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 juxtamembrane 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 ECs 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.

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 lack S100A9 in bone marrow–derived cells, including myeloid cells, are not protected against diet-induced atherosclerosis or insulin resistance. 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 atherosclerosis. 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 atherosclerosis.2

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 promoted 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.3

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 antiatherogenic 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 this observation was made in mice expressing normal amounts of systemic and macrophage apoE, it was not possible to determine whether 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.

Conclusion: Low-density lipoprotein receptor protein 1 exerts antiatherogenic effects via pathways independent of apoE involving macrophage apoptosis and monocyte recruitment.4

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 anti-inflammatory 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.5

Toll-Like Receptor-2 Mediates Inflammation and Matrix Degradation in Human Atherosclerosis

Summary: Inflammation and matrix degradation are the hallmarks of high-risk atherosclerosis that leads to myocardial infarction and stroke. Many previous studies have identified various pathways leading to atherosclerotic lesions in mice; however, this knowledge has thus far failed to translate to effective therapeutic strategies. Toll-like receptors (TLRs), key players in innate immunity, are upregulated in atherosclerotic lesions, but their functional role in human atherosclerosis is unknown. We previously showed that cytokine, chemokine, and matrix metalloproteinase production, in an in vitro model of human atherosclerosis, is dependent on nuclear factor-κB, a key transcription factor downstream of TLRs signaling. Here we have explored the effects of blocking TLR-2, TLR-4, and myeloid differentiation primary response gene 88, a signaling adaptor shared by most TLRs and interleukin-1 receptor. Blockade of TLR-2 markedly reduced the production of proinflammatory mediators and matrix metalloproteinases. Similar effects were observed with myeloid differentiation primary response gene 88 inhibition, indicating that production of proinflammatory cytokines, chemokines, and matrix metalloproteinases in human atherosclerotic lesions is myeloid differentiation primary response gene 88 dependent. Our data show for the first time that in human atherosclerosis, TLR-2 signaling through myeloid differentiation primary response gene 88 is a rate-limiting step for both inflammation and matrix degradation. This study, by identifying a specific inflammatory pathway that may mediate the instability of cardiovascular lesions, forms the basis for the development of therapeutic agents targeting TLR-2 in cardiovascular disease.

Conclusions: Our data indicate that TLR-2 signaling through MyD88 plays a predominant role in inflammation and matrix degradation in human atherosclerosis. TLR-2 blockade may represent a therapeutic strategy for atherosclerosis and its complications.5

Hyperlipidemia-Triggered Neutrophilia Promotes Early Atherosclerosis

Summary: Atherosclerosis is a chronic inflammatory disease of large arteries with prominent roles of various leukocyte subsets that are recruited from the bloodstream into the vessel wall. Although current dogma emphasizes the role of monocyte and lymphocyte subsets, we describe here a pivotal role for neutrophils in the early stages of atherosclerosis. Hypercholesterolemia is an important risk factor, and we find evidence that high levels of cholesterol induce neutrophilia by cranking up granulopoiesis and by disturbing the chemokine axes regulating neutrophil mobilization from the bone marrow. We further found that levels of circulating neutrophils correlate with the degree of atherosclerosis, which may be useful as a simple approach to cardiovascular risk prediction. In addition, neutrophils infiltrate arteries prominently through the involvement of
Inhibition of Hyaluronan Synthesis Accelerates Murine Atherosclerosis: Novel Insights Into the Role of Hyaluronan Synthesis

Summary: Hyaluronan is an integral extracellular matrix component that plays crucial roles in, for example, development and homeostasis of cartilage and skin. However, increased hyaluronan production is associated with tumor progression and vascular disease. Hyaluronan accumulates during neointimal thickening in atherosclerotic plaques and restenotic lesions. In the neointima, it contributes to volume expansion and supports the proliferative and secretory phenotype of vascular smooth muscle cells. Therefore, inhibition of hyaluronan synthesis has been considered as a strategy to limit neointimal thickening and athereoprogession. On the other hand, recent research has established hyaluronan on the luminal surface of vascular endothelial cells to be a critical constituent of the endothelial glycocalyx, which has strong vasoprotective functions. In the present study, it is shown in a murine model of atherosclerosis that inhibition of hyaluronan synthesis by an oral hyaluronan synthesis inhibitor surprisingly enhances inflammatory and thrombotic responses and in the long term increases atherosclerosis. This adverse effect was attributed to a partial loss of the endothelial glycocalyx. Of note, hyaluronan synthesis inhibitors are effective in inhibiting tumor progression in mouse models and may be tested clinically to enhance the response to antitumor strategies. In light of the present results, it may be crucial to avoid adverse effects on the endothelial glycocalyx because damage of the glycocalyx may lead to increased atherothrombotic risk and enhance inflammatory cell recruitment.

Conclusions: The data suggest that systemic inhibition of hyaluronan synthesis by 4-MU interferes with the protective function of the endothelial glycocalyx, thereby facilitating leukocyte adhesion, subsequent inflammation, and progression of atherosclerosis.

Deficiency of Antigen-Presenting Cell Invariant Chain Reduces Atherosclerosis in Mice

Summary: CD4+ T cells are the main T-cell subtypes in human atherosclerotic lesions, and their activation requires antigen presentation from antigen-presenting cells. Major histocompatibility class II molecules mediate this process in which their binding, intracellular trafficking, and peptide loading require the chaperones from invariant chains called CD74. The present study tested the hypothesis that mice lacking CD74 have reduced atherosclerosis as a result of altered T-cell activation. Using an atherogenic diet–induced mouse atherosclerosis model in low-density lipoprotein receptor–deficient (Ldlr−/−) mice, we found that mice lacking CD74 (Ldlr−/−Cd74−/−) had protection from atherosclerosis. Although Ldlr−/− mice had enhanced atherosclerosis after immunization with proatherogenic heat shock protein-65, Ldlr−/−Cd74−/− mice remained resistant, suggesting an important role of CD74 in adaptive immunity. To our surprise, Ldlr−/−Cd74−/− mice had higher serum autoantibody levels (immunoglobulin M and immunoglobulin G3 against autoantigen malondialdehyde-modified LDL) than Ldlr−/− mice, even without antigen immunization, suggesting enhanced innate-like immunity in mice lacking the invariant chain. Indeed, the absence of CD74 led to increased numbers of marginal zone B cells in the spleen and B1 cells in the peritoneal cavity, where B1 cells are enriched. These cells provide the predominant source of autoantibodies. Given the findings that proatherogenic antigen immunization promotes atherogenesis, increased autoantibody production after autoantigen vaccination reduces atherogenesis, and deficiency of CD74 impairs adaptive immunity but enhances innate-like immunity, this study provides clinical implications that regulation of invariant chain expression or processing can directly affect the progression of atherogenesis.

Conclusions: Invariant chain deficiency in Ldlr−/− mice reduces atherosclerosis. This finding was associated with an impaired adaptive immune response and that severe atherogenicity, an unexpected increase in the number of innate-like peripheral B-1 cell populations occurred, resulting in increased IgM/IgG3 titers to the oxidation-specific epitopes.

Histone Deacetylase 3 Is Critical in Endothelial Survival and Atherosclerosis Development in Response to Disturbed Flow

Summary: Atherosclerotic lesions form at distinct sites in the arterial tree, suggesting that hemodynamic forces influence the initiation of atherogenesis. In the present study, we show that disturbed flow stabilizes histone deacetylase 3 (HDAC3) protein in endothelial cells and demonstrate that HDAC3 levels correlate with Akt activity. We uncover a novel direct interaction between HDAC3 and Akt and elaborate on the molecular mechanisms involved. Our experiments indicate that the HDAC3-Akt complex formation is critical for the survival of endothelial cells. Clinically, endothelial dysfunction is identified as a major trigger of atherogenesis. Conventional treatments for vascular diseases involve lowering blood cholesterol levels, which may have a role in endothelial protection of the vessel wall. Our experiments identify HDAC3 as a crucial molecule for endothelial viability. Moreover, in vivo experiments in which the mouse aortic isograft model was used confirmed the prominent role of HDAC3 in maintaining the integrity of the vessel and provided further evidence that HDAC3 functions as a prosurvival factor in the vasculature. Therefore, HDAC3 may serve as a putative target for novel therapeutic approaches in cardiovascular diseases. Detailed elucidation of the mechanism involved would enable us to design new drugs that could interfere with HDAC3 function in endothelial cells and successfully retard disease progression.

Conclusions: Our findings demonstrated that HDAC3 serves as an essential prosurvival molecule with a critical role in maintaining the endothelial integrity via Akt activation and that severe atherosclerosis and vessel rupture in isografted vessels of apolipoprotein E-knockout mice occur when HDAC3 is knocked down.

