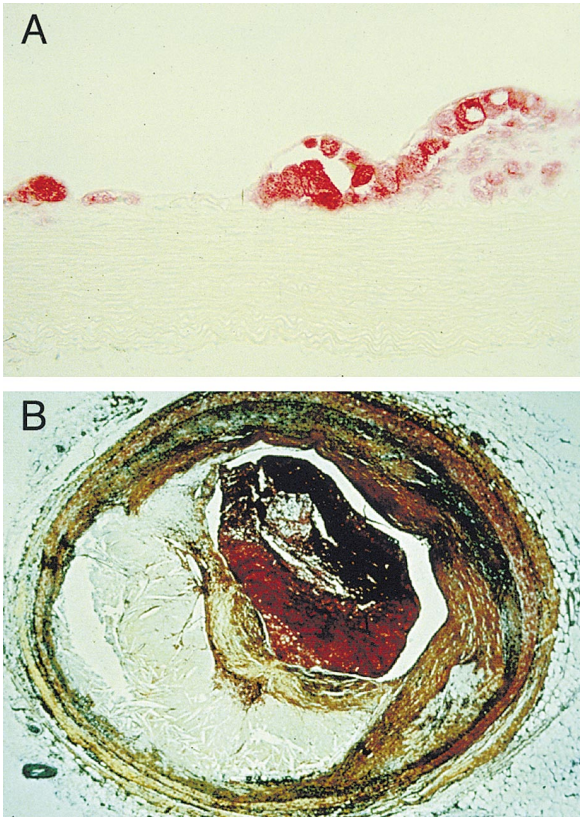

Figure 1. Early and Late Atherosclerotic Lesions

(A) Cross section of a fatty streak lesion from the aorta of a cholesterol-fed rabbit immunostained for a macrophage-specific marker. (Micrograph courtesy of Wulf Palinski.) (B) Cross section through a human coronary artery at the level of a thrombotic atherosclerotic lesion causing fatal myocardial infarction.

called scavenger receptors expressed on macrophages and smooth muscle cells. While LDL is protected from oxidation in the plasma compartment, it is thought to become susceptible to enzymatic and nonenzymatic modifications when retained by extracellular matrix proteins in the artery wall (Schwenke and Carew, 1989; Williams and Tabas, 1998). A large number of proinflammatory and proatherogenic properties have been ascribed to mmLDL and OxLDL and their components (Table 3).

There is substantial evidence that LDL oxidation occurs in both animals and in man, and numerous studies have shown that antioxidant treatment with various agents reduces the development of atherosclerosis in both nonprimate and primate hypercholesterolemic animal models (Steinberg and Witztum, 1999). Epidemiologic data in humans also supports a protective role for antioxidant supplementation (Jha et al., 1995). Despite this, prospective clinical trials with antioxidant vitamins, such as vitamin E and beta carotene, in patients with preexisting atherosclerosis have thus far been disappointing (Yusuf et al., 2000). Pinpointing the molecular mechanisms responsible for LDL oxidation in vivo and defining the most susceptible patient populations will be necessary for the development of specific and efficacious approaches to antioxidant therapy.

A number of potential oxidant-generating systems have been investigated that could directly or indirectly target LDL lipids, including myeloperoxidase, nitric oxide synthase and 15-lipoxygenase (15-LO) (Heinecke, 1998; Steinberg and Witztum, 1999). Recent evidence supporting a proatherogenic role of 15-LO has been obtained by knocking out the homologous leukocyte *12/15-LO* gene in mice and crossing these animals with apo E–deficient mice (Cyrus et al., 1999). Double knockout mice exhibited a striking reduction in atherosclerosis as compared to apo E–deficient control animals, confirming a role of 15-LO as a disease-modifying enzyme. Consistent with this, overexpression of 15-LO in the vessel wall of LDL receptor–deficient mice led to accelerated development of atherosclerosis (Harats et al., 2000).

Nitric oxide (NO) is a potent oxidant produced by both endothelial cells and macrophages that appears to exert both atherogenic and protective effects, dependent on its source of production. The vasodilator function of NO produced by endothelial NO synthase (eNOS) is protective, as deletion of the *eNOS* gene in the background of apo E deficiency results in hypertension and increased atherosclerosis (Knowles et al., 2000). In contrast, NO produced via the much higher capacity inducible NO synthase (iNOS) in macrophages serves antimicrobial functions based on its potent oxidative properties. Evidence that inducible nitric oxide synthase contributes to LDL oxidation in vivo has recently been provided by studies demonstrating that apo E–deficient mice lacking iNOS develop less atherosclerosis (although this was not observed by Knowles et al., 2000) and that inhibitors of iNOS decrease atherosclerosis in rabbits (Behr-Rousel et al., 2000; Detmers et al., 2000). In contrast, disruption of the gene encoding gp91-phox required for phagocyte NADPH oxidase activity, another potential contributor to LDL oxidation, did not reduce the development of atherosclerosis (Kirk et al., 2000). Degradation of biologically active lipids within oxLDL also appears to determine atherosclerosis susceptibility, as disruption of the gene encoding serum paraoxonase, an esterase/peroxidase carried on HDL that degrades oxidized phospholipids, resulted in increased lesion development (Shih et al., 2000).

Monocyte/Macrophage Recruitment

Although the recruitment of monocytes to the arterial wall and their subsequent differentiation into macrophages may initially serve a protective function by removing cytotoxic and proinflammatory oxLDL particles or apoptotic cells, progressive accumulation of macrophages and their uptake of oxLDL ultimately leads to development of atherosclerotic lesions. The osteopetrotic (op) mouse, which carries a naturally occurring mutation in the gene encoding macrophage colony stimulating factor (M-CSF) and exhibits a near complete absence of macrophages, is extremely resistant to the development of atherosclerosis when bred to apo E–deficient mice despite an increase in circulating cholesterol levels (Smith et al., 1995; Qiao et al., 1997).

The recruitment of monocytes to lesion-prone sites of large arteries is regulated by cell adhesion molecules that are expressed on the surface of endothelial cells

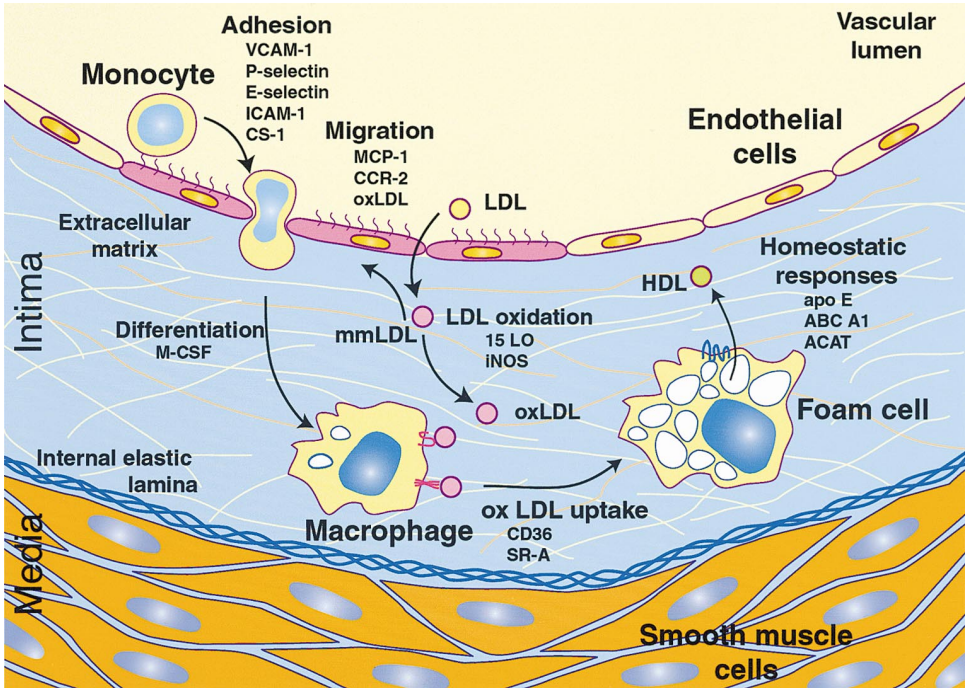

Figure 2. Initiating Events in the Development of a Fatty Streak Lesion

LDL is subject to oxidative modifications in the subendothelial space, progressing from minimally modified LDL (mmLDL), to extensively oxidized LDL (oxLDL). Monocytes attach to endothelial cells that have been induced to express cell adhesion molecules by mmLDL and inflammatory cytokines. Adherent monocytes migrate into the subendothelial space and differentiate into macrophages. Uptake of oxLDL via scavenger receptors leads to foam cell formation. OxLDL cholesterol taken up by scavenger receptors is subject to esterification and storage in lipid droplets, is converted to more soluble forms, or is exported to extracellular HDL acceptors via cholesterol transporters, such as *ABC-A1*.

in response to inflammatory stimuli (Figure 3). Several cell adhesion molecules have been suggested to play roles in macrophage recruitment. One of the first to be implicated was VCAM-1, based on its increased expression on endothelial cells over lesion-prone areas, its preferential recruitment of monocytes, and its pattern of regulation by proinflammatory stimuli (Cybulsky and Gimbrone, 1991). Studies to confirm a role of VCAM-1 in atherosclerosis in the mouse have been complicated by the fact that systemic deletion of the *VCAM-1* gene results in early embryonic lethality. E selectin and P selectin appear to play quantitative roles in monocyte entry based on a 40% to 60% decrease in atherosclerosis in apo E–deficient mice lacking both genes (Dong et al., 1998). Similarly, gene deletion of ICAM-1 resulted in small but significant reductions in monocyte recruitment to atherosclerotic lesions in apo E–deficient mice (Collins et al., 2000). Chronic delivery of a peptidomimetic

