Rho-Associated Coiled-Coil-Containing Kinase 2 Deficiency in Bone Marrow-Derived Cells Leads to Increased Cholesterol Efflux and Decreased Atherosclerosis

Summary—The retention of modified cholesterol by macrophages and their development into foam cells are critical steps in atherogenesis. Cholesterol retention in macrophages is governed by cholesterol uptake and efflux. The precise signaling pathways that regulate cholesterol uptake and efflux are not known. The Rho-associated coiled-coil-containing kinases (ROCK1 and ROCK2) are serine-threonine protein kinases that are involved in the regulation of the actin cytoskeleton. Recent studies suggest that deletion of ROCK1 in bone marrow-derived cells is atheroprotective. However, the role for ROCK2 in the pathogenesis of atherosclerosis has not been determined. In the present article, we show that ROCK2-deficient mice on a genetic atherosclerotic background developed substantially fewer atherosclerotic lesions in the aorta and subaortic sinus after consumption of a high-cholesterol diet. These findings correlated with decreased foam cell formation and increased cholesterol efflux in ROCK2-deficient mice that are mediated, in part, through the peroxisome proliferator-activated receptor-γ/liver X receptor/ATP-binding cassette transporter A1 pathway in macrophages. In contrast, cholesterol efflux was unchanged in ROCK1-deficient macrophages, indicating a distinct role for ROCK2 in the reverse cholesterol transport system. These findings provide important and novel insights into the signaling mechanism that governs cholesterol efflux, which could lead to the development of selective ROCK inhibitors as therapeutic agents for atherosclerotic vascular disease.

Conclusions—ROCK2 contributes to atherosclerosis, in part, by inhibiting peroxisome proliferator-activated receptor-γ-mediated reverse cholesterol transport in macrophages, which contributes to foam cell formation. These findings suggest that inhibition of ROCK2 in macrophages may have therapeutic benefits in preventing the development of atherosclerosis.1

Conditional Targeting of Tumor Necrosis Factor Receptor–Associated Factor 6 Reveals Opposing Functions of Toll-Like Receptor Signaling in Endothelial and Myeloid Cells in a Mouse Model of Atherosclerosis

Summary—Atherosclerosis is the major cause of death in Westernized societies, being the underlying cause of severe cardiovascular diseases such as heart attack and stroke. Toll-like receptor (TLR) signaling has been implicated as a critical pathogenic factor in atherosclerosis and has attracted increased interest as a potential therapeutic target for the treatment of atherosclerosis. Our in vivo studies showed that TLR signaling exerts surprisingly diverse functions in endothelial cells and macrophages differentially affecting the development of atherosclerosis.

Conclusions—Toll-like receptor signaling in macrophages is atheroprotective by inhibiting nuclear factor-kB activation and the expression of proinflammatory mediators by the vascular endothelium in response to oxidized lipids, resulting in less efficient recruitment of monocytes into the developing plaques. In contrast, TLR signaling in macrophages is atheroprotective by inducing interleukin-10 expression, suppressing endoplasmic reticulum stress survival responses, and effec- rocytosis capacity of macrophages. Collectively, these results revealed a previously unappreciated functional diversity of TLR signaling in 2 cell types that are critical for the development of atherosclerosis. In response to modified lipids, TLR signaling exerts proatherogenic functions in endothelial cells in female mice by inducing the expression of proinflammatory mediators facilitating monocyte recruitment. In contrast, TLR signaling in macrophages is atheroprotective by inducing interleukin-10 expression, suppressing endoplasmic reticulum stress–induced macrophage death, and stimulating effecrocytosis. These findings urge caution in the systemic application of TLR signaling inhibitors in atherosclerosis and suggest that strategies specifically targeting endothelial cells would have a greater therapeutic potential.
Cytokine Therapy With Interleukin-2/ Anti–Interleukin-2 Monoclonal Antibody Complexes Expands CD4+CD25+Foxp3+ Regulatory T Cells and Attenuates Development and Progression of Atherosclerosis

Summary—Atherosclerosis is a multifactorial inflammatory disease characterized by the accumulation of lipids and innate and adaptive immune cells, leukocytes, and lymphocytes. Activated lymphocytes are believed to be essential for both the development and exacerbation of atherosclerosis. One way to prevent their activation is to increase regulatory T cells, cells that can control both innate and adaptive immune responses. Regulatory T cells, defined by expression of CD4, CD25, and a transcription factor Foxp3, are mostly thymus derived and account for 5% to 10% of the circulating CD4+ T cell population. The cytokine interleukin-2 can stimulate their expansion but is not selective. In this article we present a novel way to chronically expand this T cell population, by complexing the cytokine interleukin-2 with an interleukin-2–neutralizing antibody, which prolongs its duration of action and specifically targets regulatory T cells, markedly increasing their numbers. Other interleukin-2–responsive lymphocytes that can accelerate atherosclerosis do not respond to this cytokine–antibody complex. Treatment with this cytokine–antibody complex inhibits the activation and proliferation of proatherogenic T cells and markedly attenuates development and progression of already developed atherosclerosis. From a clinical perspective, this finding is important because it demonstrates that by specifically targeting cells that can protect against atherosclerosis, in this case pharmacologically expanding regulatory T cell numbers, it is possible to suppress inflammation associated with atherosclerosis as well as attenuate progression of disease.