Tumor Necrosis Factor Receptor–Associated Factor 1 (TRAF1) Deficiency Attenuates Atherosclerosis in Mice by Impairing Monocyte Recruitment to the Vessel Wall

Summary: Tumor necrosis factor receptor–associated factors (TRAFs) mediate inflammatory signaling for important cytokines of the tumor necrosis factor/interleukin-1/Toll-like receptor superfamily such as CD40L, tumor necrosis factor-α, and interleukin-1β. Atherosclerosis is a chronic inflammatory disease governed by a network of such inflammatory cytokines. Although the inflammatory nature of atherosclerosis has been known for some time, cardiologists still lack a causal anti-inflammatory or immunomodulatory treatment option. The potential of such therapies is clearly suggested by the pleiotropic treatment benefits of statins, most recently demonstrated in the JUPITER trial (Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin). Although overall inhibition of cytokines may produce a variety of...
undesirable side effects, the inhibition of specific signaling intermediates potentially may overcome some of these limitations. The present study presents the novel and somewhat unexpected finding that TRAF1 deficiency potently attenuates murine atherosclerosis, most likely by impairing monocyte recruitment to the vessel wall, which suggests a proatherogenic function of TRAF1. In line with this notion, we found increased expression of TRAF1 in blood of patients who had an acute coronary syndrome. Future studies will be needed to determine whether TRAF1 targeting might indeed represent a novel treatment strategy for chronic inflammatory diseases such as atherosclerosis.

**Conclusion:** TRAF1 deficiency attenuates atherogenesis in mice, most likely owing to impaired monocyte recruitment to the vessel wall. These data identify TRAF1 as a potential treatment target for atherosclerosis.11

**Reduction of Circulating Soluble Fms-Like Tyrosine Kinase-1 Plays a Significant Role in Renal Dysfunction–Associated Aggravation of Atherosclerosis**

**Summary:** Chronic kidney disease is a worldwide public health problem not only because it leads to end-stage renal failure but also because it is an independent risk factor for atherosclerosis-related cardiovascular events. Accumulating evidence indicates atherosclerosis is usually worsened in patients with renal dysfunction, and the risk of cardiovascular disease increases sharply as the estimated glomerular filtration rate declines. Additionally, more than 50% of deaths among patients with end-stage renal failure are due to cardiovascular events. Although it is clear that most cardiovascular events in renal dysfunction result from atherosclerosis, the underlying molecular mechanism responsible for the worsening of atherosclerosis in renal dysfunction is not yet fully understood. Consequently, an effective therapeutic strategy is still lacking. Here, we examine the role played by soluble fms-like tyrosine kinase-1 (sFlt-1), an endogenous antagonist of the proatherogenic cytokine placental growth factor (PIGF), in the worsening of atherosclerosis seen in patients with renal dysfunction and in an animal model of renal failure. This report describes our novel observation that circulating sFlt-1 levels are reduced in patients with renal dysfunction in proportion to the severity of the disease, whereas there is no change in plasma PIGF levels. Moreover, renal production of sFlt-1 is also diminished in patients with renal dysfunction, and replacement treatment with recombinant human sFlt-1 reduces five-sixths-nephrectomy–induced worsening of atherosclerosis of apolipoprotein E–deficient mice. Thus, the present findings provide a new insight into the molecular mechanism for worsening of atherosclerosis in renal dysfunction and could lead to a new effective therapeutic strategy.

**Conclusions:** The present study demonstrates that a reduction in the circulating levels of sFlt-1 is associated with the worsening of atherosclerosis that accompanies renal dysfunction.12

**Long-Term Dipeptidyl-Peptidase 4 Inhibition Reduces Atherosclerosis and Inflammation via Effects on Monocyte Recruitment and Chemotaxis**

**Summary:** The incretin hormones glucagon-like peptide and glucose-dependent insulotropic polypeptide play a key role in the regulation of postprandial glycemia and satiety. Incretin hormones are inactivated by the exopeptidase dipeptidyl-peptidase 4 (DPP-4). Both small-molecule inhibitors of DPP-4 and DPP-4–resistant incretin analogs are increasingly common treatments for type II diabetes mellitus, although their effects in reducing long-term cardiovascular complications remain to be established. An expanding list of potential beneficial effects of DPP-4 inhibition on the cardiovascular system includes glucagon-like peptide–mediated effects on cardioprotective pathways, nitric oxide–dependent vasodilation, and non–glucagon-like peptide effects that relate to a pathophysiological role for DPP-4 in regulating inflammation. In this study, we investigated the net effects of long-term DPP-4 inhibition with alogliptin in a model of atherosclerosis and insulin resistance. DPP-4 activity was increased in atherosclerosis with a reduction in response to treatment. DPP-4 inhibition improved insulin resistance, blood pressure, and visceral adiposity with reductions in atherosclerosis and inflammation (evidenced by a reduction in plaque and adipose inflammatory macrophage content) and a shift to an alternately activated macrophage phenotype. DPP-4 inhibition prevented monocyte migration and actin polymerization in vitro via Rac-dependent mechanisms and prevented in vivo migration of labeled monocytes to the aorta in response to exogenously administered tumor necrosis factor–α and DPP-4. These data support a net effect of DPP-4 inhibition in reducing adipose and vascular inflammation with a concomitant reduction in atherosclerosis and support a therapeutic role for these agents in preventing cardiovascular complications in type II diabetes mellitus.

**Conclusion:** DPP-4i exerts antatherosclerotic effects and reduces inflammation via inhibition of monocyte activation/chemotaxis. These findings have important implications for the use of this class of drugs in atherosclerosis.13

**Intravital Microscopy on Atherosclerosis in Apolipoprotein E–Deficient Mice Establishes Microvessels as Major Entry Pathways for Leukocytes to Advanced Lesions**

**Summary:** The invasion of leukocytes in atherosclerosis has been thought to take place mainly from the arterial lumen. However, there has been considerable speculation regarding the potential role of lesion microvessels in the recruitment of inflammatory cells to plaques. Development of a microvascular network in lesions has been associated with the transition of stable to vulnerable plaques and thus the onset of symptomatic disease. However, there has been no direct study of leukocyte recruitment from lesion microvessels, and the quantitative role of this route of entry is unclear. In this study, adventitial microvessels in advanced lesions in atherosclerotic ApoE−/− mice were studied with intravital microscopy. The data show that there is no microvascular recruitment of leukocytes during early stages of the disease in mice. Inflammatory cells are instead recruited from the arterial lumen. However, once microvascular networks develop inside lesions, venules have a capacity to recruit leukocytes that are 100-fold stronger per endothelial area than recruitment from the arterial lumen. Recruitment involves the cell adhesion molecules P-selectin, L-selectin, and P-selectin glycoprotein ligand 1. Furthermore, studies of leukocyte extravasation from lesion venules and comparison of leukocyte density in lesions with or without adventitial microvessels suggest that these vessels contribute to overall cell recruitment to plaques. Taken together, these data establish microvessels in atherosclerosis as important entry pathways for leukocytes to lesions.

**Conclusions:** These findings provide strong data for microvascular recruitment of leukocytes in atherosclerosis and indicate roles for L-selectin and P-selectin glycoprotein ligand 1 in this process.14

**Effect of Rosiglitazone on Progression of Coronary Atherosclerosis in Patients With Type 2 Diabetes Mellitus and Coronary Artery Disease: The Assessment on the Prevention of Progression by Rosiglitazone on Atherosclerosis in Diabetes Patients With Cardiovascular History Trial**

**Summary:** The thiazolidinedione class of drugs has many favorable metabolic and vascular anatomic effects in people with type 2
diabetes mellitus. Whereas the Assessment on the Prevention of Progression by Rosiglitazone on Atherosclerosis in Diabetes Patients With Cardiovascular History (APPROACH) trial did not show a clear reduction in coronary atherosclerosis versus glipizide, its results are consistent with other studies of rosiglitazone and pioglitazone that did suggest reduced carotid and coronary atherosclerosis. Moreover, the large randomized outcomes trials of the thiazolidinediones that have been completed to date are consistent with the hypothesis that (1) pioglitazone may reduce cardiovascular outcomes compared with placebo and (2) the effect of rosiglitazone on cardiovascular outcomes is similar to that of metformin and to that of sulfonylureas. With the exception of fluid retention and pulmonary edema, these trial findings support the importance of clearly testing the cardiovascular effects of both of these drugs within 1 trial. The Thiazolidinedione Intervention With Vitamin D Evaluation (TIDE) trial is a large placebo-controlled trial of 16,000 participants that is currently assessing the cardiovascular effects of both thiazolidinediones versus placebo when added to current therapy. It will also clearly evaluate whether either of the 2 thiazolidinediones differ with respect to cardiovascular outcomes and will clearly determine whether either or both of these drugs prevents, promotes, or has a neutral effect on serious cardiovascular outcomes.

**Conclusions:** Rosiglitazone did not significantly decrease the primary end point of progression of coronary atherosclerosis more than glipizide in patients with type 2 diabetes mellitus and coronary atherosclerosis.  