Table 3. Possible Mechanisms by which oxLDL Components Can Exert Proatherogenic Effects

oxLDL Components(s)	Effect	Mechanism
lyso phosphatidyl choline (PC), oxidized phospholipids	Increased monocyte adhesion	Increased expression of adhesion molecules on endothelial cells
lyso PC, oxidized phospholipids	Increased monocyte and T cell chemotaxis	Direct effects on monocytes and T cells and indirect effects due to stimulation of chemokine production (e.g., MCP-1)
lyso PC, oxidized phospholipids	Increased scavenger receptor A expression	Activation of AP-1 and ets transcription factors
9-hydroxyeicosatetraenoic acid	Increased CD 36 expression	Activation of PPARγ
Modified apo B and oxidized phospholipids	Increased foam cell formation	Enhanced uptake of oxLDL mediated by scavenger receptor
lyso PC, oxidized phospholipids	Induction of proinflammatory genes	Activation of NFκB, AP-1, and increased cAMP
Oxidized lipids and apo B adducts	Induces cellular and humoral immune responses	Neoepitope formation
lyso PC, cholesterol, oxysterols	Increased apoptosis and necrosis	Activation of programmed cell death, formation of cholesterol crystals. Loss of membrane integrity
Oxidized lipids	Enhanced procoagulant activity	Induction of tissue factor, increased platelet aggregation

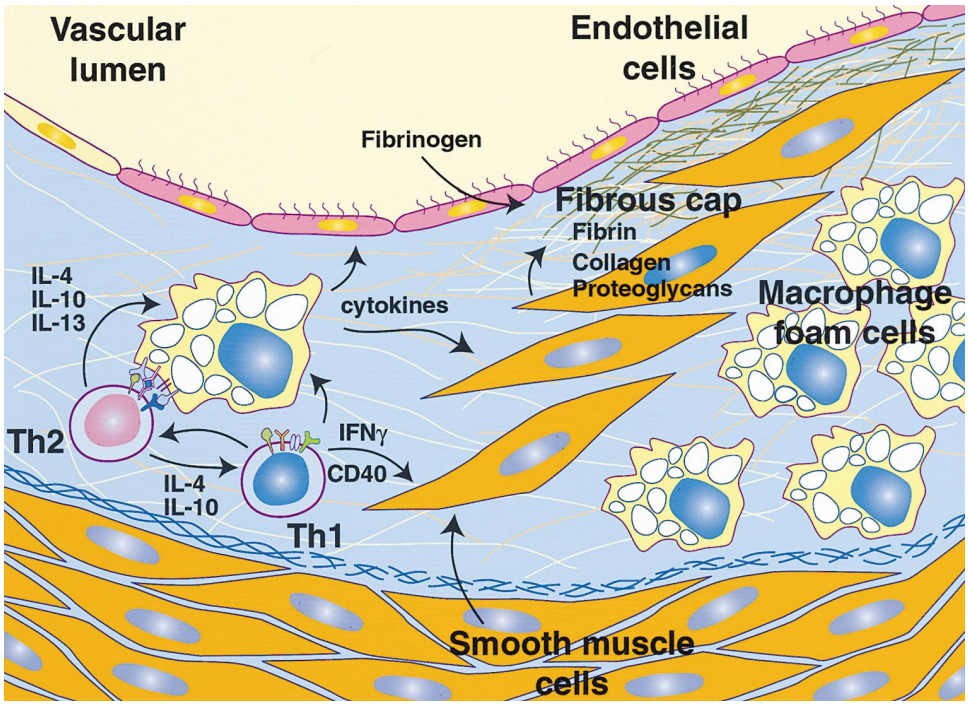

Figure 3. Lesion Progression

Interactions between macrophage foam cells, Th1 and Th2 cells establish a chronic inflammatory process. Cytokines secreted by lymphocytes and macrophages exert both pro- and antiatherogenic effects on each of the cellular elements of the vessel wall. Smooth muscle cells migrate from the medial portion of the arterial wall, proliferate and secrete extracellular matrix proteins that form a fibrous plaque.

corresponding to connecting segment 1 (CS-1) domain of fibrinectin 1, which blocks function of the adhesion molecule VLA-4 on the leukocyte surface, reduced lipid accumulation in C57BL/6J mice fed an atherogenic diet (Shih et al., 1999). Together, these findings suggest that many cell adhesion molecules contribute to the recruitment of monocytes and T cells to the atherosclerotic lesion. Neutrophils, which normally contribute to most inflammatory responses, are notably absent in lesions, although the mechanisms leading to their exclusion remain to be defined.

Migration of monocytes into the artery wall is likely to be stimulated in part by oxLDL, which can directly attract monocytes (Steinberg et al., 1989) and can also induce the expression of chemotactic molecules by endothelial cells, such as monocyte chemotactic protein 1 (MCP-1) (Navab et al., 1996). Intriguingly, monocyte expression of CCR2, the receptor for MCP-1, is stimulated by hypercholesterolemia and monocytes derived from hypercholesterolemic patients exhibit increased chemotactic responses to MCP-1 (Han et al., 1999). Disruption of the *MCP-1* or *CCR2* genes markedly reduces the development of atherosclerosis in apoE$^{-/-}$ or apo B–overexpressing mice, respectively (Boring et al., 1998; Gu et al., 1998; Gosling et al., 1999). IL-8, which is present in human atherosclerosis lesions, may also play a role in monocyte-macrophage trafficking. Although a clear homolog of IL-8 has not been established in the mouse, reconstitution of the hematopoietic system of LDL R–deficient mice with bone marrow cells lacking CXCR2, one two high-affinity receptors for IL-8 and other CXC chemokines, resulted in significantly less atherosclerosis than mice reconstituted with wild-type bone marrow cells (Boisvert et al., 1998). In concert, these findings suggest that inhibition of macrophage chemotaxis mediated by MCP-1 and/or interference with CXCR-2 activity may be of therapeutic benefit.

Foam Cell Formation

The development of macrophage "foam cells" that contain massive amounts of cholesterol esters is a hallmark of both early and late atherosclerotic lesions. Cholesterol accumulation in these cells is thought to be mediated primarily by uptake of modified forms of LDL via so-called scavenger receptors (Yamada et al., 1998) (Figure 2). Recognition of oxLDL by scavenger receptors is mediated, at least in part by oxidized phospholipids in both the lipid phase and as covalent adducts on apo B (Hörkkö et al., 2000). Although several proteins may contribute to this overall process, scavenger receptors A (SR-A) and CD36 have been demonstrated to play quantitatively significant roles. Apo E–deficient mice lacking the SR-A or CD36 scavenger receptors developed significantly less atherosclerosis than control apo E–deficient mice, with quantitatively greater reductions in lesion area observed in CD36/apo E–deficient mice (Suzuki et al., 1997; Febraio et al., 2000).

OxLDL-derived cholesterol brought into the macrophage via scavenger receptors consists of free cholesterol as well as cholesterol esters that are hydrolyzed in lysosomes. Free cholesterol has a number of potential metabolic fates, including esterification by acyl CoA: cholesterol acyltransferase-1 (ACAT-1) and storage in the lipid droplets that characterize foam cells. Choles-

terol esters within these lipid droplets can in turn be hydrolyzed by hormone-sensitive lipase, generating free cholesterol for incorporation into membranes and transport out of the cell. Membrane incorporation of excess cholesterol inhibits the proteolytic activation of the SREBP transcription factors required for cholesterol biosynthesis and LDL receptor expression (Brown and Goldstein, 1999). While this prevents further accumulation of cholesterol via these pathways, it does not alter cholesterol uptake via scavenger receptors or via phagocytic mechanisms. Thus, mechanisms mediating cholesterol efflux are critical for maintenance of cholesterol homeostasis in the macrophage. Disruption of ACAT-1 results in marked systemic abnormalities in lipid homeostasis in hypercholesterolemic apo E–deficient and LDL-R-deficient mice, leading to extensive deposition of free cholesterol in skin and brain (Accad et al., 2000; Yagu et al., 2000). ACAT-1 deficiency did not prevent development of atherosclerosis in these models, but reduced the lipid and macrophage content of lesions. Although these alterations could result in more stable lesions, the systemic lipid abnormalities observed in ACAT-1-deficient mice suggest that therapeutic inhibition of ACAT-1 could have detrimental effects.

The macrophage has two potential mechanisms for disposing of excess cholesterol: enzymatic modification to more soluble forms and efflux via membrane transporters. The enzyme cholesterol 27 hydroxylase is expressed in macrophages at relatively high levels and could potentially play a role in cholesterol excretion by converting it to the more soluble 27-OH-cholesterol (Bjorkhem, 1992). The major mechanism for cholesterol efflux, however, is likely to be via membrane transporters, with HDL serving as the primary extracellular acceptor. This role of HDL is thought to be critical for physiologic "reverse cholesterol transport" and to at least partially explain why risk of atherosclerosis is inversely correlated with HDL cholesterol levels (Tall et al., 2000). Although low HDL levels are a major risk factor for development of atherosclerosis, deletion of the *apo A-I* gene, which encodes the major protein component of HDL, is not sufficient to cause atherosclerosis in mice. However, apo A-I deficiency results in more severe atherosclerosis in hypercholesterolemic mouse models, while adenovirus-mediated overexpression of apo A-I is protective (Voyiaziakis et al., 1998; Benoit et al., 1999; Tangirala et al., 1999). There is also evidence that macrophages may contribute directly to the availability of extracellular cholesterol acceptors through secretion of apo E (Linton et al., 1995), which is capable of contributing to the formation of HDL particles.