Conclusions—Interleukin (IL)-2/anti–IL-2 mAb treatment in vivo attenuates atherosclerosis via selective Tregs expansion. The findings suggest that cytokine-based IL-2/anti–IL-2 mAb complex therapy could represent an attractive approach for treating atherosclerosis, because it markedly attenuates progression as well as development, by modulating its immunoinflammatory component.1

Loss of Perivascular Adipose Tissue on Peroxisome Proliferator–Activated Receptor–y Deletion in Smooth Muscle Cells Impairs Intravascular Thermoregulation and Enhances Atherosclerosis

Summary—Recent confirmation that in addition to white adipose tissue, brown adipose tissue (BAT) is found in adult humans, where it plays a distinct role in adaptive thermoregulation and energy metabolism, has sparked active research on adipose tissues as bioactive organs, mostly focusing on their pathophysiological aspects for treatment of metabolic disorders. This revival led to the recognition of perivascular adipose tissue (PVAT) not just as a support structure but rather as a biologically active regulatory component of the vasculature. Despite increased understanding of PVAT autocrine and paracrine vascular roles, there is a paucity of knowledge regarding its involvement in atherosclerosis. PVAT shares some characteristics with BAT, but to date, its specific roles remain obscure because of limited animal models. We present experimental proteomics evidence that in vivo chronic cold stimulus results in activation of PVAT characterized by cellular metabolic pathways shared with BAT. A peroxisome proliferator–activated receptor–y–knockout strategy targeting smooth muscle cells results in loss of PVAT surrounding the vasculature. Use of this unique model in combination with our newly developed surgical removal of BAT reveals, for the first time, robust atheroprotection mediated by adaptive thermogenic activation of PVAT in chronic cold adaptation associated with a reduction of plasma lipid profiles and improvement of the endothelial dysfunction. Endothelial protective effects likely result from increased prostacyclin release by activated PVAT. Thus, we uncovered a new paradigm that directly links PVAT to the beneficial effects of cold exposure and thermogenesis in atherosclerosis likely to mimic those observed in humans.

Conclusions—PVAT is a vasoactive organ with functional characteristics similar to BAT and is essential for intravascular thermoregulation of cold acclimation. This thermogenic capacity of PVAT plays an important protective role in the pathogenesis of atherosclerosis.7

Toll-Like Receptor 7 Protects From Atherosclerosis by Constraining “Inflammatory” Macrophage Activation

Summary—Atherosclerosis underlying cardiovascular mortality is the leading cause of death in developed countries. Efforts are therefore concentrating on unwinding the pathophysiological mechanisms controlling its development and clinical complications. Among them, Toll-like receptors (TLRs) have taken center stage in atherosclerosis research by virtue of their ability to sense danger in response to hypercholesterolemia, tissue stress, or necrosis, and drive macrophage activation and inflammation in the vessel wall. TLR2 and TLR4, in particular, have been shown to play a critical role in promoting plaque development and vulnerability leading to the view that all TLRs are pathogenic for this disease. This article now reports the surprising finding that TLR7, an endosomal TLR that recognizes viral single-stranded RNA and self-RNA released from necrotic cells, is protective. In experimental atherosclerosis in mice, TLR7 prevented lesion development, stenosis, and plaque vulnerability by constraining monocyte chemoattractant protein-1 production, Ly6C+ “inflammatory” monocyte expansion and M1 inflammatory macrophage accumulation in developing atherosclerotic lesions. In human carotid endarterectomy specimens, TLR7 was positively associated with an M2 anti-inflammatory macrophage signature and collagen genes and inversely related/unrelated to proinflammatory mediators and platelet markers, whereas TLR7 activity in human atheroma cultures selectively suppressed the production of monocyte chemoattractant protein-1. Altogether, these findings reveal that TLR7 is part of a protective response that limits atherosclerotic plaque development and vulnerability and challenge the prevailing concept that all TLRs are pathogenic. They also provide new insight about the complex interplay of innate immunity in atherosclerosis and support the exploitation of the TLR7 pathway for therapy.