### Endothelial-Specific Deletion of Connexin40 Promotes Atherosclerosis by Increasing CD73-Dependent Leukocyte Adhesion

**Summary:** Endothelial dysfunction, the initiating event of atherosclerosis, is characterized by increased expression of adhesion molecules and cytokines, which promotes the transmigration of leukocytes into the atherosclerotic lesion. This study provides evidence that connexin40 (Cx40), a gap junction protein expressed in endothelial cells, regulates the activity of the membrane-bound 5’-ecto-nucleotidase (CD73). The activity of endothelial CD73 generates adenosine from the hydrolysis of adenine nucleotides. Adenosine, in turn, activates surface membrane receptors to trigger antiadhesion signals for leukocytes to the endothelium. We have generated an atherosclerosis-susceptible mouse line in which Cx40 is specifically deleted from the endothelium. Endothelial deletion of Cx40 accelerated atherosclerotic lesion formation and coincided with increased expression of vascular cell adhesion molecule-1 as well as decreased expression of CD73. The antiadhesive role of Cx40 was confirmed in an endothelial cell line by specific targeting of Cx40 with antisense and small interfering RNA. We also found that functional Cx40 intercellular channels convey antiadhesion signals for leukocytes. Thus, Cx40-dependent regulation of CD73 may contribute to the spatial propagation of anti-inflammatory and antiadhesive responses within the endothelium. These findings provide a molecular basis for therapeutic modulation of Cx40-mediated intercellular communication, which may be beneficial not only in atherosclerosis but for other inflammatory diseases as well. Of note, Cx40 gene polymorphisms affecting protein expression levels have been associated recently with hypertension in humans. Future investigations should determine whether these polymorphisms might be of use in atherosclerosis risk assessment.

**Conclusions:** Cx40-mediated gap junctional communication contributes to a quiescent nonactivated endothelium by propagating adenosine-evoked anti-inflammatory signals between endothelial cells. Alteration in this mechanism by targeting Cx40 promotes leukocyte adhesion to the endothelium, thus accelerating atherosclerosis.  

### CD137 (4–BB) Deficiency Reduces Atherosclerosis in Hyperlipidemic Mice

**Summary:** The tumor necrosis factor receptor superfamily, which includes CD40, OX40, and LIGHT, plays important roles in atherogenesis. CD137 (4–BB), a member of the tumor necrosis factor receptor superfamily, has been reported to be expressed in human atherosclerotic lesions. In this study, we show that CD137 deficiency leads to attenuation of atherosclerosis in 2 different mouse atherosclerosis models. We propose a model mechanism for the roles of CD137/CD137 ligand signaling in atherosclerosis on the basis of the results of the present study. CD137/CD137 ligand signaling can induce activation of macrophages and lymphocytes through its bidirectional signaling. Endothelial CD137 activation also can induce the production of monocyte chemotactic protein-1 and cell adhesion molecules, which leads to enhanced monocyte attachment to endothelium. Taken together, the blocking of CD137/CD137 ligand signaling can reduce atherosclerosis by attenuating the immune responses in lymphocytes, macrophages, and endothelial cells. Our results suggest that CD137 can be an effective therapeutic target for the development of antiatherogenic drugs.

**Conclusions:** CD137/CD137 ligand signaling plays multiple roles in the progression of atherosclerosis, and thus, blockade of this pathway is a promising therapeutic target for the disease.  

### Blockade of Interleukin-17A Results in Reduced Atherosclerosis in Apolipoprotein E–Deficient Mice

**Summary:** Atherosclerotic lesion progression depends on chronic inflammation within the artery wall, and T cells are involved in the immune response that accompanies atherogenesis. Interleukin (IL)-17A is a recently discovered cytokine that plays a protective role in host defenses against extracellular pathogens and a pathogenic role in several autoimmune diseases, including multiple sclerosis, inflammatory bowel disease, and arthritis. In the present study, we showed that plasma levels of IL-17A and aortic IL-17A–producing γδ and CD4+ (Th17) T cells were significantly elevated in the atherosclerosis-prone conditions found in apolipoprotein E–deficient (ApoE–) mice. We confirmed a proatherogenic role of IL-17A using adenosine-delivered soluble IL-17 receptor against IL-17A, which caused a significant decrease in plasma levels of IL-6 and granulocyte colony-stimulating factor, diminished aortic macrophage content and CXCL1 expression, and led to a reduction in plaque burden in treated ApoE– mice. Conversely, the treatment of isolated ApoE– aortas with recombinant IL-17A increased CXCL1 expression and monocyte adhesion to vessel wall. Our findings highlight a proatherogenic role for IL-17A in coronary atherosclerosis and suggest that future therapies targeting IL-17A could potentially reduce vascular wall infiltrates and lesion size and attenuate atherosclerosis and other forms of vascular disease.

**Conclusions:** These results demonstrate that atherosclerosis-prone conditions induce the differentiation of IL-17A–producing T cells. IL-17A plays a proatherogenic inflammatory role during atherogenesis by promoting monocyte/macroage recruitment into the aortic wall.  

### The Phosphodiesterase Inhibitor Cilostazol Induces Regression of Carotid Atherosclerosis in Subjects With Type 2 Diabetes Mellitus: Principal Results of the Diabetic Atherosclerosis Prevention by Cilostazol (DAPC) Study: A Randomized Trial

**Summary:** Antiplatelet agents are widely reported to be effective in preventing the recurrence of cardiovascular events. Clinical guidelines have recommended that individuals with risk factors for coronary heart disease (eg, diabetes mellitus) take aspirin for both primary and secondary prevention. Cilostazol, a phosphodiesterase III inhibitor with antiplatelet, antiarrhythmic, vasodilatory, and antiproliferative effects, is currently indicated for the treatment of intermittent claudication and/or ischemic signs and symptoms asso-
associated with chronic arterial occlusion around the world and secondary prevention of cerebral infarction in Asian countries. However, there was no report comparing the efficacy and usefulness of 2 different antiplatelet drugs, aspirin and cilostazol, in the prevention of occurrence or progression of atherosclerosis in diabetic patients. This is the first study to directly compare the effect of cilostazol and aspirin on the progression of atherosclerosis in diabetic patients. In this international, prospective, randomized, open, blinded end point study involving a total of 329 Asian type 2 diabetic patients suspected of peripheral artery disease, we found that cilostazol treatment (100–200 mg/d) potently and safely inhibited the progression of carotid intima-media thickness, an established surrogate marker of cardiovascular events, compared with aspirin treatment (81–100 mg/d) during a 2-year observation period. Our findings suggest that cilostazol is a more effective antiatherosclerotic agent than aspirin in patients with type 2 diabetes mellitus. A large-scale prospective trial is needed to establish the usefulness of cilostazol for primary prevention of cardiovascular events in patients with type 2 diabetes mellitus.

**Conclusions:** Compared with aspirin, cilostazol potently inhibited progression of carotid intima-media thickness, an established surrogate marker of cardiovascular events, in patients with type 2 diabetes mellitus.19

**Long-Term Administration of Endothelin Receptor Antagonist Improves Coronary Endothelial Function in Patients With Early Atherosclerosis**

**Summary:** Endothelial dysfunction is considered critical in the initiation, progression, and complications of coronary artery disease and is independently associated with cardiovascular events. It is a reversible process that represents the functional expression of an individual's overall cardiovascular risk factor burden, and many therapies that restore endothelial function also decrease cardiovascular events. Coronary endothelial function is regulated by the balance of endothelium-derived vasodilator and vasoconstrictor factors such as endothelin-1. This study provides evidence that long-term ET\(_{A}\) receptor antagonist administration improves coronary microvascular endothelial function in humans and supports a role for endogenous endothelin in the mechanism and potentially the treatment of coronary endothelial function in humans.

**Conclusions:** This study demonstrates that 6-month treatment with atrasentan improves coronary microvascular endothelial function and supports the role of the endogenous endothelin system in the regulation of endothelial function in early atherosclerosis in humans.20

**Early Atherosclerosis Exhibits an Enhanced Procoagulant State**

**Summary:** Apart from their well-established role in coagulation, several hemostatic factors (eg, tissue factor/activated factor VII complex, activated factor X, thrombin) have been reported to evoke multiple proatherogenic events on a wide range of arterial wall constituents. While exploring the presence and distribution of all coagulation proteins in both early and advanced human atherosclerotic plaques, we found a colocalization of key procoagulant proteins with smooth muscle cells and macrophages, thus suggesting an active cell-based coagulation network within the atherosclerotic plaque. Furthermore, we provide new evidence pointing toward local synthesis of several coagulation factors within the atherosclerotic vessel wall. The principal finding of this study, indicating enhanced procoagulant activity of early atherosclerotic plaques versus stable advanced plaques, suggests a role for the hemostatic proteins and hypercoagulability in regulating the onset and progression of atherosclerosis. These findings may become clinically relevant in the new era of selective oral anticoagulants, in which such agents may have effects on the complex process of atherosclerosis beyond their direct antithrombotic action.

**Conclusions:** This study shows an enhanced procoagulant state of early-stage atherosclerotic plaques compared with advanced-stage plaques, which may provide novel insights into the role of coagulation during atherosclerotic plaque progression.21

**Natural History of Experimental Coronary Atherosclerosis and Vascular Remodeling in Relation to Endothelial Shear Stress: A Serial, In Vivo Intravascular Ultrasound Study**

**Summary:** Knowledge of the natural history of atherosclerosis and of the determinants of heterogeneous atherosclerotic manifestations is a precondition for the early identification of coronary regions most likely to evolve to culprit lesions of acute coronary events. This experimental study indicates that the in vivo combined assessment of local low endothelial shear stress, excessive expansive remodeling, and advanced plaque severity at earlier stages of the disease course may enable the prediction of lesions most likely to possess or to acquire a high-risk phenotype. Assessment of local hemodynamic and arterial wall characteristics might thereby be used as a clinical tool for individual plaque risk stratification and prognosis. Percutaneous coronary interventions are currently performed either in severely obstructive plaques causing myocardial ischemia or in culprit lesions of acute coronary syndromes, the majority of which are minimally obstructive. Early in vivo identification of high-risk lesions before they actually become prone to rupture may guide the appropriate application of a focused systemic treatment or highly selective local prophylactic interventions to avert the thrombotic complications of coronary plaque rupture. A large-scale, human natural history study currently underway (the PREDICTION Trial) may confirm the applicability of evaluating shear stress and arterial wall morphology to predict the development of lesions that cause new coronary events.