A key insight into the molecular mechanisms responsible for cholesterol efflux resulted from studies of patients with Tangier disease, which is characterized by extremely low levels of HDL and cholesterol accumulation in macrophages. Several different approaches led to the identification of null mutations in *ABC A1,* encoding a member of the ATP binding cassette family of transporters, as the cause of Tangier disease (Bodzioch et al., 1999; Brooks-Wilson et al., 1999; Lawn et al., 1999; Rust et al., 1999). Although mechanistic details remain to be defined, in vitro studies indicate that ABC A1 mediates transport of cholesterol from cells to HDL acceptors. In the absence of proper lipidation, HDL particles are rapidly cleared, suggesting a probable explanation for the extremely low HDL cholesterol levels in Tangier patients. Variability in ABC A1 expression and function due to more subtle mutations may account for at least some of the variability of HDL levels in human populations. Once the free cholesterol has been taken up from peripheral cells by HDL, it is esterified to cholesterol esters by lecithin-cholesterol acyltransferase (LCAT). HDL can subsequently exchange cholesterol esters for triglycerides carried by other lipoproteins via cholesterol ester transfer protein (CETP). Alternatively, HDL can selectively deliver cholesterol esters to the liver for excretion by binding to the HDL receptor SR-B1. The physiologic importance of the SR-B1 receptor for reverse cholesterol transport is suggested by the findings that hypercholesterolemic mice homozygous for a hypomorphic SR-B1 allele develop increased atherosclerosis, while mice that overexpress SR-B1 exhibit reduced atherosclerosis (Huszar et al., 2000; Kozarsky et al., 2000).

Lesion Progression and Immunologic Responses

The transition from the relatively simple fatty streak to the more complex lesion is characterized by the immigration of smooth muscle cells from the medial layer of the artery wall past the internal elastic lamina and into the intimal, or subendothelial, space (Figure 3). Intimal smooth muscle cells may proliferate and take up modified lipoproteins, contributing to foam cell formation, and synthesize extracellular matrix proteins that lead to the development of the fibrous cap (Ross, 1999; Steinberg and Witztum, 1999; Paulsson et al., 2000). This phase of lesion development is influenced by interactions between monocyte/macrophages and T cells that result in a broad range of cellular and humoral responses and the acquisition of many features of a chronic inflammatory state. Significant cross talk appears to occur among the cellular elements of developing lesions. Lesional T cells appear to be activated, expressing both Th1 and Th2 cytokines (Hansson, 1997). Similarly, macrophages, endothelial cells, and smooth muscle cells appear to be activated based on their expression of MHC class II molecules and numerous inflammatory products, such as TNFα, IL-6, and MCP-1 (Figure 3).

Lymphocytes do not appear to be required for the development of atherosclerosis. When apoE-deficient mice were crossed with RAG-1 recombinase-deficient mice, which eliminates both T and B cell compartments, and then fed cholesterol to achieve plasma cholesterol levels of 1600 mg/dl or greater, lesion formation was unaffected. However, if these double KO mice were fed a normal diet, so that cholesterol levels of "only" 600 mg/dl were achieved, lesion formation was inhibited by 42% (Dansky et al., 1997). These data imply that once early lesions develop, immune responses modulate progression. Immune responses appear to exert both atherogenic and antiatherogenic effects. Even a single immunomodulatory cytokine can exert both positive and negative effects. For example, the potent Th1-derived cytokine, interferonγ (IFNγ), reduces scavenger receptor expression on macrophages, decreases collagen synthesis, and inhibits smooth muscle cell proliferation, all potentially antiatherogenic effects. On the other hand,

IFNγ also stimulates macrophage production of proinflammatory cytokines and increases expression of MHC class 2 molecules. Together, these effects of IFNγ would be predicted to increase accumulation of macrophages within lesions and enhance their ability to present antigen to T cells. The net effect of IFNγ in mice is atherogenic, as apo E–deficient mice lacking the IFNγ receptor (R0) exhibited significantly less atherosclerosis than control apo E–deficient mice (Gupta et al., 1997). Lesions were less cellular and had decreased collagen content. Recent studies suggest that the interaction of CD40 ligand with its receptor promotes expression of a diverse set of atherogenic molecules by macrophages, smooth muscle cells, and endothelial cells, including cytokines, matrix metalloproteinases, adhesion molecules and tissue factor (Mach et al., 1998a). Inhibition of CD40 signaling using genetic or immunologic approaches inhibited development of atherosclerosis in LDL receptor–deficient mice and resulted in a more stable plaque phenotype (Mach et al., 1998b; Lutgens et al., 1999).

Th2-derived cytokines also appear to have complex effects on lesion development. Interleukin-4 (IL-4) exerts a number of effects that are predicted to be antiatherogenic, including antagonistic effects on INFγ activity in macrophages and inhibition of Th1 cell function (Figure 3). However, IL-4 is also a potent inducer of 15-LO, which promotes LDL oxidation and development of atherosclerosis in mice. IL-10, which cross-regulates Th1 cells (Figure 3), has potent deactivating properties in macrophages and modulates several other cellular processes that may interfere with the development and stability of the atherosclerotic plaque. IL-10-deficient mice exhibited a 3-fold increase in lipid accumulation, indicating an antiatherogenic role (Mallat et al., 1999; Pinderski et al., 1999). Consistent with this, transgenic overexpression of IL10 in T cells using the human IL-2 promoter resulted in significant inhibition of lesion development in C57BL/6J mice fed an atherogenic diet (Pinderski et al., 1999).

These observations indicate that immune activation is ongoing in atherosclerotic lesions. The most significant antigens responsible for immune activation are not known with certainty. Most attention has focused on the role of bacterial and viral antigens, heat shock proteins, and neoepitopes (antigenic epitopes resulting from the formation of adducts between oxidized lipids in oxLDL and apo B or arterial wall components). Substantial evidence supports an important role for epitopes of oxLDL as dominant immunogens (Hörkkö et al., 2000). The ability of macrophages to function as antigen presenting cells (APCs) is undoubtedly related to the generation of a variety of autoantibodies to oxidatively modified lipids and proteins that occurs with progressive atherosclerosis. Up to 10% of $CD4^+$ T cell clones from human carotid lesions proliferated specifically in response to oxLDL in an HLA-DR-dependent manner (Hansson, 1997). There are now many reports in mice and humans of a strong correlation between autoantibody titers to epitopes of oxLDL and extent of atherosclerosis (reviewed in Hörkkö et al., 2000) It is not yet clear whether such autoantibodies are simply markers for the extent of disease, or if they have any physiological or pathophysiological role.

Cloning autoantibodies to oxLDL from apoE-deficient mice revealed that many recognized epitopes of oxidized phospholipids either as free lipids or as protein adducts. These antibodies, mostly IgM, had the ability to block the binding and degradation of oxLDL by macrophages, suggesting that this epitope was a ligand for oxLDL uptake. Intriguingly, a genetic analysis of these antibodies revealed that they were 100% homologous to T15 natural antibodies to phosphorylcholine, which provide optimal protection to mice against lethal infection with *S. pneumoniae* (Shaw et al., 2000). Thus, these evolutionarily conserved antibodies, which are present even in mice raised in germ-free environments, were probably selected for their ability to bind to oxidized membranes, and may have a protective role.

These observations suggest that it might be possible to modulate the development of atherosclerosis by immunization or other immune-based interventions. Elimination of T cells or immunization of experimental animals with antigens that are homologous to endogenously expressed proteins present in lesions, such as certain heat shock proteins, has been found to accelerate lesion formation. In contrast, intravenous administration of polyspecific immunoglobulin *decreased* lesion formation, as did hyperimmunization of hypercholesterolemic rabbit and murine models with homologous oxLDL (Hörkkö et al., 2000).