Conclusions—These findings provide evidence for a beneficial role of TLR7 in atherosclerosis by constraining inflammatory macrophage activation and cytokine production. This challenges the prevailing concept that all TLRs are pathogenic and supports the exploitation of the TLR7 pathway for therapy.9

Cyclin-Dependent Kinase 5–Mediated Hyperphosphorylation of Sirtuin-1 Contributes to the Development of Endothelial Senescence and Atherosclerosis

Summary—Aging is a major risk factor for cardiovascular diseases. Endothelial senescence represents one of the early aging characteristics during the development of atherosclerosis in human arteries. SIRT1 is an enzyme responsible for deacetylating a diverse range of protein targets. The role of its yeast homolog, Sir2p, in lifespan extension was reported in the late 1990s. Since then, a vast amount of information has supported the potential antiaging activity of SIRT1
in rodents and humans. This study identified an important posttranslational modification (S47 phosphorylation) of SIRT1 that was augmented during endothelial senescence. By inhibition of the upstream kinase (cyclin-dependent kinase 5), the development of atherosclerosis and vascular inflammation in mice was significantly halted. This evidence demonstrates that SIRT1 is an anti-vascular aging factor and that it may be a promising therapeutic target for cardiovascular diseases.

Conclusions—Cyclin-dependent kinase 5-mediated hyperphosphorylation of SIRT1 facilitates the development of endothelial senescence and atherosclerosis.

Prediction of Progression of Coronary Artery Disease and Clinical Outcomes Using Vascular Profiling of Endothelial Shear Stress and Arterial Plaque Characteristics: The PREDICTION Study

Summary—Coronary plaques progresses in a highly individual manner. Identification of early stages of high-risk plaque may enable development of preemptive strategies to alter the natural history of high-risk plaque and avert adverse cardiac events. The purposes of the PREDICTION Study were to determine the role of local vascular characteristics on coronary plaque progression in humans and to relate plaque changes to clinical events. Five hundred six patients with an acute coronary syndrome treated with a percutaneous coronary intervention were enrolled. Vascular profiling using coronary angiography and intravascular ultrasound was used to reconstruct the artery and calculate endothelial shear stress and plaque/remodeling characteristics in vivo. Each reconstructed artery was divided into sequential 3-mm segments for serial analysis. Three-vessel vascular profiling was performed at baseline and in a subset of 374 patients 6 to 10 months later to assess plaque natural history. Clinical follow-up was performed at 1 year. Symptomatic clinical events were infrequent. Increase in plaque area at follow-up was predicted by baseline large plaque burden; decrease in lumen area was independently predicted by baseline large plaque burden and low endothelial shear stress. Large plaque size and low endothelial shear stress independently predicted increased plaque burden and worsening of luminal obstructions treated with a percutaneous coronary intervention at follow-up. Large plaque burden and low local endothelial shear stress provide independent and additive prediction to identify plaques that develop progressive enlargement and lumen narrowing. These observations may justify prospective, randomized trials to identify early stages of high-risk plaque and investigate the value of local preemptive interventions.

Conclusions—Large plaque burden and low local endothelial shear stress provide independent and additive prediction to identify plaques that develop progressive enlargement and lumen narrowing.

Phospholipase A2 Enzymes, High-Dose Atorvastatin, and Prediction of Ischemic Events After Acute Coronary Syndromes

Summary—Secretory phospholipase A2 (sPLA2) and lipoprotein-associated phospholipase A2 (Lp-PLA2) are enzyme biomarkers of increased cardiovascular risk and targets of emerging therapeutic agents. This study demonstrates that sPLA2 mass independently predicts death during a 16-week period after acute coronary syndrome (ACS). High-dose atorvastatin significantly reduces sPLA2 and Lp-PLA2 mass and activity after ACS and mitigates the risk of death associated with sPLA2 mass. Atorvastatin may exert antiinflammatory effects on phospholipases that contribute to its therapeutic benefit after ACS. These findings, derived from a large, randomized, placebo-controlled study and measuring sPLA2 and Lp-PLA2 mass and activity for the first time in the same data set, provide an important roadmap for interpreting future studies on sPLA2 and Lp-PLA2 inhibitors. The observations in this analysis are timely because novel inhibitors of Lp-PLA2 and sPLA2 are being evaluated for clinical efficacy in large, prospective, randomized trials involving patients with recent ACS (SOLID, VISTA-16). These inhibitors may reduce the mass and/or activity of these phospholipases. Since the completion of the Myocardial Ischemia Reduction With Aggressive Cholesterol Lowering (MIRACL) and Pravastatin or Atorvastatin Evaluation and Infection Therapy (PROVE-IT) trials, intensive statin treatment has become the standard of care after ACS. Therefore, future data sets will not provide the opportunity of the placebo-controlled MIRACL trial to assess both the natural history and prognostic value of phospholipase A2 biomarkers after ACS in the placebo arm and the modulation of these patterns by intensive statin treatment.

Conclusions—sPLA2 mass independently predicts death during a 16-week period after acute coronary syndrome. High-dose atorvastatin significantly reduces sPLA2 and Lp-PLA2 mass and activity after acute coronary syndrome and mitigates the risk of death associated with sPLA2 mass. Atorvastatin may exert antiinflammatory effects on phospholipases that contribute to its therapeutic benefit after acute coronary syndrome.