**Conclusions:** The synergistic effect of local endothelial shear stress (ESS) and the remodeling response to plaque formation determine the natural history of individual lesions. Combined in vivo assessment of ESS and remodeling may predict the focal formation of high-risk coronary plaque.22

**Dietary Intervention to Reverse Carotid Atherosclerosis**

**Summary:** The main findings in this study are as follows: (1) Diet-mediated weight loss over a 2-year period can induce a significant regression of carotid vessel wall volume. (2) Low-fat, low-carbohydrate, and Mediterranean diets provide similar degrees of carotid vessel wall volume regression. Thus, a low-carbohydrate diet is an alternative to low-fat and Mediterranean diets in reversing carotid atherosclerosis. (3) Over 2 years, changes in carotid intima-media thickness and 3-dimensional ultrasound are more clearly predicted by diet-induced changes in blood pressure than by changes in lipoprotein levels. For the practicing clinician, this study demonstrates that carotid atherosclerosis is reversible by long-term adherence to dietary strategies to induce weight loss. This effect is more pronounced among mildly obese persons who lose >5.5 kg body weight within 12 months and whose systolic blood pressure decreases by >7 mm Hg within 9 months. Increase in apolipoprotein A1 and decrease in plasma total homocysteine levels are also associated with subsequent success in reversing carotid atherosclerosis.

**Conclusions:** Two-year weight loss diets can induce a significant regression of measurable carotid vessel wall volume. The effect is similar in low-fat, Mediterranean, or low-carbohydrate strategies and appears to be mediated mainly by the weight loss–induced decline in blood pressure.23
Pediatric Metabolic Syndrome Predicts Adulthood Metabolic Syndrome, Subclinical Atherosclerosis, and Type 2 Diabetes Mellitus But Is No Better Than Body Mass Index Alone: The Bogalusa Heart Study and the Cardiovascular Risk in Young Finns Study

Summary: In a recent Scientific Statement from the American Heart Association on metabolic syndrome (MetS) in children and adolescents, the need for additional research examining the efficacy of pediatric MetS to predict adult health was highlighted. In the present analyses based on 2 population-based prospective cohorts, the Bogalusa Heart Study and the Cardiovascular Risk in Young Finns Study, we examined the utility of youth MetS and its components in predicting adult high carotid intima-media thickness and type 2 diabetes mellitus among 1781 participants aged 9 to 18 years at baseline who were reexamined 14 to 27 years later. We observed that youth with MetS were at 2 to 3 times the risk of having high carotid intima-media thickness and type 2 diabetes mellitus as adults compared with those free of MetS. However, the prediction of adult high carotid intima-media thickness and type 2 diabetes mellitus with the use of youth body mass index was either equivalent or superior to classification based on pediatric MetS. Our findings have direct clinical relevance because they suggest that in the clinical setting, efforts to identify youth with heightened future risk of meaningful outcomes can be minimally achieved with the use of body mass index only, thus avoiding cost and other barriers associated with testing and classification of youth MetS. However, clinicians who use high body mass index to identify youth at increased future risk need to keep in mind that a large proportion of contemporary youth will be classified as at risk and that our analyses are unable to discount that youth MetS may be useful in identifying and possibly treating other cardiometabolic disorders.

Conclusions: Youth with MetS are at increased risk of meaningful adult outcomes; however, the simplicity of screening for high BMI or overweight and obesity in the pediatric setting offers a simpler, equally accurate alternative to identifying youth at risk of developing adult MetS, high cIMT, or T2DM.24

C5a Receptor Targeting in Neointima Formation After Arterial Injury in Atherosclerosis-Prone Mice

Summary: Despite the success of antiproliferative therapies, restenosis remains a common problem after vascular intervention. The receptor for complement C5a (C5aR) has been identified in human atherosclerotic tissue, and adverse cardiovascular events have been correlated with C5aR plasma levels. Receptor binding of C5a leads to proinflammatory activation of many cell types, but the role of receptor-mediated action during arterial remodeling after injury has not been studied. In the present study, we examined the contribution of the C5aR to neointima formation in apolipoprotein E–deficient mice employing a human peptidomimetic C5aR antagonist and a C5aR-blocking monoclonal antibody. Our results indicate a strong protective effect by short-term (1 week) blockade of C5aR with C5aR antagonist or anti-C5aR monoclonal antibody on neointimal plaque formation, leukocyte recruitment, and vascular cell adhesion molecule-1 expression. In contrast, long-term treatment (3 weeks) reduced inflammatory cell recruitment and vascular cell adhesion molecule-1 expression but also induced plasminogen activator inhibitor-1–dependent smooth muscle cell migration to the neointima, thus stabilizing but not reducing plaques. Further studies confirmed that protective effects of short-term C5aR antagonist treatment persisted over 3 weeks after injury. On the basis of these properties, C5aR targeting can be considered an attractive pharmacological strategy for short-term treatment of vascular injury and inflammation. However, long-term approaches should account for direct or indirect effects of C5aR signaling on adaptive immune responses, cell apoptosis, migration, and tissue regeneration or fibrosis that may affect such treatment. Thus, our study provides the first evidence that intervention of the complement cascade at the level of C5aR may be clinically feasible to limit restenosis after vascular injury.

Conclusions: One-week treatment with C5aRA or anti-C5aR-blocking monoclonal antibody limited neointimal hyperplasia and inflammatory cell content and was associated with reduced vascular cell adhesion molecule-1 expression. However, treatment for 3 weeks failed to reduce but rather stabilized plaques, likely by reducing vascular plasminogen activator inhibitor-1 and increasing vascular smooth muscle cell migration.25

High-Resolution Magnetic Resonance Imaging Enhanced With Superparamagnetic Nanoparticles Measures Macrophage Burden in Atherosclerosis

Summary: Accumulation of macrophages influences clinical outcomes of various inflammatory diseases, including atherosclerosis. Particularly, these proinflammatory phagocytes promote not only development of atherosclerosis but also its acute thrombotic complications. Development of novel circulating or imaging biomarkers targeting macrophages should thus help to identify patients with subclinical inflamed lesions and to provide new and important insights into preventive cardiovascular medicine. Molecular or functional imaging, a rapidly emerging technology, visualizes biological or pathological processes in specific organs or disease contexts, in addition to providing anatomic information. The present study represents modern molecular imaging that can assess vascular inflammation. We demonstrate that high-resolution 3-T MRI enhanced with superparamagnetic iron nanoparticles can measure inflammatory burden in atherosclerosis of hypercholesterolemic rabbits. Macrophages internalize such iron particles, most likely via phagocytosis, thus changing the magnetic field in inflamed tissues. The present study demonstrates that the magnitude of T2-weighted signal intensity loss, reflecting phagocytic activity of macrophages, is associated positively with the extent of accumulation of these immune cells in atherosclerotic plaques. Such a noninvasive imaging approach might also offer a powerful tool to monitor the effects of anti-inflammatory therapies in clinical practice or during clinical trials for new drugs. Our study indeed reports that the magnitude of T2-weighted signal intensity loss significantly decreased after statin treatment and correlated well with the reduction of lesional macrophage accumulation. Furthermore, such macrophage-targeted molecular imaging should provide novel insight into the mechanisms of atherosclerosis and its acute complications.

Conclusion: The magnitude of T2 signal intensity reduction in high-resolution MRI after administration of superparamagnetic phagocytosable nanoparticles can assess macrophage burden in atheromata, providing a clinically translatable tool to identify inflamed plaques and to monitor therapy-mediated changes in plaque inflammation.26

Circulating Endothelial Progenitor Cells Do Not Contribute to Plaque Endothelium in Murine Atherosclerosis

Summary: The idea that endothelial progenitor cells (EPCs) circulate in blood and contribute to endothelial cell turnover and regeneration in the cardiovascular system has become a widely accepted paradigm. Clinical studies have measured the level of putative EPCs in the blood of patients and found that it correlates inversely with atherogenic risk factors, established atherosclerotic disease, and future atherosclerotic events. This has fostered the theory that circulating EPCs provide protection against atherosclerosis by their innate ability to replace dysfunctional ECs and to regenerate senescent and damaged endothelium. Direct evidence that EPCs contribute to plaque endothelium at all is sparse, however. In the present
study, we investigated whether circulating EPCs home and differentiate into endothelial cells in murine atherosclerotic plaques using a series of transplantation techniques, specific markers for cell type and origin, and high-resolution microscopy. We report that endothelial cells in both intact and healed disrupted plaques are replenished by local proliferation with no or extremely rare contributions from circulating cells. Although one should always be cautious about extrapolating from animal models to human disease, our data suggest that if the circulating cell populations known as EPCs in humans have a role in the development or progression of atherosclerosis, then it is not by differentiating into plaque endothelial cells.