Plaque Stability

Although advanced atherosclerotic lesions can lead to ischemic symptoms as a result of progressive narrowing of the vessel lumen, acute cardiovascular events that result in myocardial infarction and stroke are generally thought to result from plaque rupture and thrombosis (Davies et al., 1993; Lee and Libby, 1997). Plaque rupture exposes plaque lipids and tissue factor to blood components, initiating the coagulation cascade, platelet adherence, and thrombosis (Figure 4). Analysis of human atherosclerosis suggests that the evolution of advanced plaques may involve repetitive cycles of microhemorrhage and thrombosis. Plaque ruptures associated with acute myocardial infarction generally occur at the shoulder regions of the plaque and are more likely to occur in lesions with thin fibrous caps, a relatively high concentration of lipid-filled macrophages within the shoulder region, and large necrotic cores (Davies et al., 1993; Lee and Libby, 1997). Studies of human and animal models of atherosclerosis suggest that programmed cell death plays a quantitatively important role in formation of the necrotic core (Bennet, 1999). Apoptosis of macrophages and vascular smooth muscle cells appears to result from cell–cell interactions and the local cytokine environment within the arterial wall, involving the actions of pro- and antiapoptotic proteins that include death receptors, proto-oncogenes, and tumor suppressor genes. Oxidized sterols present in oxLDL also appear to promote apoptosis and necrosis in lesions (Colles et al., 1996). In addition, the accumulation of apoptotic cells in the lesions suggest that both increased production and decreased disposal of such cells occur. Indeed the cholesterol-loaded apoptotic cell may be akin to a trojan horse, with macrophage uptake resulting in death of the ingesting cell. The release of oxidized and insoluble lipid from necrotic cells undoubtedly contributes to the formation

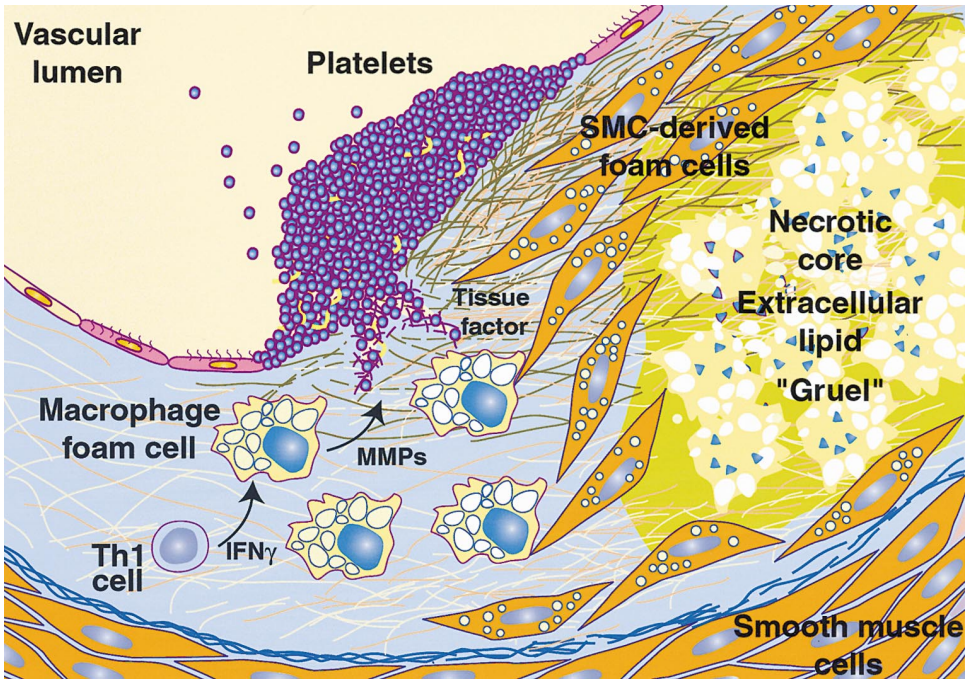

Figure 4. Plaque Rupture and Thrombosis

Necrosis of macrophage and smooth muscle cell–derived foam cells leads to the formation of a necrotic core and accumulation of extracellular cholesterol. Macrophage secretion of matrix metalloproteinases and neovascularization contribute to weakening of the fibrous plaque. Plaque rupture exposes blood components to tissue factor, initiating coagulation, the recruitment of platelets, and the formation of a thrombus.

of the "gruel" characteristic of advanced lesions (Figure 4). Apoptosis of macrophages and smooth muscle cells may not only be important in determining the ability of lesions to undergo regression, but may also influence plaque stability, as such lipids in the necrotic core are suggested to increase the potential for thrombosis.

Matrix metalloproteinases secreted by macrophages have been detected in regions of plaque rupture and are suggested to influence plaque stability by degrading extracellular matrix proteins (Galis et al., 1994; Carmeliet, 2000). Thus, methods to alter the expression or activities of metalloproteinases would be of potential clinical benefit. To date, animal models in which plaque rupture and the coagulation system reliably contribute to lesion progression and acute thrombotic events have not been developed. Variation in levels of several coagulation factors, including fibrinogen and factor VII, are associated with increased risk of cardiovascular disease (Table 1). Extensive fibrin deposition is observed in most complex human lesions (Figure 3), and decreased fibrinolytic activity has been proposed to accelerate arterial atherogenesis by facilitating thrombosis and fibrin deposition within developing atherosclerotic lesions (Lee and Libby, 1997). However, Apo E–deficient mice lacking fibrinogen develop lesions that are similar in size and complexity to control apo E–deficient mice (Xiao et al., 1998). Similarly, LDL receptor–deficient mice lacking plasminogen activator inhibitor-1 (PAI-1), which inhibits the activator of plasminogen and thus should shift the balance toward greater fibrin hydrolysis, also develop typical atherosclerotic lesions (Sjoland et al., 2000).

Neovascularization is prevalent in human atherosclerotic lesions associated with plaque rupture, hemorrhage or unstable angina (progressive episodes of temporary cardiac ischemia due to transient thrombus formation). Angiogenesis occurs in association with remodeling and protease activation in surrounding tissues, suggesting that neovascularization could contribute to plaque instability and rupture. Intimal or subendothelial neovascularization has been observed in advanced atherosclerotic lesions in apo E–deficient mice and treatment of these mice with the angiogenesis inhibitors endostatin and TNP-470 significantly decreased lesion size (Moulton et al., 1999).

Although it has been difficult to establish animal models of spontaneous acute myocardial infarction (i.e., not requiring direct interventions such as ligation of a coronary artery), the recent observation that it is possible to reproducibly induce myocardial infarctions in mice with combined LDL R and apo E deficiency by exposure to mental stress or hypoxia (Caligiuri et al., 1999) should provide new opportunities for investigation of later stages of lesion development. While myocardial infarctions in this model do not appear to result from plaque rupture, the ischemic consequences of transient hypoxia could be prevented by treatment with an endothelin A receptor antagonist. These observations suggest an important role of endothelin-dependent vasoconstriction in determining the extent of hypoxic injury that may also be relevant to ischemic events resulting from coronary thrombosis.

Toward New Therapies

As atherosclerosis is a chronic disease requiring lifetime preventive therapy, new antiatherogenic drugs should meet the criteria of being safe, palatable, inexpensive

Table 4. Potential Targets for Development of Small Molecule Inhibitors of Atherosclerosis

Target	Drug	Mechanism of Action
15-lipoxygenase	Inhibitor	Inhibition of LDL oxidation within the arterial wall
MTP	Inhibitor	Inhibition of VLDL assembly and secretion from liver
CCR2	Antagonist	Inhibition of monocyte recruitment to vessel wall
Endothelin A receptor	Antagonist	Inhibition of hypoxia-induced vasoconstriction
MMPs	Inhibitor	Prevention of plaque rupture
CETP	Inhibitor	Increased HDL cholesterol levels and reverse cholesterol transport
IκB kinase	Inhibitor	Inhibition of NFκB activation, resulting in decreased inflammatory responses
PPARα	Agonist	Decreased TG-rich lipoproteins, increased HDL, anti-inflammatory effects in vessel wall
PPARγ	Selective modulator	Decreased insulin resistance and anti-inflammatory effects in macrophage foam cells
LXRα	Selective modulator	Decreased cholesterol absorption, increased hepatic cholesterol excretion and increased reverse cholesterol transport from macrophage foam cells
RXR	Selective modulator	Activation of PPAR and LXR pathways. Current RXR ligands induce hypertriglyceridemia and central hypothyroidism
ERα,β	Selective modulator	Improved lipoprotein profiles and beneficial effects on arterial wall biology.

and of significant benefit beyond currently available therapies. Small molecule–based approaches targeting enzymes or signal-dependent transcription factors are thus much more likely to be of general clinical utility than approaches requiring the delivery of genes or polypeptides. Multiple steps in the atherogenic process could theoretically serve as the basis for intervention, including steps that control LDL levels and LDL oxidation, monocyte recruitment, scavenger receptor expression and reverse cholesterol transport (Table 4). We consider here selected examples of potential targets for new classes of antiatherogenic drugs that illustrate some of the opportunities and practical challenges that lie ahead.

New approaches to lowering LDL cholesterol levels and increasing HDL cholesterol levels remain attractive avenues for development of novel classes of antiatherogenic drugs. Inhibitors of specific enzymes required for lipoprotein synthesis and metabolism have already been shown to exert beneficial effects on lipoprotein profiles in animal models. For example, inhibitors of microsomal triglyceride transfer protein (MTP), which is required for the assembly of apo B–containing lipoproteins, have been shown to reduce plasma LDL and VLDL cholesterol levels in rabbits (Wetterau et al., 1998). Overexpression of human cholesterol ester transfer protein (CETP) which mediates exchange of cholesterol esters in HDL for triglycerides in VLDL, increases atherosclerosis in apo E–deficient and LDL receptor–deficient mice (Plump et al., 1999). Consistent with this, CETP inhibitors have been shown to increase plasma HDL cholesterol levels, decrease VLDL and LDL cholesterol, and inhibit development of atherosclerosis in rabbits (Okamoto et al., 2000).