Extramedullary Hematopoiesis Generates Ly-6Chigh Monocytes That Infiltrate Atherosclerotic Lesions

Summary—Atherosclerosis is an inflammatory disease characterized by the accumulation of lipids and leukocytes in the arterial wall. Monocytes are large circulating leukocytes believed to be essential
to the development and exacerbation of atherosclerosis. As disease worsens, the number of circulating monocytes increases, whereas in models with monocyte depletion, atherosclerosis neither develops nor evolves. It is believed that hematopoietic progenitors give rise to circulating monocytes exclusively in the bone marrow. These medullary monocytes circulate, accumulate in tissue, and differentiate to macrophages or dendritic cells. Extramedullary sites such as the spleen maintain reservoirs of undifferentiated monocytes that can exit en masse in response to acute inflammation. In this study, we show that during atherosclerosis the bone marrow outsources the production of monocytes to the spleen. These extramedullary monocytes accumulate in the growing atheroma. From a clinical perspective, this finding is important because it identifies the spleen as a possible biomarker organ and therapeutic target for cardiovascular disease, and it proposes that inflammatory hematopoiesis could be targeted therapeutically in atherosclerosis.

Conclusions—Our findings indicate that extramedullary sites supplement the hematopoietic function of the bone marrow by producing circulating inflammatory cells that infiltrate atherosclerotic lesions.10

**Loss of Myeloid Related Protein-8/14 Exacerbates Cardiac Allograft Rejection**

Summary—Graft arterial disease (GAD) limits long-term survival in cardiac transplant recipients. GAD shares some pathophysiological features with conventional atherosclerosis; APCs present alloantigens (eg, antigens on donor ECs) to T cells, initiating differentiation and activation of T cells, B cells, and inflammatory cell responses with costimulatory signaling. Nevertheless, there are important differences in the pathology and distribution of these diseases. Although hyperlipidemia, a well-established risk factor for conventional atherosclerosis, is common after transplantation, GAD lesions tend to be lipid poor. GAD involves large and medium-sized vessels and the microvasculature and affects the media, adventitia, and intima. MRP-14 deficiency can attenuate wire injury–induced vascular lesions and atherosclerotic lesions in atherogenic animals. In contrast, the presence of MRP-14 deficiency can attenuate wire injury–induced vascular lesions and atherosclerotic lesions in atherogenic animals. In contrast, the present study demonstrates that recipient MRP-14 deficiency exacerbates allograft vasculopathy. This study examined the effect of MRP-14 deficiency on parenchymal rejection and GAD after MHC class II–mismatched murine heart transplantation without immunosuppression. Clinical application requires future studies evaluating the effects of MRP-14 expression in the setting of immunosuppressive therapy.

Conclusions—Our results indicate that MRP-14 regulates B7 molecule expression and reduces antigen presentation by dendritic cells and subsequent T-cell priming. The absence of MRP-14 markedly increased T-cell activation and exacerbated allograft rejection, indicating a previously unrecognized role for MRP-14 in immune cell biology.11

**Acute Coronary Syndrome and Khat Herbal Amphetamine Use: An Observational Report**

Summary—Chewing the leaves of the plant *Catha edulis* (khat) likely dates to times of antiquity and may precede the use of coffee. Twenty million people worldwide are believed to be using khat for its stimulant effects. The use of khat was previously confined to East Africa and the Arabian Peninsula. It initially was thought to be of limited concern to Western populations because of its complicated cultivation and distribution systems. However, overnight delivery systems and the immigration of khat chewers contributed to the globalized distribution of khat. Moreover, to make distribution of khat easier and to preserve its efficacy for a longer time, several synthetic forms, including *hagigat* and *graba*, were made. Although khat chewing is illegal in the United States, numerous seizures of fresh and dried khat have been made recently. Cathinone, cathine, and norephedrine are the main ingredients of the plant. Cathinone is structurally similar to amphetamine and functionally similar to cocaine and ecstasy (3,4- methylenedioxymethylamphetamine). Cathinone, the most active khat alkaloid, has been shown to have multiple cardiovascular effects, including increasing heart rate and blood pressure and inducing coronary artery spasm. The present study suggests that khat chewers presenting with acute coronary syndrome have fewer cardiovascular risk factors and present late compared with non–khat chewers. This late presentation may be attributed to the analgesic effect of khat. Khat chewers presenting with acute coronary syndrome have increased risk of morbidity and mortality. Increased awareness of endemic practices is paramount in the context of increasing global migration.