Conclusions: Circulating EPCs rarely, if ever, contribute to plaque endothelium in apoE−/− mice. These findings bring into question the prevailing theory that circulating EPCs play an important role in atherogenesis.27

The Alternative Pathway Is Critical for Pathogenic Complement Activation in Endotoxin- and Diet-Induced Atherosclerosis in Low-Density Lipoprotein Receptor-Deficient Mice

Summary: Previous experiments in animals have suggested that the early components of the classical and lectin complement pathways may have protective effects against the development of atherosclerosis. In this study, we have addressed the role of the alternative pathway by crossing the low-density-lipoprotein receptor–deficient mouse model of atherosclerosis (Ldlr−/−) with mice that lack complement factor B (Bf−/−), the initiator of the alternative pathway. Under 2 different proatherogenic conditions, administration of lipopolysaccharide and high-fat diet, Bf−/−/Ldlr−/− mice showed markedly reduced atherosclerotic lesion formation compared with Ldlr−/− mice. The protective effects of factor B deficiency were associated with significant reductions in systemic and lesional complement activation. Overall, our data provide the first direct evidence of the proatherogenic role of the amplification of complement activation by the alternative pathway in response to lipopolysaccharide or high-fat diet. This work lends support for developing therapeutic strategies aiming to inhibit the complement system by blocking the alternative pathway without interfering with the protective effect(s) mediated by the classical and lectin pathways.

Conclusions: These data demonstrate that amplification of complement activation by the alternative pathway in response to lipopolysaccharide or high-fat diet plays a proatherogenic role.28

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 the central cell of plaques, macrophages, with the change in such lipid reduction were decreases in the content and inflammatory status was augmented by treatment with pioglitazone, consistent with the effects of peroxisome proliferator-activated receptor-γ agonists for 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.29

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 postangioplasty restenosis of human coronary arteries, this study provides potential strategies for the prevention of cardiovascular diseases and the advancement of coronary intervention therapies.

Conclusions: In addition to the well-known signaling for apoptosis induction, CHOP may play important roles in augmenting potentially pathological biological stress responses. This noncanonical role of CHOP, especially that expressed in vascular cells, may contribute to the progression of vascular remodeling and atherosclerosis.30

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.

Conclusion: 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.31

**Notch Signaling Regulates Endothelial Progenitor Cell Activity During Recovery From Arterial Injury in Hypercholesterolemic Mice**

Summary: Abundant evidence has linked hypercholesterolemia with the advent and progression of atherosclerosis. To facilitate the study of the mechanisms by which hypercholesterolemia leads to atherosclerosis, animal models have been developed. Most notably, the apolipoprotein E (ApoE)−/−null and low-density lipoprotein receptor−null mice have been used extensively in attempts to gain a better understanding of the effects of high lipid levels on atheroma formation. In recent years, traditional cardiovascular risk factors have also been associated with decreased numbers of circulating endothelial progenitor cells (EPCs), which have been suggested to participate in endothelial repair and maintenance. Ample experimental evidence suggests that EPCs are mobilized from the bone marrow to the peripheral circulation after arterial injury (eg, percutaneous transluminal coronary angioplasty or stent implantation) and promote reendothelialization at the injury site, thereby speeding endothelial recovery and reducing the risk of restenosis and atherosclerotic plaque formation. Here, we compared EPC recruitment, reendothelialization, and plaque formation after carotid artery injury in ApoE−/− mice and ApoE−/− mice. Our findings indicate that EPC recruitment was higher in ApoE−/− mice than in wild-type mice and that the enhanced recruitment likely evolves through a moderate decline in Notch expression. Reendothelialization was also greater in ApoE−/− mice, which was somewhat surprising because ApoE−/− mice are prone to atherosclerosis. Furthermore, transplanted, bone marrow–derived EPCs were found at the border of the atherosclerotic lesions, which could suggest that EPCs contribute to the early stage of plaque formation. However, the clinical implications of these observations must be interpreted with caution, particularly because circulating EPC levels were higher in ApoE−/− mice than in wild-type mice, whereas hypercholesterolemia, hypertension, and many other risk factors for cardiovascular disease are associated with declines in human EPC levels. Collectively, our findings emphasize the need for additional experiments in other hypercholesterolemic animal models and underscore the potential for genetic mouse models of human disease to yield data that do not necessarily reflect clinical reality. To clarify the paradoxical observation on the direct effect of cholesterol on EPC contribution to reendothelialization versus plaque formation in Apo E knockout mice after arterial injury, further investigations using different types of hypercholesterolemic animals and, importantly, additional clinical correlation are required.

Conclusion: The results presented here provide novel insights into the role of EPCs during atherosclerosis and suggest that cholesterol and Notch1 may be involved in the regulation of EPC activity.32

**Burden of Cardiovascular Risk Factors, Subclinical Atherosclerosis, and Incident Cardiovascular Events Across Dimensions of Religiosity: The Multi-Ethnic Study of Atherosclerosis**

Summary: A variety of behaviors can benefit or harm one's cardiovascular health, and it has been observed that religious beliefs and practices may influence one's health behaviors significantly. We sought to determine whether and to what extent different aspects of religiosity may be associated with cardiovascular health. Using a large, ethnically diverse, community-based sample of men and women ages 45 to 84 years who were asymptomatic at baseline, we compared the prevalence of cardiovascular risk factors, the presence and burden of subclinical cardiovascular disease, and the incidence of cardiovascular events across different levels of religiosity. We adjusted for sociodemographic factors to ensure that these potential confounders were not responsible for associations found between religion and cardiovascular health. We observed that those who attended services frequently and those who prayed frequently were significantly less likely to smoke than those who never attended services or prayed. Perhaps more surprisingly, we found that frequent service attendees and those who prayed often were significantly more likely to be obese. We did not observe any significant associations between religiosity and presence/extent of subclinical disease or incident cardiovascular events. The consistent and significant association found between religiosity and obesity in this cross-sectional study raises some interesting questions. What is the temporal nature of the association? If being more religious makes one more likely to be obese, then why does this occur? Ultimately, this observation should encourage interaction between healthcare providers, public health officials, and the religious community in an effort to improve obesity education and prevention.

Conclusions: Our results do not confirm those of previous studies associating greater religiosity with overall better health risks and status, at least with regard to CVD. There was no reduction in risk for CVD events associated with greater religiosity.33

**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 (O2−) 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.44
Chronic Kidney Disease Is Associated With the Incidence of Atrial Fibrillation: The Atherosclerosis Risk in Communities (ARIC) Study

**Summary:** Previous research has shown that individuals with end-stage renal disease have a higher risk of developing atrial fibrillation, and some cross-sectional studies have found higher prevalence of atrial fibrillation among those with decreased kidney function. However, evidence from prospective studies in the general population is limited. In an analysis of 10,328 men and women participating in the Atherosclerosis Risk in Communities Study, we observed that impaired kidney function, measured by lower cystatin-based or creatinine-based estimated glomerular filtration rate, was strongly associated with a higher risk of atrial fibrillation. Similarly, individuals with increased levels of urinary albumin-to-creatinine ratio, a marker of kidney damage, had a higher risk of developing atrial fibrillation. Our study highlights the potential role of chronic kidney disease as a risk factor for atrial fibrillation. Interventions aimed at preventing and treating chronic kidney disease could also contribute to reduce the burden of atrial fibrillation in the population.

**Conclusion:** In this large population-based study, reduced kidney function and presence of albuminuria were strongly associated with the incidence of atrial fibrillation independently of other risk factors.

Rapid, Direct Effects of Statin Treatment on Arterial Redox State and Nitric Oxide Bioavailability in Human Atherosclerosis via Tetrahydrobiopterin-Mediated Endothelial Nitric Oxide Synthase Coupling

**Summary:** Statin treatment reduces cardiovascular risk. Although statins exert their antiatherogenic effects mainly by reducing low-density lipoprotein, experimental studies demonstrated a number of pleiotropic effects directly on the vascular cells. However, mechanistic studies examining the pleiotropic effect of statins on endothelial nitric oxide (NO) bioavailability and the vascular redox state in human arteries are remarkably limited. In the present study, we first demonstrate that in a real-life population of 492 patients undergoing coronary artery bypass graft surgery, the use of statins was a predictor of both improved endothelial function and reduced vascular superoxide (O$_2^-$) in internal mammary arteries (IMAs). Next, in a randomized, clinical trial with 42 patients undergoing coronary artery bypass graft surgery, we demonstrated that 3 days of treatment with atorvastatin 40 mg/d before coronary artery bypass graft surgery improved endothelial function and reduced vascular O$_2^-$ in internal mammary arteries of these patients by improving endothelial NO synthase coupling with increased vascular levels of the endothelial NO synthase cofactor tetrahydrobiopterin. We then performed a number of mechanistic ex vivo experiments in which atorvastatin rapidly upregulated GTP-cyclohydrolase I gene expression in the arterial wall, increased GTP cyclohydrolase I activity, and stimulated the synthesis of vascular biopterins, resulting in an improvement of endothelial NO synthase coupling, a reduction of endothelium-derived vascular O$_2^-$, and an improvement in endothelial function in a low-density lipoprotein–free environment. These effects were due to a direct inhibition of hydroxymethylglutaryl-coenzyme A reductase in the vascular wall. Therefore, our study demonstrates for the first time in humans that high-dose treatment with atorvastatin rapidly modifies the vascular redox state and endothelial function via tetrahydrobiopterin-mediated endothelial NO synthase coupling, providing one of the first direct reports of a pleiotropic effect of statins on the human arterial endothelium.