Inhibitors of enzymes that play critical roles in LDL oxidation are potentially novel classes of antiatherogenic drugs that could work synergistically with cholesterol lowering agents. 15-LO is an attractive target for drug development, as genetic experiments in mice clearly indicate that it is atherogenic and small molecule inhibitors of 15-LO have already been shown to reduce development of atherosclerosis in rabbits (Sendobry et al., 1997). Although 15-LO is expressed in human lesions, a critical unanswered question is whether it exerts atherogenic effects comparible to those observed in animal models . Epidemiologic studies linking 15-LO expression levels or specific 15-LO polymorphisms to risk of atherosclerosis could provide helpful information along these lines. A challenge in carrying out intervention studies in humans is the need to link a measurable parameter of the inhibitor's activity with disease outcome. In the case of statins, effectiveness of inhibition of HMG CoA reductase activity was readily assessed by measurement of serum cholesterol levels, reductions of which were highly correlated with reductions in atherosclerotic complications. In contrast, the relevant site of action of 15-LO inhibitors (as well as some of the other potential targets listed in Table 4) is presumably within the artery wall and not readily accessible for measurement of inhibitory effects on LDL oxidation. In the 15-LO deficient mice, decreased excretion of isoprostanes, a breakdown product of arachidonic acid peroxidation, as well as autoantibodies to oxLDL correlated strongly with the decreased extent of atherosclerosis, and could potentially be used to monitor inhibitor activity in humans.

Nuclear Targets

Transcription factors that regulate cholesterol homeostasis, vascular wall biology, and/or inflammatory responses represent a potentially large class of therapeutic targets. Transcription factors represent more complex targets for intervention than metabolic enzymes because they are usually expressed in multiple tissues and potentially regulate large numbers of genes. For example, members of the NFκB family coordinately regulate gene clusters that control inflammatory responses and have been implicated in the development of atherosclerosis (Thurberg and Collins, 1998). The development of inhibitors of NFκB, for example by targeting the recently identified IκB kinases that are required for NFκB activation, will be of interest with respect to their potential antiatherogenic properties. While such inhibitors are likely to be useful in acute inflammation, clinical use in chronic disease settings such as atherosclerosis may be more limited if therapeutic effects come at the expense of compromised immune function.

Several members of the nuclear receptor superfamily have emerged as targets of drugs that have direct or indirect effects on the development of atherosclerosis (Table 4). Estrogen receptors are important targets of drugs for breast cancer and hormone replacement therapy and the impact of these agents on the cardiovascular system is an important consideration in their clinical use (Roe et al., 2000). Estrogens exert a number of protective effects on lipoprotein profiles and the vascular wall, effects that are lost following menopause. Selective estrogen receptor modulators (SERMs), including tamoxifen and raloxifene, have been developed that exert proestrogenic effects in some tissues and antiestrogenic effects in others. Studies of postmenopausal women given tamoxifen as adjuvant therapy after operable breast cancer found a significant decrease in myocardial infarction and tamoxifen has been shown to dramatically reduce cholesterol levels and development of atherosclerosis in apo E–deficient mice (Reckless et al., 1997 and references therein). The selective effects of these ligands have been linked to their ability to recruit specific nuclear receptor coactivators and corepressors that exhibit tissue-specific patterns of expression and that appear to integrate the activities of nuclear receptors with other transcription factors in a promoter-specific manner (Glass and Rosenfeld, 2000). The ability to selectively modulate the biological actions of the estrogen receptor with structurally distinct ligands suggests that it will be possible to differentially modulate the activities of other members of the nuclear receptor family.

The peroxisome proliferator-activated receptor subfamily (PPARα, γ, and δ) also appears to play metabolic and immunologic roles that influence development of atherosclerosis (Fruchart et al., 1999). PPARα regulates several aspects of fatty acid metabolism, including beta oxidation and uptake of fatty acids from triglyceride-rich lipoproteins. Intriguingly, PPARα stimulates expression of apoA-I and raises HDL levels in humans but not rodents due to differences in *cis* active regulatory elements in the apoA-I promoter (Staels and Auwerx, 1998). The recognition that PPARα is the molecular target of the fibrate class of lipid-lowering drugs has facilitated the development of increasingly powerful agonists that are in current clinical development. The observation that PPARα is expressed in endothelial and smooth muscle cells and that PPARα ligands can exert anti-inflammatory effects in some settings (Staels et al., 1998) should stimulate further efforts to evaluate their effects on the development of atherosclerosis.

PPARγ regulates fat cell development and glucose homeostasis and is the molecular target of the thiazolidinedione (TZD) class of insulin sensitizers used in the treatment of type 2 diabetes mellitus (Spiegelman, 1998). Recent studies indicate that PPARγ is also highly expressed in macrophage foam cells and exerts both pro- and antiatherogenic effects on macrophage gene expression (Jiang et al., 1998; Nagy et al., 1998; Ricote et al., 1998a, 1998b; Tontonoz et al., 1998). On the one hand, treatment of macrophages with TZDs has been shown to increase expression of the scavenger receptor CD36, suggesting that these drugs might promote foam cell formation. On the other hand, TZDs have been shown to inhibit expression of TNFα, gelatinase B, and other inflammatory mediators, suggesting that they might inhibit inflammatory components of atherosclerosis. In addition, PPARγ has recently been shown to increase ABC A1 expression and cholesterol efflux in macrophages by a mechanism involving induction of LXRα (Chawla et al., 2001). Consistent with these observations, PPARγ agonists exert potent antiatherogenic effects in LDL $R^{-/-}$ mice (Li et al., 2000), and reconstitution of the hematopoietic system of LDL $R^{-/-}$ mice with PPAR$\gamma^{-/-}$ bone marrow progenitor cells results in increased atherosclerosis compared to LDL $R^{-/-}$ mice reconstituted with wild-type progenitor cells (Chawla et al., 2001). Because TZDs are now widely used in diabetic patients, who are at high risk for developing complications of atherosclerosis, these observations have important clinical implications. The therapeutic benefits of future generations of PPARγ ligands in patients with type 2 diabetes mellitus could presumably be greatly enhanced by selecting compounds that retain insulin-sensitizing activities and are optimized for their antiatherogenic activities. The use of microarrays to assess genome-wide responses of adipocytes, muscle cells and macrophages to structurally distinct PPARγ ligands should be useful in selecting candidates that have these properties for testing in vivo.

LXRs have more recently emerged as transcription factors that function in concert with SREBPs to regulate cholesterol homeostasis (Repa et al., 2000a). While elevated cellular levels of cholesterol and oxysterols suppress the transcription of SREBP target genes, they stimulate transcription of LXR target genes. LXR target genes include the 7α-hydroxylase gene in rodents, which encodes an enzyme catalyzing the rate-limiting step required for bile acid synthesis and thus cholesterol excretion from liver. In addition, the *ABC-A1* gene is regulated in the intestine and in macrophages by LXRs (Costet et al., 2000; Repa et al., 2000b). Mice lacking LXRα develop massive hepatomegaly when placed on a high cholesterol diet due to increased cholesterol absorption in the gut and impaired excretion from liver. Administration of potent RXR or LXR agonists to wild-type mice substantially reduces cholesterol absorption, suggesting that this is a regulated step that is subject to pharmacologic intervention. However, LXR and RXR agonists also stimulate fatty acid biosynthesis in liver and an increase in circulating triglyceride levels by inducing the expression and activity of SREBP-1c (Repa et al., 2000a; Schultz et al., 2000). These observations suggest that it may be necessary to develop selective modulators of RXRs and LXRs if they are to become useful targets of therapeutic intervention.

Identification and Validation of New Atherosclerosis Susceptibility Genes

Although atherosclerosis has long been the subject of intensive epidemiologic and pathophysiologic investigation, there is considerable evidence that quantitatively important determinants of disease susceptibility remain to be identified (Hennekens, 1998). Family history of heart disease remains an important risk factor after removing other previously identified independent factors, implying additional genetic factors. In mice, atherosclerosis susceptibility varies dramatically in different strain backgrounds (Dansky et al., 1999). The majority of this

variation cannot as yet be attributed to changes in known risk factors, such as lipoprotein levels, again suggesting yet to be discovered modifying genes.

Studies of rare Mendelian disorders such as familial hypercholesterolemia and Tangier disease should continue to lead to the identification of genes that influence the development of atherosclerosis. The recent discovery of mutations in the *ABC-A1* gene as the cause of low HDL levels in Tangier patients provides a case in point. However, most common forms of atherosclerosis are multifactorial in origin and pure genetic approaches have been less successful in pinning down significant determinants of risk (Risch, 2000). For example, the major genetic determinants of the metabolic syndrome, characterized by insulin resistance, hypertension, abdominal obesity, low HDL, and mild hypertriglyceridemia, remain to be defined. The marked variance in atherosclerosis susceptibility among different inbred strains of mice should allow relevant genes to be identified through generation of congenic lines and positional cloning. The cataloging of common single nucleotide polymorphisms (SNPs) as part of human and murine genome sequencing efforts may allow the development of new genetic strategies for identifying atherosclerosis-susceptibility genes (Risch, 2000).

Clearly, the sequencing of expressed sequence tags (ESTs) and the entire genomes of humans and model organisms has already had an enormous impact on a broad range of investigation. In addition to facilitating genetic studies, this information has enabled the rapid isolation of full-length cDNA clones based on partial protein or cDNA sequence information, the recognition of new members of gene families based on conserved functional domains, and the fabrication of microarrays used for large-scale gene expression profiling. In addition, comparisons of large regions of noncoding sequence across species has recently been shown to allow the identification of conserved regulatory elements (Loots et al., 2000). Variations in such transcriptional control regions are likely to account for much of the variation in levels of gene expression that control such complex traits as serum cholesterol levels and blood pressure.