Conclusions—Our data confirm earlier observations of worse inhospital outcome among acute coronary syndrome patients who chew khat. This worse outcome persists up to 1 year from the index event. This observational report underscores the importance of improving education concerning the cardiovascular risks of khat chewing.12

**m-Calpain Induction in Vascular Endothelial Cells on Human and Mouse Atheromas and Its Roles in VE-Cadherin Disorganization and Atherosclerosis**

Summary—One of the earliest pathological features of atherosclerosis is barrier dysfunction in vascular endothelial cells, which triggers the infiltration of monocytes/macrophages or plasma active constituents into subendothelial space, allowing further increase in the atherosclerosis susceptibility in the large artery. Although VE-cadherin–mediated adherence junctions, a dominant determinant of endothelial cell barrier functions, are known to decay in the early phase of atherosclerosis, the molecular mechanism underlying this disorder remains unknown. Here, we show that m-calpain, an intracellular cysteine protease, is induced in vascular endothelial cells in murine and human atherosclerotic aortas. Furthermore, this study provides direct evidence that m-calpain proteolytically cleaves VE-cadherin at its juxtapembrane regions, leading to endothelial cell barrier dysfunction. Importantly, atherosclerosis in mouse models is ameliorable by calpain inhibition trials. Thus, m-calpain can be regarded as a unique molecular target for controlling atherosclerosis. Subtype-selective m-calpain inhibitor may be highly desirable to achieve a better therapeutic outcome.

Conclusions—Subtype-selective induction of m-calpain in aortic endothelial cells during atherosclerotic progression is associated with proteolytic disorganization of VE-cadherin and proatherogenic hyperpermeability in cells. Thus, a strategy to selectively inhibit m-calpain may be useful for the therapeutic treatment of patients with atherosclerosis.15

**Identification of a Danger-Associated Peptide From Apolipoprotein B100 (ApoBDS-1) That Triggers Innate Proatherogenic Responses**

Summary—Accumulated low-density lipoprotein particles in arterial wall are the primary cause of atherosclerosis by triggering...
chronic vascular inflammation, characterized by local activation of cellular inflammatory responses including lesional macrophages. The molecular identities of low-density lipoprotein–derived inflammatory components have been poorly defined. Apolipoprotein B100 (ApoB100) is the only unchangeable protein constituent of the low-density lipoprotein particle. Both clinical and preclinical studies suggest that ApoB100 protein fragments are implicated in modulation of immune responses in the process of disease development, implying that ApoB100 is not a bystander to the inflammation of atherosclerosis. In the present study, by screening a peptide library of human ApoB100, we have identified a previously unrecognized native peptide fragment that exhibits distinctive activity triggering inflammatory responses of human monocytes/macrophages and atherosclerotic plaques ex vivo. Given its capacity to induce innate immune responses, we named the peptide ApoB100 danger-associated signal 1, signifying the first identified ApoB100–derived danger-associated signal. Importantly, the peptides with specific ApoB100 danger-associated signal 1 activity are present in atherosclerosis. These findings shed light on the pathobiological role of low-density lipoprotein in atherosclerosis.

Conclusions—Our data show that ApoBDS-1 is a previously unrecognized peptide with robust proinflammatory activity, contributing to the disease-promoting effects of low-density lipoprotein in the pathogenesis of atherosclerosis.

Induction of Vascular GTP-Cyclohydrolase I and Endogenous Tetrahydrobiopterin Synthesis Protect Against Inflammation-Induced Endothelial Dysfunction in Human Atherosclerosis

Summary—Vascular tetrahydrobiopterin (BH4) is an essential cofactor of endothelial nitric oxide synthase (eNOS), and its deficiency induces “uncoupling” of this enzyme, which leads to production of superoxide \( \left( O_2^- \right) \) instead of nitric oxide (NO). We hypothesized that BH4 synthesis is stimulated by inflammation, and this may serve as an endogenous defense mechanism of the vascular wall against systemic inflammation. To address this hypothesis, we performed 4 sequential studies. In Study 1, acute inflammation (induced by vaccination with Salmonella typhi vaccine) rapidly increased circulating BH4 in parallel with systemic inflammatory markers and impaired endothelial function in healthy individuals. In Study 2, we observed that a functional haplotype (X haplotype) in the \( GCH1 \) gene, encoding GTP-cyclohydrolase I, the rate-limiting enzyme in BH4 biosynthesis, was associated with endothelial dysfunction in subjects with increased background inflammation. In Study 3, we screened a large cohort of patients with coronary atherosclerosis and recruited patients on the basis of their \( GCH1 \) genotype to receive proinflammatory stimulation with \( S \) typhi vaccine. We observed that those with XX genotype were unable to increase plasma biopterins after vaccination, and these patients had a greater reduction of FMD than those with OO genotype. In Study 4, we used ex vivo models of human arteries and veins to demonstrate that the ability of these vessels to increase \( GCH1 \) gene expression and improve BH4 bioavailability in response to cytokine stimulation preserves vascular endothelial function. These novel findings suggest that vascular BH4 may constitute a novel therapeutic target for the treatment of endothelial dysfunction in inflammatory states such as human atherosclerosis.

Conclusions—The ability to increase vascular \( GCH1 \) expression and BH4 synthesis in response to inflammation preserves endothelial function in inflammatory states. These novel findings identify BH4 as a vascular defense mechanism against inflammation-induced endothelial dysfunction.