**Conclusions:** This study demonstrates for the first time in humans the direct effects of statin treatment on the vascular wall, supporting the notion that this effect is independent of low-density lipoprotein lowering. Atorvastatin directly improves vascular NO bioavailability and reduces vascular O$_2^-$ through tetrahydrobiopterin-mediated endothelial NO synthase coupling. These findings provide new insights into the mechanisms mediating the beneficial vascular effects of statins in humans.

Sex and Race Differences in Right Ventricular Structure and Function: The Multi-Ethnic Study of Atherosclerosis–Right Ventricle Study

**Summary:** Right ventricular (RV) morphology is an important predictor of outcomes in heart and lung disease; however, demographic differences in RV structure or function have not been studied. We found that age, sex, and race/ethnicity had important associations with RV mass and volumes and RV ejection fraction. These associations may explain differences in outcomes in a variety of common heart and lung diseases that affect the RV. We derived normative equations for RV parameters that could be used to define and diagnose RV dysfunction in adults of various races and ethnicities in research and clinical practice.

**Conclusion:** Age, sex, and race are associated with significant differences in RV mass, RV volumes, and RV ejection fraction, potentially explaining distinct responses of the RV to cardiopulmonary disease.

Absolute and Attributable Risks of Atrial Fibrillation in Relation to Optimal and Borderline Risk Factors: The Atherosclerosis Risk in Communities (ARIC) Study

**Summary:** This study, which is based on >17 years of follow-up of >14,500 men and women, represents an important contribution to the literature on the major and modifiable causes of atrial fibrillation (AF). Atrial fibrillation is an important risk factor for stroke and overall mortality and affects between 0.4% and 1.0% of the US population. Unlike other forms of cardiovascular disease, information about the preventable burden of AF is lacking. Therefore, the aim of this study was to determine what proportion of the burden of AF in blacks and whites could theoretically be avoided by the maintenance of an optimal risk profile. Previously established modifiable AF risk factors, namely high blood pressure, elevated body mass index, diabetes mellitus, cigarette smoking, and prior cardiac disease, were categorized into optimal, borderline, and elevated levels. On the basis of their risk factor levels, individuals were classified into 1 of these 3 groups. Overall, 57% of AF cases could be explained by having ≥1 borderline or elevated risk factors, of which suboptimal blood pressure was the most important contributor, accounting for one quarter of the burden of AF. In comparison, only 5% of AF cases were attributable to diabetes mellitus. These findings illustrate that, as with other forms of cardiovascular disease, more than half of the AF burden is potentially avoidable through the maintenance of optimal levels of classic cardiovascular risk factors, further reinforcing the need for effective primary prevention strategies that enable individuals to adopt and maintain healthy diet and behavioral patterns.

**Conclusion:** As with other forms of cardiovascular disease, more than half of the AF burden is potentially avoidable through the optimization of cardiovascular risk factors levels.

Cardiac Troponin T Measured by a Highly Sensitive Assay Predicts Coronary Heart Disease, Heart Failure, and Mortality in the Atherosclerosis Risk in Communities Study

**Summary:** Cardiac troponin T (cTnT) and troponin I are the biomarkers of choice in the evaluation of patients with acute coronary syndromes. Cardiac troponin has been independently associated with adverse outcomes after acute coronary syndrome, in patients with
chronic heart failure, and in the general population, although cTnT levels are detectable in only a small fraction of the general population with the use of current assays. A precommercial highly sensitive cTnT assay can detect 10-fold lower concentrations than currently available assays. We sought to define the prevalence of measurable cTnT using this newly highly sensitive assay and the associations of cTnT levels with adverse cardiovascular events in participants in the Atherosclerosis Risk in Communities (ARIC) Study, aged 54 to 74 years, who were initially free of cardiovascular disease. We found that the new highly sensitive cTnT assay detected circulating cTnT in 66.5% of the ARIC sample (<1% would have been detected by the currently available cTnT assays) and that measured cTnT had strong associations with coronary heart disease events, mortality, and heart failure and improved risk prediction. cTnT appears to be an important marker of coronary heart disease, mortality, and heart failure risk even in a healthy middle-aged population without manifest cardiovascular disease.

Conclusions: cTnT detectable with a highly sensitive assay was associated with incident coronary heart disease (CHD), mortality, and heart failure in individuals from a general population without known CHD/stroke.40

Association of Colony-Forming Units With Coronary Artery and Abdominal Aortic Calcification

Summary: Endothelial progenitor cells are made up of circulating cells that originate from the bone marrow and are believed to contribute to arterial homeostasis. Although experimental studies suggest that decreased endothelial progenitor cell quantity may promote atherosclerosis, data in humans are limited. Therefore, among 889 participants of the Framingham Heart Study, we examined the association of endothelial progenitor cell-related cell types with the presence of subclinical atherosclerosis as evidenced by coronary artery calcification or abdominal aortic calcification detected by multidetector computed tomography. We observed that a lower quantity of colony-forming units was significantly associated with greater coronary artery calcification and abdominal aortic calcification, even after adjustment for cardiovascular risk factors. In contrast, neither the CD34+/KDR− nor CD34− cell type was associated with differences in coronary artery calcification or abdominal aortic calcification. These results are consistent with the theory that colony-forming units and CD34+−related cells represent different functional types of endothelial progenitor cells, with likely distinct roles in mediating the vascular response to atherogenic exposures. Overall, these findings suggest that decreased angiogenic potential, as represented by colony-forming unit quantity, could contribute to the development of atherosclerosis in humans.

Conclusions: In this large, community-based sample of men and women, lower colony-forming unit number was associated with a higher burden of subclinical atherosclerosis in the coronary arteries and aorta. Decreased angiogenic potential could contribute to the development of atherosclerosis in humans.40

Major Contribution of the P2Y<sub>1</sub> Receptor in Purinergic Regulation of TNFα-Induced Vascular Inflammation

Summary: The P2Y<sub>1</sub> receptor plays a key role in platelet activation and arterial thrombosis, as has been shown in P2Y<sub>1</sub>-deficient mice and through the use of selective P2Y<sub>1</sub> antagonists in vitro in platelet function studies and in vivo in animal models of thrombosis. It is thus a potentially promising target for new antiplatelet drugs. In addition, the P2Y<sub>1</sub> receptor is involved in atherosclerosis, and the present study demonstrates the role of the endothelial receptor in inflammatory events in arteries. Altogether, these findings add to interest in simultaneously targeting separate aspects of atherothrombosis (ie, platelet activation, vascular inflammation, and development of atherosclerosis). Moreover, because the P2Y<sub>1</sub> receptor plays a minor role in normal hemostasis when compared with the P2Y<sub>12</sub> receptor, one can expect a smaller risk of bleeding with P2Y<sub>1</sub>-targeting drugs, bleeding being the major limitation of aggressive antiplatelet therapy, especially when targeting the P2Y<sub>12</sub> receptor. The pharmacological inhibition of the leukocyte recruitment in vivo using the selective antagonist MRK2500 is very promising. P2Y<sub>1</sub>-targeting drugs might therefore be efficient in patients requiring long-term treatment.

Conclusions: The data highlight a key role of the endothelial P2Y<sub>1</sub> receptor in acute vascular inflammation. Pharmacological targeting the P2Y<sub>1</sub> receptor could represent a promising approach for the treatment of vascular inflammation.41

Chemokine Receptor 7 Knockout Attenuates Atherosclerotic Plaque Development

Summary: Modulation of both innate and adaptive immune responses has become an attractive and, in some clinical settings, successful tool to treat a variety of diseases (eg, rheumatic arthritis and different forms of cancer). The perception that the interaction between innate and adaptive immunity is also important for the development of atherosclerosis has already resulted in some attempts to influence atherosclerotic plaque development by immunomodulation. Our study strengthens the importance of secondary lymphoid organs and the impact of T-cell recirculation for the transition from innate to adaptive immune system activation during atherogenesis. Together with the fact that our findings, together with others, underline the antigenic potential of oxidized low-density lipoprotein, the study presented may lead the way to novel therapeutic strategies to fight the detrimental clinical end points of atherosclerosis. For instance, blockage of costimulatory molecules on oxidized low-density lipoprotein–pulsed dendritic cells ex vivo might positively influence atherosclerotic plaque development. In addition, artificial modulation of oxidized low-density lipoprotein presentation or interaction of dendritic cells with T cells in secondary lymphoid organs may be new ways to attenuate atherogenesis.