The emerging ability to profile the expression of thousands of genes in a single experiment is revolutionizing the study of developmental, homeostatic, and pathological programs of gene expression (Lockhart and Winzeler, 2000; Young, 2000). This technology is now being applied to determine genome-wide responses of smooth muscle cells, endothelial cells, and macrophages to a variety of atherogenic stimuli (Shiffman et al., 2000). Analysis of the data emerging from these experiments will undoubtedly lead to the identification of new candidate genes that can be tested for roles in the development of atherosclerosis. These experimental approaches are also likely to prove extremely useful in the search for novel therapeutic agents that have desirable activity profiles, particularly for drugs that act to alter the function of transcription factors or other regulatory proteins. In addition, one can envision using microarray technologies as epidemiologic and diagnostic tools, for example by correlating risk of disease or response to a specific therapy with gene expression profiles in circulating monocytes.

Regular exercise, a healthy diet and management of established risk factors such as hypercholesterolemia with available methods is accomplishing a great deal in the prevention of atherosclerosis. For many individuals, however, these measures are not enough. The combination of robust animal models, emerging genomic and proteomic technologies, and the dividends of genome sequencing efforts suggest that the stage is set for an accelerated pace of discovery of the mechanisms contributing to the pathogenesis of atherosclerosis and of the development of powerful new antiatherogenic drugs.

Acknowledgments

We thank our colleagues at the La Jolla Specialized Center of Research on Molecular Medicine and Atherosclerosis for many fruitful discussions, and in particular to Dan Steinberg for critical review of the manuscript. We apologize to our colleagues for being unable to provide primary citations for much of the work described in this review due to space limitations.

References

Accad, M., Smith, S., Newland, D., Sanan, D., King, J.L., Linton, M., Fazio, S., and Farese, J.R. (2000). Massive xanthomatosis and altered composition of atherosclerotic lesions in hyperlipidemic mice lacking acyl CoA:cholesterol acyltransferase 1. J. Clin. Invest. *105*, 711–719.

Behr-Roussel, D., Rupin, A., Simonet, S., Bonhomme, E., Coumailleau, S., Cordi, A., Serkiz, B., Fabiani, J.N., and Verbeuren, T.J. (2000). Effect of chronic treatment with the inducible nitric oxide synthase inhibitor N-iminoethyl-L-lysine or with L-arginine on progression of coronary and aortic atherosclerosis in hypercholesterolemic rabbits. Circulation *102*, 1033–1038.

Bennet, M.R. (1999). Apoptosis of vascular smooth muscle cells in vascular remodelling and atherosclerotic plaque rupture. Cardiovascular Res. *41*, 361–368.

Benoit, P., Emmanuel, F., Caillaud, J.M., Bassinet, L., Castro, G., Gallix, P., Fruchart, J.C., Branellec, D., Denefle, P., and Duverger, N. (1999). Somatic gene transfer of human apo A-I inhibits atherosclerosis progression in mouse models. Circulation *99*, 105–110.

Bjorkhem, I. (1992). Mechanism of degradation of the steroid side chain in the formation of bile acids. J. Lipid Res. *33*, 455–471.

Bodzioch, M., Orso, E., Klucken, J., Langmann, T., Bottcher, A., Diederich, W., Drobnik, W., Barlage, S., Buchler, C., Porsch-Ozcurumez, M., et al. (1999). The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. Nat. Genet. *22*, 347–351.

Boisvert, W.A., Santiago, R., Curtiss, L.K., and Terkeltaub, R.A. (1998). A leukocyte homologue of the IL-8 receptor CXCR-2 mediates the accumulation of macrophages in atherosclerotic lesions of LDL receptor-deficient mice. J. Clin. Invest. *101*, 353–363.

Boring, L., Gosling, J., Cleary, M., and Charo, I.F. (1998). Decreased lesion formation in $CCR2^{-/-}$ mice reveals a role for chemokines in the initiation of atherosclerosis. Nature *394*, 894–897.

Breslow, J.L. (1996). Mouse models of atherosclerosis. Science *272*, 685–688.

Brooks-Wilson, A., Marcil, M., Clee, S.M., Zhang, L.H., Roomp, K., van Dam, M., Yu, L., Brewer, C., Collins, J.A., Molhuizen, H.O., et al. (1999). Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nat. Genet. *22*, 336–345.

Brown, M.S., and Goldstein, J.L. (1997). The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell *89*, 331–340.

Brown, M.S., and Goldstein, J.L. (1999). A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. Proc. Natl. Acad. Sci. USA *96*, 11041–11048.

Caligiuri, G., Levy, B., Pernow, J., Thoren, P., and Hansson, G.K. (1999). Myocardial infarction mediated by endothelin receptor sig-

naling in hypercholesterolemic mice. Proc. Natl. Acad. Sci. USA *96*, 6920–6924.

Carmeliet, P. (2000). Proteins in cardiovascular aneurysms and rupture: targets for therapy? J. Clin. Invest. *105*, 1519–1520.

Chawla, A., Boisvert, W.A., Lee, C.-H., Lafitte, B.A., Barak, Y., Joseph, S.B., Liao, D., Nagy, L., Edwards, P.A., Curtiss, L.K., et al. (2001). A PPARγ-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. Mol. Cell *7*, 161–171.

Colles, S.M., Irwin, K.C., and Chisolm, G.M. (1996). Roles of multiple oxidized lipids in cellular injury. J. Lipid Res. *37*, 2018–2028.

Collins, R.G., Velji, R., Guevara, N.V., Hicks, M.J., Chan, L., and Beaudet, A.L. (2000). P-selectin or intercellular adhesion molecule (ICAM)-1 deficiency substantially protects against atherosclerosis in apolipoprotein E–deficient mice. J. Exp. Med. *191*, 189–194.

Costet, P., Luo, Y., Wang, N., and Tall, A.R. (2000). Sterol-dependent transactivation of the ABC1 promoter by the liver X Receptor/Retinoid X receptor. J. Biol. Chem. *275*, 28240–28245.

Cybulsky, M.I., and Gimbrone, M.A., Jr. (1991). Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. Science *251*, 788–791.

Cyrus, T., Witztum, J.L., Rader, D.J., Tangirala, R., Fazio, S., Linton, M.F., and Funk, C.D. (1999). Disruption of the 12/15-lipoxygenase gene diminishes atherosclerosis in apo E-deficient mice. J. Clin. Invest. *103*, 1597–1604.

Dansky, H.M., Charlton, S.A., Harper, M.M., and Smith, J.D. (1997). T and B lymphocytes play a minor role in atherosclerotic plaque formation in the apolipoprotein E–deficient mouse. Proc. Natl. Acad. Sci. USA *94*, 4662–4646.

Dansky, H.M., Charlton, S.A., Sikes, J.L., Heath, S.C., Simantov, R., Levin, L.F., Shu, P., Moore, K.J., Breslow, J.L., and Smith, J.D. (1999). Genetic background determines the extent of atherosclerosis in apo E–deficient mice. Arterioscler. Thromb. Vasc. Biol. *19*, 1960–1968.

Davies, M.J., Richardson, P.D., and Woolf, N. (1993). Risk of thrombosis in human atherosclerotic plaques: role of extracellular lipid, macrophage, and smooth muscle cell content. Br. Heart J. *69*, 377–381.

Detmers, P.A., Hernandez, M., Mudgett, J., Hassing, H., Burton, C., Mundt, S., Chun, S., Fletcher, D., Card, D.J., Lisnock, J., et al. (2000). Deficiency in inducible nitric oxide synthase results in reduced atherosclerosis in apolipoprotein E-deficient mice. J. Immunol. *165*, 3430–3435.

Dong, Z., Chapman, S., Brown, A., Frenette, P., Hynes, R., and Wagner, D. (1998). The Combined role of P- and E-selectins in atherosclerosis. J. Clin. Invest. *102*, 145–152.

Febraio, M., Podrez, E.A., Smith, J.D., Hajjar, D.P., Hazen, S.L., Hoff, H.F., Sharma, K., and Silverstein, R.L. (2000). Targeted disruption of the class B scavenger receptor CD36 protects against atherosclerotic lesion development in mice. J. Clin. Invest. *105*, 1049–1056.

Fruchart, J.C., Duriez, P., and Staels, B. (1999). Peroxisome proliferator-activated receptor-alpha activators regulate genes governing lipoprotein metabolism, vascular inflammation and atherosclerosis. Curr. Opin. Lipidology *10*, 245–257.

Galis, Z.S., Sukhova, G.K., Lark, M.W., and Libby, P. (1994). Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J. Clin. Invest. *94*, 2493–2503.

Glass, C.K., and Rosenfeld, M.G. (2000). The coregulator exchange in transcriptional functions of nuclear receptors. Genes and Dev. *14*, 121–141.

Goldstein, J.L., and Brown, M.S. (1977). The low-density lipoprotein pathway and its relation to atherosclerosis. Ann. Review Biochem. *46*, 897–930.

Gosling, J., Slaymaker, S., Gu, L., Tseng, S., Zlot, C.H., Young, S.G., Rollins, B.J., and Charo, I.F. (1999). MCP-1 deficiency reduces susceptibility to atherosclerosis in mice that overexpress human apolipoprotein B. J. Clin. Invest. *103*, 773–778.

Gould, L.A., Rossouw, J.E., Santanello, N.C., Heyse, J.F., and Furberg, C.D. (1998). Cholesterol reduction yields clinical benefit: Impact of statin trials. Circulation *97*, 946–952.