Wnt Signaling Mediates Pathological Vascular Growth in Proliferative Retinopathy

Summary—Pathological neovascularization in ischemic proliferative retinopathies such as retinopathy of prematurity and diabetic retinopathy is a major cause of blindness in children and working-age adults. Although anti–vascular endothelial growth factor therapy has been proven partially successful in the suppression of neovascularization, identification of other signaling mechanisms involved in this disease process is essential for developing therapies specifically targeting pathological vessels while sparing normal vessels. Here, we found that the Wnt signaling pathway, a pathway important for cardiac development and differentiation, is a major component in regulating pathological neovascularization in retinopathy. Using a mouse model of oxygen-induced retinopathy, we found that Wnt ligands and receptors are highly upregulated in retinas with induced retinopathy and pathological neovessels, respectively. Mutant mice lacking Wnt coreceptor Lrp5 or downstream signaling molecule dishevelled2 have significantly decreased levels of neovascularization. Importantly, the proangiogenic effect of Wnt signaling is mediated through tight junction protein claudin5, which is highly downregulated in Lrp5-null vessels. Suppression of claudin5 significantly inhibits Wnt-mediated vascular growth in vitro and pathological vessel growth in vivo. Our data suggest that Wnt signaling pathway plays a significant role in mediating pathological vascular growth in ischemic proliferative retinopathy, and selectively targeting this pathway might be a potentially useful strategy to develop future therapies for retinopathy.

Conclusions—These results demonstrate an important role of Wnt signaling in pathological vascular development in retinopathy and show a novel function of claudin5 in promoting angiogenesis.

Donor Simvastatin Treatment Abolishes Rat Cardiac Allograft Ischemia/Reperfusion Injury and Chronic Rejection Through Microvascular Protection

Summary—Ischemia/reperfusion injury after heart transplantation may result in primary graft dysfunction or initiation of fibroproliferative cascades, leading to the development of cardiac fibrosis, allograft arteriosclerosis, and compromised long-term survival. Vascular dysfunction, including permeability and perfusion disturbances, plays a central role in ischemia/reperfusion injury. Statins are widely used to lower cholesterol levels, but they also have cholesterol-independent pleiotropic effects through Rho GTPase inhibition. We used heterotopic rat heart transplantation models to investigate whether a single dose of simvastatin administered to cardiac allograft donors perorally 2 hours before graft removal protects the cardiac allograft through direct vasculoprotective effects. Donor simvastatin treatment abolished cardiac allograft ischemia/reperfusion injury by preventing the no-reflow phenomenon and reducing vascular permeability, inflammation and cardiomyocyte injury. These early vasculoprotective and cardioprotective effects were mirrored with sustained antiinflammatory, antifibrotic, and antiarteriosclerotic effects in a chronic rejection heart transplantation model. Mechanistic studies indicated that donor simvastatin treatment decreased cardiac allograft microvascular endothelial cell and pericyte RhoA activation, modified the expression of vasculoprotective genes, and improved endothelial barrier function. In contrast to donor simvastatin treatment, recipient simvastatin treatment did not protect against ischemia/reperfusion injury.
In vitro studies also showed that simvastatin decreased endothelial-mesenchymal transition, a recently characterized mechanism participating in cardiac fibrosis. Collectively, our results highlight the rapid vasculoprotective effects of simvastatin during ischemia/reperfusion injury and suggest donor simvastatin as a novel, clinically feasible strategy to protect cardiac allografts.

Conclusions—Our results demonstrate that donor simvastatin treatment prevents microvascular endothelial cell and pericyte dysfunction, ischemia/reperfusion injury, and chronic rejection and suggest a novel, clinically feasible strategy to protect cardiac allografts.17

Involvement of Endoplasmic Stress Protein C/EBP Homologous Protein in Arteriosclerosis Acceleration With Augmented Biological Stress Responses

Summary—Complex interactions among numerous biological pathways are implicated in the pathogenesis of arteriosclerosis such as atherosclerosis and vascular remodeling. In particular, responses to inflammation and oxidative stress have been considered to play central roles in arteriosclerosis development. In addition, recent studies revealed endoplasmic reticulum stress to be associated with atherosclerosis involving free cholesterol-induced macrophage apoptosis. However, details of the molecular mechanisms of interactions among classic atherogenic actions and endoplasmic reticulum stress responses remained to be elucidated. This study focused on the transcription factor C/EBP homologous protein (CHOP), which is well known to be induced by endoplasmic reticulum stress, mediating apoptotic cell death. Here, using CHOP-deficient mice, we show that CHOP plays important roles in accelerating 2 types of arteriosclerosis: cuff injury–induced neointimal formation and hypercholesterolemia-induced atherosclerosis. Augmented inflammatory and oxidative stress responses mediated by CHOP are important underlying mechanisms. Furthermore, CHOP, especially that expressed in hematopoietic and vascular cells, is involved in inflammatory interactions among macrophages, endothelial cells, and vascular smooth muscle cells, acting in a coordinated fashion to promote arteriosclerosis development. Thus, these observations of this noncanonical role of CHOP may lead to a better understanding of the molecular pathogenesis of vascular remodeling and atherosclerosis. Furthermore, given that neointimal formation is an important feature of postanastomotic lesions, our results suggest the rapid progression of vascular remodeling and atherosclerosis.18