Conclusion: These results demonstrate that both CCR7-dependent T-cell priming in secondary lymphoid organs and CCR7-dependent recirculation of T cells between secondary lymphoid organs and inflamed tissue are crucially involved in atherosclerotic plaque development.42

Influence of Age on Associations Between Childhood Risk Factors and Carotid Intima-Media Thickness in Adulthood: The Cardiovascular Risk in Young Finns Study, the Childhood Determinants of Adult Health Study, the Bogalusa Heart Study, and the Muscatine Study for the International Childhood Cardiovascular Cohort (i3C) Consortium

Summary: The pediatric origin of atherosclerosis is now well accepted, with several authorities issuing guidelines and consensus statements for the assessment and management of cardiovascular disease risk factors, including lipids and lipoprotein, blood pressure, and adiposity, in childhood. Despite this, there have been scant data that have assessed the optimal age when childhood risk exposure begins to associate with adult atherosclerosis, and thus the optimal age for risk factor screening. In the present analyses based on 4 population-based, prospective childhood cohorts—the Cardiovascular Risk in Young Finns Study (Finland), the Childhood Determinants of Adult Health study (Australia), the Bogalusa Heart Study (United States), and the Muscatine Study (United States)—we examined the influence of age on the associations between childhood risk factors and adult
Carotid artery intima-media thickness, a subclinical marker of atherosclerosis, among 4380 participants 3 to 18 years old at baseline who were reexamined 13 to 28 years later. On the basis of our findings, risk factors measured before the age of 9 years had only weak or nonsignificant associations with carotid intima-media thickness. Our data have direct clinical and public health importance because they suggest that risk factor screening from the age of 9 years onward allows youth who are at increased risk of subclinical atherosclerosis in adulthood to be identified. However, care providers need to keep in mind that although the optimal age for pediatric risk factor screening may commence at 9 years of age, primordial prevention of cardiovascular disease should begin earlier in the life course.

Conclusions: Our analyses from 4 longitudinal cohorts showed that the strength of the associations between childhood risk factors and carotid intima-media thickness is dependent on childhood age. On the basis of these data, risk factor measurements obtained at or after 9 years of age are predictive of subclinical atherosclerosis in adulthood.

Pharmacological Suppression of Hepatic ATP-Binding Cassette Transporter 1 Activity in Mice Reduces High-Density Lipoprotein Cholesterol Levels But Promotes Reverse Cholesterol Transport

Summary: Plasma levels of high-density lipoprotein cholesterol (HDL-C) do not always reflect the dynamic process of reverse cholesterol transport (RCT) from macrophage to bile and feces and the risk of atherosclerosis. For example, mice lacking the hepatic HDL receptor scavenger receptor class B type I have markedly elevated HDL-C levels but impaired RCT and increased atherosclerosis. The ATP-binding cassette transporter 1 (ABCA1) is expressed in the liver, and by exporting cholesterol out of the liver to the HDL protein, apolipoprotein A-I plays a critical role in maintaining plasma HDL-C levels. However, the relationship of hepatic ABCA1 to RCT and atherosclerosis remains poorly understood. Because hepatic ABCA1 pumps cholesterol from the liver into the blood instead of the bile, it might reduce the rate at which the liver excretes HDL-derived cholesterol. Probucol is a drug that reduces HDL-C levels but also, paradoxically, reduces atherosclerosis and xanthomas. We tested the hypothesis that probucol inhibits hepatic ABCA1 activity, thereby reducing HDL-C levels but promoting RCT from macrophages. In studies in mice lacking the hepatic HDL receptor scavenger receptor class B type I, probucol substantially reduced HDL-C but significantly increased macrophage RCT. Furthermore, probucol significantly enhanced the excretion of HDL-derived cholesterol into the feces. Probucol markedly inhibited ABCA1-dependent cholesterol efflux from mouse primary hepatocytes, and this effect was shown to be responsible for the effect of probucol on increasing the fecal excretion of HDL-derived cholesterol in vivo. These results provide an explanation for the beneficial effects of probucol on atherosclerosis despite its HDL-lowering effects and suggest that inactivation of hepatic ABCA1 leads to increased RCT despite reducing plasma HDL-C levels.

Conclusions: We demonstrate that pharmacological inhibition of hepatic ABCA1 activity with probucol reduced HDL-C levels but promoted RCT through diversion of HDL-derived cholesterol from efflux back into plasma instead to excretion in the bile. These results explain the beneficial effects of probucol on atherosclerosis and xanthomas despite its HDL-lowering effects and suggest that inactivation of hepatic ABCA1 leads to increased RCT despite reducing plasma HDL-C levels.

Microvascular Function Predicts Cardiovascular Events in Primary Prevention: Long-Term Results From the Firefighters and Their Endothelium (FATE) Study

Summary: The endothelium plays a prominent role in vascular homeostasis and in health inhibits atherosclerosis formation and its complications. Conduit vessel function can be readily measured noninvasively by assessing flow-mediated dilation after a hyperemic stimulus. Blood flow at the tissue level determines organ viability and is controlled mainly at the microvascular level. Thus, there has been recent interest in measures of microvascular function and its clinical relevance. In the present study, we demonstrated in a large, long-term, prospective cohort of healthy men a number of important and novel findings. First, unlike some previous studies, flow-mediated dilation was not associated with vascular events. However, the hyperemic stimulus was an independent risk marker after Framingham risk score was considered. Second, carotid intima-media thickness was also associated with events, and this association remained after correction for Framingham risk and the hyperemic stimulus. This suggests that measures of both structure and microvascular function are risk predictors for adverse outcomes and provide synergistic information. The present study adds further evidence to the literature that these end points are surrogate measures of atherosclerosis outcomes and can be used in clinical research. Finally, because hyperemic velocity is a readily measured outcome variable, it has the potential to be incorporated into clinical care if further studies support our reported association. This would be most applicable to intermediate-risk individuals in whom additional testing has been advocated for clinical decision making.

Conclusions: In men, hyperemic velocity, the stimulus for flow-mediated dilation, but not flow-mediated dilation itself was a significant risk marker for adverse cardiovascular outcomes. The prognostic value was additive to traditional risk factors and carotid intima-media thickness was also associated with events, and this association remained after correction for Framingham risk and the hyperemic stimulus. This suggests that measures of both structure and microvascular function are risk predictors for adverse outcomes and provide synergistic information. The present study adds further evidence to the literature that these end points are surrogate measures of atherosclerosis outcomes and can be used in clinical research. Finally, because hyperemic velocity is a readily measured outcome variable, it has the potential to be incorporated into clinical care if further studies support our reported association. This would be most applicable to intermediate-risk individuals in whom additional testing has been advocated for clinical decision making.

Targeted Deletions of Cyclooxygenase-2 and Atherogenesis in Mice

Summary: Nonsteroidal anti-inflammatory drugs specific for the inhibition of cyclooxygenase-2 (COX-2) confer a risk of myocardial infarction, stroke, hypertension, and heart failure. Rodent models have established that suppression of COX-2–derived prostacyclin (PGI2) augments thrombogenesis, hypertension, and heart failure. An unanswered question is whether delayed emergence of the nonsteroidal anti-inflammatory drug hazard in chemoprevention trials selected for patients at low initial cardiovascular risk reflects the risk transformation during long-term drug administration. Deletion of the PGI2 receptor fosters initiation and early development of atherosclerosis in mice. Attempts to address this issue with COX-2 knockout mice and inhibitors have yielded conflicting results. This may reflect different times of drug initiation and varied selectivity of the regimens used, restricted utility of the knockouts resulting from the developmental effects of COX-2 deletion, and contrasting effects of COX-2 products formed by different cells during disease evolution. To begin to address the last possibility, we report that although knockout of T-cell COX-2 has no effect, deletion of myeloid COX-2, dominant in macrophages, restrains atherosclerosis. Interestingly, this removes a restraint on enzyme expression in other lesional cells. These are not macrophages and have some hallmarks of vascular smooth muscle cells. If they are, this finding likely explains the impact on atherogenesis because it would shift from proatherogenic COX-2 products of macrophages—thromboxane and prostaglandin E2—to PGI2 formed by vascular smooth muscle cell COX-2. Elucidation of the cell-specific biology of COX-2 deletion may advance the prospect of cell targeted nonsteroidal anti-inflammatory drug delivery.
Conclusions: Macrophage–COX-2, primarily a source of thromboxane A2 and prostaglandin (PG)E2, promotes atherogenesis and exerts a restraint on enzyme expression by lesional cells suggestive of vascular smooth muscle cells, a prominent source of atheroprotective prostacyclin. TC COX-2 does not detectably influence TC development or function or atherogenesis in mice.46

Cholesterol Ester Transfer Protein and Mortality in Patients Undergoing Coronary Angiography: The Ludwigshafen Risk and Cardiovascular Health Study

Summary: Cholesterol ester transfer protein (CETP) is the major player in reverse cholesterol transport, but its role in the development of atherosclerosis continues to be in question since its discovery nearly 20 years ago. After the ahead-of-schedule termination of the large phase III clinical trial Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events (ILLUMINATE) of the CETP inhibitor torcetrapib, the dispute reached a fervent revival. The authors of the ILLUMINATE trial proposed 2 explanations for the higher mortality in the torcetrapib group: a side effect of the drug (eg, increased blood pressure) or suppressed CETP activity per se. In the work submitted herein, we present a large prospective observation on a very similar study population relating variation of CETP mass to mortality. Our data suggest that endogenous low CETP plasma levels constitute an independent risk factor for all-cause and cardiovascular mortality and thus strongly point to the latter explanation for the ILLUMINATE results (ie, CETP inhibition per se causing increased mortality). We believe that our study provides a serious caveat to CETP inhibition in general.