Gu, L., Okada, Y., Clinton, S.K., Gerard, C., Sukhova, G.K., Libby, P., and Rollins, B.J. (1998). Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein-deficient mice. Mol. Cell *2*, 275–281.

Gupta, S., Pablo, A., Jiang, X., Wang, N., Tall, A., and Schindler, C. (1997). IFN-γ potentiates atherosclerosis in apoE knock-out mice. J. Clin. Invest. *99*, 2752–2761.

Han, K.H., Han, K.O., Green, S.R., and Quehenberger, O. (1999). Espression of hte monocyte chemoattractant protein-1 receptor CCR2 is increased in hypercholesterolemia: differential effects of plasma lipoproteins on monocyte function. J. Lipid Res. *40*, 1053–1063.

Hansson, G.K. (1997). Cell-mediated immunity in atherosclerosis. Curr Opin Lipidol *8*, 301–311.

Harats, D., Shaish, A., George, J., Mulkins, M., Kurihara, H., Levkovitz, H., and Sigal, E. (2000). Overexpression of 15-Lipoxygenase in Vascular Endothelium Accelerates Early Atherosclerosis in LDL Receptor-Deficient Mice. Arterioscler. Thromb. Vasc. Biol. *20*, 2100–2105.

Heinecke, J.W. (1998). Oxidants and antioxidants in the pathogenesis of atherosclerosis: implications for the oxidized low density lipoprotein hypothesis. Atherosclerosis *141*, 1–15.

Hennekens, C.H. (1998). Increasing burden of cardiovascular disease: Current knowledge and future directions for research on risk factors. Circulation *97*, 1095.

Hobbs, H.H., Russell, D.W., Brown, M.S., and Goldstein, J.L. (1990). The LDL receptor locus in familial hypercholesterolemia: Mutational analysis of a membrane protein. Ann. Rev. Genet. *24*, 133–170.

Hörkkö, S., Binder, C.J., Shaw, P.X., Chang, M., Silverman, G., Palinski, W., and Witztum, J.L. (2000). Immunological responses to oxidized LDL. Free Radic. Biol. Med. *28*, 1771–1779.

Huszar, D., Varban, M.L., Rinninger, F., Feeley, R., Arai, T., Fairchild-Hubtress, V., Donavan, M.J., and Tall, A.R. (2000). Increased LDL cholesterol and atherosclerosis in LDL receptor-deficient mice with attenuated expression of scavenger receptor B1. Arterioscler. Thromb. Vasc. Biol. *4*, 1068–1073.

Jha, P., Flather, M., Lonn, E., Farkouh, M., and Yusuf, S. (1995). The antioxidant vitamins and cardiovascular disease. A critical review of epidemiologic and clinical trial data. Ann. Intern. Med. *123*, 860–872.

Jiang, C., Ting, A.T., and Seed, B. (1998). PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. Nature *391*, 82–86.

Kirk, E.A., Dinauer, M.C., Rosen, H., Chait, A., Heinecke, J.W., and LeBoeuf, R.C. (2000). Impaired superoxide production due to a deficiency in phagocyte NADPH oxidase fails to inhibit atherosclerosis in mice. Arterioscl. Thromb. Vasc. Biol. *20*, 1529–1535.

Knowles, J.W., Reddick, R.L., Jennette, J.C., Shesely, E.G., Smithies, O., and Maeda, N. (2000). Enhanced atherosclerosis and kidney dysfunction in eNOS(−/−)ApoE(−/−) mice are ameliorated by enalapril treatment. J. Clin. Invest. *105*, 451–458.

Kozarsky, K.F., Donahee, M.H., Glick, J.M., Krieger, M., and Rader, D.J. (2000). Gene transfer and hepatic overexpression of the HDL receptor SR-B1 reduces atherosclerosis in the cholesterol-fed LDL receptor-deficient mouse. Arterioscler. Thromb. Vasc. Biol. *3*, 721–727.

Lawn, R.M., Wade, D.P., Garvin, M.R., Wang, X., Schwartz, K., Porter, J.G., Seilhamer, J.J., Vaughan, A.M., and Oram, J.F. (1999). The Tangier disease gene product ABC1 controls the cellular apolipoprotein-mediated lipid removal pathway. J. Clin. Invest. *104*, R25–R31.

Lee, R.T., and Libby, P. (1997). The unstable atheroma. Ateriosclerosis. Thromb. Vasc. Biol. *17*, 1859–1867.

Li, A., Brown, K., Silvestre, M., Willson, T., Palinski, W., and Glass, C. (2000). Periosome proliferator-activated receptor γ ligands inhibit development of atherosclerosis in LDL receptor-deficient mice. J. Clin. Invest. *106*, 523–531.

Linton, M.F., Atkinson, J.B., and Fazio, S. (1995). Prevention of atherosclerosis in apolipoprotein E–deficient mice by bone marrow transplantation. Science *267*, 1034–1037.

Lockhart, D.J., and Winzeler, E.A. (2000). Genomics, gene expression and DNA arrays. Nature *405*, 827–836.

Loots, G.G., Locksley, R.M., Blankespoor, C.M., Wang, Z.E., Miller, W., Rubin, E.M., and Frazer, K.A. (2000). Identification of a coordinate regulator of interleukins 4, 13 and 5 by cross-species sequence comparisons. Science *288*, 136–140.

Lutgens, E., Gorelik, L., Daemen, M.J., de Muinck, E.D., Grewal, I.S., Koteliansky, V.E., and Flavell, R.A. (1999). Requirement for CD154 in the progression of atherosclerosis. Nat. Med. *5*, 1313–1316.

Mach, F., Schoenbeck, U., and Libby, P. (1998). CD40 signaling in vascular cells: a key role in atherosclerosis? Atherosclerosis *137*, S89–S95.

Mach, F., Schönbeck, U., Sukhova, G.K., Atkinson, E., and Libby, P. (1998). Reduction of atherosclerosis in mice by inhibition of CD40 signalling. Nature *394*, 200–203.

Mallat, Z., Besnard, S., Duriez, M., Deleuze, V., Emmanuel, F., Bureau, M.F., Soubrier, F., Esposito, B., Duez, H., Fievet, C., et al. (1999). Protective role of interleukin-10 in atherosclerosis. Circ. Res. *85*, 17–24.

Moulton, K.S., Heller, E., Konerding, M.A., Flynn, E., Palinski, W., and Folkman, J. (1999). Angiogenesis inhibitors endostatin or TNP-470 reduce intimal neovascularization and plaque growth in apolipoprotein E–deficient mice. Circulation *99*, 1726–1732.

Nagy, L., Tontonoz, P., Alvarez, J.G.A., Chen, H., and Evans, R.M. (1998). Oxidized LDL regulates macrophage gene expression through ligand activation of PPAR-gamma. Cell *93*, 229–240.

Napoli, C., D'Armiento, F.P., Mancini, F.P., Postiglione, A., Witztum, J.L., Palumbo, G., and Palinski, W. (1997). Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. J. Clin. Invest. *100*, 2680–2690.

Navab, M., Berliner, J.A., Watson, A.D., Hama, S.Y., Territo, M.C., Lusis, A.J., Shih, D.M., Van Lenten, B.J., Frank, J.S., Demer, L.L., et al. (1996). The yin and yang of oxidation in the development of the fatty streak. Arterioscler. Thromb. Vasc. Biol. *16*, 831–842.

Okamoto, H., Yonemori, F., Wakitani, K., Minowa, T., Maeda, K., and Shinkai, H. (2000). A cholesteryl ester transfer protein inhibitor attenuates atherosclerosis in rabbits. Nature *406*, 203–206.

Paulsson, G., Zhou, X., Torrielli, M., and Hansson, G.K. (2000). Oligoclonal T cell expansion in atheroslcerotic lesions of apoliprotein E-deficient mice. Aterioscler. Thromb. Vasc. Biol. *20*, 10–17.

Pinderski, L., Hedrick, C., Olvera, T., Hagenbaugh, A., Territo, M., Berliner, J., and Fyfe, A. (1999). Interleukin-10 blocks atherosclerotic events in vitro and in vivo. Arteroscler. Thromb. Vasc. Biol. *19*, 2847–2853.

Plump, A., Mascucci-Magoulas, L., Bruce, C., Bisgaier, Breslow, J., and Tall, A. (1999). Increased atherosclerosis in ApoE and LDL receptor gene knock-out mice as a result of human cholesteryl ester transfer protein transgene expression. Arterioscler. Thromb. Vasc. Biol. *19*, 1105–1110.

Qiao, J.H., Tripathi, J., Mishra, N.K., Cai, Y., Tripathi, S., Wang, X.P., Imes, S., Fishbein, M.C., Clinton, S.K., Libby, P., Luis, A.J., and Rajavashisth, T.B. (1997). Role of macrophage colony-stimulating factor in atherosclerosis: studies of osteoporotic mice. Am. J. Path. *150*, 1687–1699.

Reckless, J., Metcalfe, J.C., and Grainger, D.J. (1997). Tamoxifen decreases cholesterol sevenfold and abolishes lipid lesion development in apolipoprotein E knockout mice. Circulation *95*, 1542–1548.

Repa, J.J., Liang, G., Ou, J., Bashmakov, Y., Lobaccaro, J.-M.A., Shimomura, I., Shan, B., Brown, M.S., Goldstein, J.L., and Mangelsdorf, D.J. (2000a). Regulation of mouse sterol regulatory element-binding protein-1c (SREBP-1c) by oxysterol receptors LXRα and LXRβ. Genes Dev. *14*, 2819–2830.