Conclusions—Prolonged hypercholesterolemia impairs Treg but not effector T cell accumulation in lesions, but reversal of hypercholesterolemia can prevent loss of lesional Treg. Therefore, cholesterol-lowering therapies may induce dynamic and beneficial changes in Treg:effector T cell ratios in atherosclerotic lesions.19

Nonmuscle Myosin Light-Chain Kinase Deficiency Attenuates Atherosclerosis in Apolipoprotein E–Deficient Mice via Reduced Endothelial Barrier Dysfunction and Monocyte Migration

Summary—Endothelial dysfunction and monocyte migration have been implicated in the pathogenesis of atherosclerosis. Nonmuscle myosin light chain kinase (nmMLCK) is known to contribute to inflammation-associated endothelial barrier dysfunction by activating the cytoskeletal contractile response via its kinase activity on myosin light chain phosphorylation. The specific contribution of nmMLCK to atherosclerotic injury and its mechanism of action have not been evaluated. In this study, we tested the hypothesis that nmMLCK promotes atherosclerotic lesion development by altering endothelial barrier properties. In the aorta of apolipoprotein E–deficient mice fed an atherogenic diet, nmMLCK deficiency significantly reduced lesion size, intimal hyperplasia, and macrophage deposition in the vascular wall, indicating a pathogenic role of nmMLCK in atherosclerosis. Consistent with the in vivo observations, nmMLCK expression was detected in both AECs and peripheral monocytes, and nmMLCK deficiency attenuated endothelial hyperpermeability and monocyte transendothelial migration caused by atherosclerosis-relevant inflammatory stimuli, including thrombin, oxidized low-density lipoprotein, tumor necrosis factor α, and monocyte chemoattractant protein-1. Further mechanistic studies demonstrated that, in addition to myosin light chain phosphorylation, Src signaling contributed to nmMLCK-induced cellular responses. Pharmacological blockade or genetic manipulation of Src inhibited nmMLCK-mediated hyperpermeability and monocyte transmigration. Taken together, the data suggest a novel function of nmMLCK in atherosclerosis that involves a nonconventional signaling pathway independent of myosin light chain phosphorylation. Further characterization of specific cellular responses to isoform-specific MLCK kinase activity and kinase-independent mechanisms would contribute to the development of new therapeutic targets for treating atherosclerosis.

Conclusions—Nonmuscle myosin light-chain kinase contributes to atherosclerosis by regulating endothelial barrier function and monocyte migration via mechanisms involving not only kinase-mediated MLC phosphorylation but also Src activation.20
S100A9 Differentially Modifies Phenotypic States of Neutrophils, Macrophages, and Dendritic Cells: Implications for Atherosclerosis and Adipose Tissue Inflammation

Summary—It has previously been demonstrated that elevated plasma levels of S100A9 (also known as myeloid related protein-14) in complex with its binding partner S100A8 (myeloid related protein-8) predict increased risk of future cardiovascular events in healthy postmenopausal women and recurrent events in patients with acute coronary syndromes. Furthermore, apolipoprotein E–deficient mice that are also deficient in S100A9 exhibit reduced atherosclerosis. These important findings suggest that S100A9 is both a biomarker and a mediator of atherosclerosis and cardiovascular events. Most of the constitutively secreted S100A9 is believed to be derived from myeloid cells. We demonstrate that low-density lipoprotein receptor–deficient mice that lack S100A9 in bone marrow–derived cells, including myeloid cells, are not protected against diet-induced atherosclerosis. Furthermore, S100A9 deficiency differentially modifies phenotypic states of myeloid cell populations. S100A9-deficient neutrophils exhibit a reduced secretion of cytokines, whereas S100A9-deficient dendritic cells show an exacerbated release of cytokines. The effect of S100A9 deficiency on atherosclerosis and other inflammatory diseases is therefore predicted to depend on the relative contribution of these cell types at different stages of disease progression. Furthermore, S100A9 expression in nonmyeloid cells is likely to contribute to atherogenesis. Further study is needed to fully understand the functions of S100A8/A9 in specific cell populations and disease states before S100A8/A9 are considered therapeutic targets.