Repa, J.J., Turley, S.D., Lobaccaro, J.A., Medina, J., Li, L., Lustig, K., Shan, B., Heyman, R.A., Dietschy, J.M., and Mangelsdorf, D.J. (2000b). Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers. Science *289*, 1524–1529.

Ricote, M., Huang, J., Fajas, L., Li, A., Welch, J., Najib, J., Witztum, J.L., Auwerx, J., Palinski, W., and Glass, C.K. (1998a). Expression of the peroxisome proliferator-activated receptor γ (PPARγ) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein. Proc. Natl. Acad. Sci. USA *95*, 7614–7619.

Ricote, M., Li, A.C., Willson, T.M., Kelly, C.J., and Glass, C.K. (1998b). The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation. Nature *391*, 79–82.

Risch, N.J. (2000). Searching for genetic determinants in the new millennium. Nature *405*, 847–856.

Roe, E.B., Chiu, K.M., and Arnaud, C.D. (2000). Selective estrogen receptor modulators and postmenopausal health. Adv. Internal Med. *45*, 257–278.

Ross, R. (1999). Atherosclerosis—an inflammatory disease. N. Eng. J. Med *340*, 115–126.

Rust, S., Rosier, M., Funke, H., Real, J., Amoura, Z., Piette, J.C., Deleuze, J.F., Brewer, H.B., Duverger, N., Denefle, P., and Assmann, G. (1999). Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. Nat. Genet. *22*, 352–355.

Schultz, J.R., Tu, H., Luk, A., Repa, J.J., Medina, J.C., Li, L., Schwendner, S., Wang, S., Thoolen, M., Mangelsdorf, D.J., et al. (2000). Role of LXRs in control of lipogenesis. Genes Dev. *14*, 2831–2838.

Schwenke, D.C., and Carew, T.E. (1989). Initiation of atherosclerotic lesions in cholesterol-fed rabbits. I. Focal increases in arterial LDL concentration precede development of fatty streak lesions. Arteriosclerosis *9*, 895–907.

Sendobry, S.M., Conricelli, J.A., Welch, K., Bocan, T., Tait, B., Trivedi, B.K., Colbry, N., Dyer, R.D., Feinmark, S.J., and Daugherty, A. (1997). Attenuation of diet-induced atherosclerosis in rabbits with a highly selective 15-lipoxygenase inhibitor lacking significant antioxidant properties. Br. J. Pharmacol. *120*, 1199–1206.

Shaw, P., Hörkkö, S., Chang, M., Curtiss, L., Palinski, W., Silverman, G., and Witztum, J. (2000). Natural antibodies with the T15 idiotype may act in atherosclerosis, apoptotic clearance, and protective immunity. J. Clin. Invest. *105*, 1731–1740.

Shiffman, D., Mikita, T., Tai, J.T., Wade, D.P., Porter, J.G., Seilhamer, J.J., Somogyi, R., Liang, S., and Lawn, R.M. (2000). Large scale gene expression analysis of cholesterol-loaded macrophages. J. Biol. Chem. *275*, 37324–37332.

Shih, P.T., Brennan, M.L., Vora, D.K., Territo, M.C., Strahl, D., Elices, M.J., Lusis, A.J., and Berliner, J.A. (1999). Blocking very late antigen-4 integrin decreases leukocyte entry and fatty streak formation in mice fed an atherogenic diet. Circ. Res. *84*, 345–351.

Shih, D.M., Xia, Y.R., Wang, X.P., Miller, E., Castellani, L.W., Subbanagounder, G., Cheroutre, H., Faull, K.F., Berliner, J.A., Witztum, J.L., and Lusis, A.J. (2000). Combined serum paraoxonase knockout/apolipoprotein E knockout mice exhibit increased lipoprotein oxidation and atherosclerosis. J. Biol. Chem. *275*, 17527–17535.

Sjoland, H., Eitzman, D., Gordan, D., Westrick, R., Nabel, E., and Ginsberg, D. (2000). Atherosclerosis progression in LDL receptor-deficient and apolipoprotein E-deficient mice is independent of genetic alterations in plasminogen activator inhibitor-1. Arteriosclerosis *20*, 846–852.

Smith, J.D., Trogan, E., Ginsberg, M., Grigaux, C., Tian, J., and Miyata, M. (1995). Decreased atherosclerosis in mice deficient in both macrophage colony stimulating factor (op) and apolipoprotein E. Proc. Natl. Acad. Sci. USA *92*, 8264–8268.

Smithies, O., and Maeda, N. (1995). Gene targeting approaches to complex genetic disease: atherosclerosis and essential hypertension. Proc. Natl. Acad. Sci. USA *92*, 5266–5272.

Spiegelman, B.M. (1998). PPAR-gamma: adipogenic regulator and thiazolidinedione receptor. Diabetes *47*, 507–514.

Staels, B., and Auwerx, J. (1998). Regulation of apoA-I gene expression by fibrates. Atherosclerosis *137*, S19–23.

Staels, B., Koenig, W., Habib, A., Merval, R., Lebret, M., Torra, I.P., Delerive, P., Fadel, A., Chinetti, G., Fruchart, J.C., et al. (1998). Activation of human aortic smooth-muscle cells is inhibited by PPARalpha but not by PPARgamma activators. Nature *393*, 790–793.

Steinberg, D., and Witztum, J.L. (1999). Lipoproteins, Lipoprotein,

Oxidation, and Atherogenesis, K.R. Chien, ed. (Philadelphia: W.B. Saunders Co.).

Steinberg, D., Parthasarathy, S., Carew, T.E., Khoo, J.C., and Witztum, J.L. (1989). Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N. Engl. J. Med. *320*, 915–924.

Suzuki, H., Kurihara, Y., Takeya, M., Kamada, N., Kataoka, M., Jishage, K., Ueda, O., Sakaguchi, H., Higashi, T., Suzuki, T., et al. (1997). A role for macrophage scavenger receptors in atherosclerosis and susceptibility to infection. Nature *386*, 292–296.

Tall, A.R., Jiang, X., Lou, Y., and Silver, D. (2000). 1999 George Lyman Duff memorial lecture: lipid transfer proteins, HDL metabolism and atherogenesis. Arterioscler. Thromb. Vasc. Biol. *20*, 1185–1188.

Tangirala, R.K., Tsukamoto, K., Chun, S.H., Usher, D., Pure, E., and Rader, D.J. (1999). Regression of atherosclerosis induced by liver-directed gene transfer of apolipoprotein A-I in mice. Circulation *100*, 1816–1822.

Thurberg, B.L., and Collins, T. (1998). The nuclear factor-kappa B/inhibitor of kappa B autoregulatory system and atherosclerosis. Curr. Opin. Lipidol. *9*, 387–396.

Tontonoz, P., Nagy, L., Alvarez, J.G.A., Thomazy, V.A., and Evans, R.M. (1998). PPARγ promotes monocyte/macrophage differentiation and uptake of oxidized LDL. Cell *93*, 241–252.

Voyiaziakis, E., Goldberg, I.J., Plump, A.S., Rubin, E.M., Breslow, J.L., and Huang, L.S. (1998). ApoA-I deficiency causes both hypertriglyceridemia and increased atherosclerosis in human apoB transgenic mice. J. Lipid Res. *39*, 313–321.

Wetterau, J.R., Gregg, R.E., Harrity, T.W., Arbeeny, C., Cap, M., Connolly, F., Chu, C.H., George, R.J., Gordon, D.A.J.H., Jolibois, K.G., et al. (1998). An MTP inhibitor that normalizes atherogenic lipoprotein levels in WHHL rabbits. Science *282*, 751–754.

Williams, K.J., and Tabas, I. (1998). The response-to-retention hypothesis of atherogenesis reinforced. Curr. Opin. Lipidol. *9*, 471–474.

Witztum, J.L., and Steinberg, D. (1995). The Hyperlipoproteinemias. In Cecil Textbook of Medicine, L. Goldman and J.C. Bennett, eds. (Philadelphia: W.B. Saunders), pp. 1090–1100.

Xiao, Q., Danton, M., Witte, D., Kowala, M., Valentine, M., and Degen, J. (1998). Fibrinogen deficiency is compatible with the development of atherosclerosis in mice. J. Clin. Invest. *101*, 1184–1194.

Yagu, H., Kitamine, T., Osuga, J., Tozawa, R., Chen, Z., Kaji, Y., Oka, T., Perrey, S., Tamura, Y., Ohyashi, K., et al. (2000). Absence of ACAT-1 attenuates atherosclerosis but causes dry eye and cutaneous xanthomatosis in mice with congenital hyperlipidemia. J. Biol. Chem. *275*, 21324–21330.

Yamada, Y., Doi, T., Hamakubo, T., and Kodama, T. (1998). Scavenger receptor family proteins: roles for atherosclerosis, host defence and disorders of the central nervous system. Cell. Mol. Life Sci. *54*, 628–640.

Young, R.A. (2000). Biomedical discovery with DNA arrays. Cell *102*, 9–15.

Yusuf, S., Dagenais, G., Pogue, J., Bosch, J., and Sleight, P. (2000). Vitamin E supplementation and cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evalulation Study Investigators. N. Engl. J. Med. *342*, 154–160.