Conclusions—S100A9 differentially modifies phenotypic states of neutrophils, macrophages, and dendritic cells. The effect of S100A9 deficiency on atherosclerosis and other inflammatory diseases is therefore predicted to depend on the relative contribution of these cell types at different stages of disease progression. Furthermore, S100A9 expression in nonmyeloid cells is likely to contribute to atherogenesis.21

Immunotherapy With Tolerogenic Apolipoprotein B-100–Loaded Dendritic Cells Attenuates Atherosclerosis in Hypercholesterolemic Mice

Summary—In recent years, the perception of atherosclerotic cardiovascular disease has changed from that of a vascular lipid disorder to a chronic inflammatory condition elicited by lipoprotein retention in the vessel wall. Components of the accumulating low-density lipoprotein particles are immunogenic and can activate T cells and macrophages that promote inflammation, lesion growth, and plaque vulnerability. We devised a cell-therapy strategy to dampen inflammation and reduce atherosclerosis by injecting tolerogenic dendritic cells into hypercholesterolemic mice. Before transfer, dendritic cells were loaded with apolipoprotein B100, the protein part of low-density lipoprotein, and subsequently exposed to the antiinflammatory cytokine interleukin-10. Such dendritic cells suppressed the activity of apolipoprotein B100–reactive T cells. A single injection of tolerogenic dendritic cells loaded with apolipoprotein B100 significantly reduced atherosclerosis and increased plaque-stabilizing collagen in hypercholesterolemic mice. Because similar immune responses occur in human atherosclerosis, tolerogenic dendritic cell therapy may represent a new strategy for reduction and stabilization of atherosclerotic lesions in humans.

Conclusions—Tolerogenic dendritic cells pulsed with ApoB100 reduced the autoimmune response against low-density lipoprotein and may represent a novel possibility for treatment or prevention of atherosclerosis.22

Reversal of Hyperlipidemia With a Genetic Switch Favorably Affects the Content and Inflammatory State of Macrophages in Atherosclerotic Plaques

Summary—The ultimate cure for atherosclerosis would be the regression of arterial plaques. Discovery research toward this goal has been hampered by limited and sometimes cumbersome animal models. The Reversa mouse combines a standard model of human atherosclerosis, the hyperlipidemic low-density lipoprotein receptor-deficient mouse, with a genetic switch that electively shuts off low-density lipoprotein production. In the present study, arterial plaques were allowed to develop in Reversa mice to a stage mimicking advanced human coronary artery disease, and then the elevated low-density lipoprotein level was severely reduced, thereby simulating aggressive lipid management. The major findings after such lipid reduction were decreases in the content and inflammatory state of the central cell of plaques, macrophages, with the change in total plaque size more modest because of compensatory increases in collagen content. The improvement in macrophage inflammatory status was augmented by treatment with pioglitazone, consistent with the effects of peroxisome proliferator-activated receptor-γ agonists on macrophages in vitro. The results may explain why plaque volume decreases have been modest in recent statin trials despite significant reduction in events and may provide one basis for the cardioprotective effects of pioglitazone in clinical studies. Continued study of this convenient model should lead to an improved understanding of plaque regression at the molecular level.

Conclusions—The Reversa mouse is a new model of atherosclerosis regression. After lipid lowering, favorable changes in plaque composition were independent of changes in size. In addition, plaque CD68+ cells became less inflammatory, an effect enhanced by treatment with pioglitazone.23

Low-Density Lipoprotein Receptor–Related Protein 1 Prevents Early Atherosclerosis by Limiting Lesional Apoptosis and Inflammatory Ly-6C<sup>high</sup> Monocytosis: Evidence That the Effects Are Not Apolipoprotein E Dependent

Summary—Apolipoprotein E (apoE) is a plasma protein that regulates both clearance of very low–density lipoprotein and maturation of high-density lipoprotein. It is also expressed at high levels by macrophages and has been found to have strong anti-atherogenic effects in mouse models. In humans, high-density lipoprotein–associated apoE correlates with presence of coronary artery disease and may become a biomarker for this common disease. Apolipoprotein E binds to multiple receptors, including low-density lipoprotein receptor protein 1 (LRP1), a member of the low-density lipoprotein receptor family. Low-density lipoprotein receptor protein 1 binds multiple ligands and can both internalize cargo and trigger signaling-mediated downstream effects. Both proteins control cellular cholesterol trafficking and plaque volume via regulation of cell death. These functions are key targets for the development of therapeutic strategies aiming at inducing plaque regression, an elusive and highly prized objective. We previously determined that macrophages lacking LRP1 cause
accelerated atherosclerosis, a paradoxical finding given that in these cells (a) atherogenic lipoproteins are internalized with reduced efficiency and (b) secretion of apoE is significantly upregulated. Because the negative effect of LRP1 removal was either caused by the interruption of an apoE–LRP1 axis or attenuated by the overexpression of apoE. The current studies clearly show that most functions of apoE and LRP1 in the artery wall occur through mutually independent pathways and that the absence of apoE greatly magnifies the effects of LRP1 deficiency on cell death. Our results help understand the forces controlling plaque volume expansion or contraction and may inform development of regression-inducing agents.

Conclusions—Low-density lipoprotein receptor protein 1 exerts anti-atherogenic effects via pathways independent of apoE involving macrophage apoptosis and monocyte recruitment.24

References
Circulation Editors' Picks: Most Read Articles on the Topic of Atherosclerosis
The Editors

Circulation. 2013;128:e328-e335
doi: 10.1161/CIRCULATIONAHA.113.006354

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/128/16/e328

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/