Conclusions: We interpret our data to suggest that low endogenous CETP plasma levels per se are associated with increased cardiovascular and all-cause mortality, challenging the rationale of pharmacological CETP inhibition.47

Association of Physical Activity With Vascular Endothelial Function and Intima-Media Thickness

Summary: The development and progression of subclinical atherosclerosis in adolescence are associated with functional and structural changes of the arteries, including impairment of the arterial vasodilatory function and thickening of the arterial wall. Flow-mediated dilatation of the brachial artery, assessed noninvasively by ultrasound, is a widely used marker of systemic arterial endothelial function. Although the carotid artery has commonly been the target for ultrasonic assessment of early structural changes, the earliest morphological alterations emerge in the abdominal aorta. Intima-media thickness of the abdominal aorta may therefore be a better surrogate marker of atherosclerosis than carotid intima-media thickness especially at young age. Physical activity has a beneficial effect on vascular function and structure by enhancing endothelial function and decreasing the progression of carotid intima-media thickness. To date, data on the effect of physical activity on vascular function and structure in adolescents are scarce and longitudinal studies are lacking. Our study shows the favorable effect of leisure-time physical activity on flow-mediated dilatation and intima-media thickness in nearly 500 adolescents studied repeatedly at 13, 15, and 17 years of age. Importantly, even a moderate increase in physical activity among those who were sedentary was related to a decreased progression of intima-media thickness. These results emphasize the potential of promoting physical activity, especially in inactive adolescents, to support cardiovascular health. Clinicians should encourage physical activity among adolescents, and enjoyable ways to increase physical activity should be paid attention to, especially for those who are sedentary.

Conclusions: Physical activity is favorably associated with endothelial function and intima-media thickness (IMT) in adolescents. Importantly, a moderate increase in physical activity is related to decreased progression of IMT. A physically active lifestyle seems to prevent the development of subclinical atherosclerotic vascular changes in healthy adolescents.48

Nox Activator 1: A Potential Target for Modulation of Vascular Reactive Oxygen Species in Atherosclerotic Arteries

Summary: After decades of investigation, there remains a need for preventive strategies that can reduce atherosclerosis and atherothrombosis, which are the most common causes of death and disability in the United States. Despite many important advances in the treatment of cardiovascular diseases, elucidation of specific therapies that target vascular wall cells has been elusive. An important limitation of targeting intracellular signaling pathways in dysfunctional vascular cells is that most of them are present ubiquitously and are necessary for normal cellular function. Many investigators have studied the hypothesis that regulated production of reactive oxygen species and oxidative stress in vascular cells are important in atherogenesis and that it may be possible to specifically inhibit upregulation of reactive oxygen species production in vascular cells, and in doing so, limit atherogenesis and atherothrombosis. The pathways that produce reactive oxygen species—NADPH oxidase, xanthine oxidase, cyclooxygenase, lipopigase, and others—are necessary, however, for normal cellular function. The experiments described here were designed to test the strategy of targeting a specific component of NADPH oxidase, arguably the most important regulated system for reactive oxygen species production in vascular cells. To do so, we examined modulation of NoxA1, an NADPH oxidase component necessary for upregulation of superoxide production in vascular smooth muscle cells. Our studies indicate that NoxA1 is critically important in the NADPH oxidase-mediated overexpression of reactive oxygen species characteristic of vascular diseases. Although specific inhibitors of NoxA1 are not known, this work suggests that the strategy of cell-specific modulation of NADPH oxidase function is a therapeutic approach worthy of further investigation.

Conclusions: NoxA1 is the functional homolog of p67phox in vascular smooth muscle cells (VSMC) that regulates redox signaling and VSMC phenotype. These findings support the potential for modulation of NoxA1 expression as a viable approach for the treatment of vascular diseases.49

Dynamic Changes in Regulatory T Cells Are Linked to Levels of Diet-Induced Hypercholesterolemia

Summary: CD4+ effector T cells have multiple proinflammatory properties that contribute to the chronic inflammatory phenotype of evolving atherosclerotic lesions, as well as to the destabilization of plaques associated with acute coronary events. Regulatory T cells (Treg) actively suppress T cell–mediated immune responses, and reduced Treg function or numbers are associated with immune-mediated inflammatory disease. The influence of Treg in atherosclerosis has become a central area of interest because of potential therapeutic implications. This study demonstrates that in a mouse model of atherosclerosis, a Treg response is induced by hypercholesterolemia, but the response declines while the effector T cell response is maintained when hypercholesterolemia is prolonged. The decline in the Treg response is associated with selective decrease in homing properties and increased apoptosis of Treg but not Teff during prolonged hypercholesterolemia. The Treg response is sustained by dietary reversal of hypercholesterolemia early after the initial response is induced. Our data suggest that an important therapeutic goal in atherosclerotic patients is to reestablish favorable lesional Treg:Teff ratios, and this may be one of the mechanisms of benefit of profound cholesterol lowering.

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.50
Erythropoietin Suppresses the Formation of Macrophage Foam Cells: Role of Liver X Receptor \(\alpha\)

**Summary:** Atherosclerosis is a major cause of death resulting from cardiovascular diseases. Atherosclerosis starts with an increase in circulating cholesterol levels and subsequently involves a complex cascade of events, including lipid modification, foam cell formation, and recurrent inflammation within the artery wall. Currently, the most widely prescribed medications for treating atherosclerosis are statins, a group of drugs that mainly aim to lower circulating cholesterol levels. Although clinical trials have favorably shown a reduction in atherogenic events by statins, this therapeutic strategy is not optimal. Foam cells derived from macrophages play a critical role in the initiation and progression of atherosclerosis. They not only accumulate lipids but also release inflammatory and chemotactic cytokines as atherogenic factors. Thus, therapeutic approaches for reducing foam cell formation may also represent an important strategy for the prevention and treatment of atherosclerosis. Erythropoietin is known as a glycoprotein hormone that controls erythropoiesis. Erythropoietin has been used as a therapeutic agent to treat anemia in patients with chronic kidney disease or cancer. Although interest in other beneficial functions of erythropoietin has recently emerged, its role in atherogenesis is unknown. This study demonstrates that erythropoietin is increased mainly in the macrophage foam cells present in atherosclerotic lesions. Either treatment with exogenous erythropoietin or overexpression of erythropoietin markedly reduces foam cell formation by increasing the cholesterol efflux from macrophages, suggesting a novel antiatherogenic function of erythropoietin. Our findings may shed new light on the potential therapeutic application of erythropoietin to treat atherosclerosis.

**Conclusion:** Our data suggest that erythropoietin suppresses foam cell formation via the liver X receptor \(\alpha\)--dependent upregulation of ABCA1 and ABCG1.51

Overexpression of Urokinase by Plaque Macrophages Causes Histological Features of Plaque Rupture and Increases Vascular Matrix Metalloproteinase Activity in Aged Apolipoprotein E–Null Mice

**Summary:** Rupture of previously stable atherosclerotic plaques causes unstable angina, myocardial infarctions, and strokes. However, the mechanisms that cause plaque rupture are not yet understood. If these mechanisms could be identified, therapies might be developed that prevent plaque rupture. The most widely held mechanistic hypothesis relative to plaque rupture is that it is caused by increased activity of plaque proteolytic enzymes including urokinase, plasmin, and matrix metalloproteinases. This hypothesis is supported by histological analyses of human plaques that show active proteases in ruptured plaques and by clinical studies that associate higher levels of proteolytic activity (in plasma) with major cardiovascular events. However, these studies do not prove causality, and a large number of animal studies have not revealed a reliable cause-and-effect relationship between plaque proteolytic activity and histological features of plaque rupture. Here we generated a new animal model in which we introduce macrophages that express high levels of urokinase into advanced atherosclerotic plaques in mice. Arteries from these mice have elevated proteolytic activity, including high levels of both plasminogen activator and matrix metalloproteinase activity. Compared with control mice, mice with urokinase-type plasminogen activator–overexpressing macrophages have a significantly higher (61% versus 13%) prevalence of intraplaque hemorrhage as well as a higher prevalence of plaque cap disruption and fibrinogen staining (features found in ruptured human plaques). These data provide experimental support for a mechanistic connection between macrophage urokinase-type plasminogen activator expression, matrix metalloproteinase activity, and plaque rupture. These data also support a causal link between elevated plaque plasminogen activator expression, plaque rupture, and cardiovascular events in humans.

**Conclusions:** In advanced plaques of Apoe\(^{-/-}\) mice, macrophage uPA overexpression causes intraplaque hemorrhage and fibrous cap disruption, features associated with human plaque rupture. uPA overexpression also increases vascular matrix metalloproteinase activity. These data provide a mechanism that connects macrophage uPA expression, matrix metalloproteinase activity, and plaque rupture features in mice. The data also suggest that elevated plaque plasminogen activator expression and plasminogen activation in humans may be causally linked to plaque rupture and cardiovascular events.52

Altered Mitochondrial Dynamics Contributes to Endothelial Dysfunction in Diabetes Mellitus

**Summary:** Type 2 diabetes mellitus is an increasingly prevalent risk factor for atherosclerotic cardiovascular disease, and dysfunction of the vascular endothelium contributes to the development of diabetic vascular disease. We studied a previously unrecognized mechanism of endothelial dysfunction in human diabetes mellitus and show that an alteration in mitochondrial homeostasis is important. In addition to serving as the primary source of ATP in the cell, mitochondria participate in many other cellular functions. In the past, mitochondrial were viewed as discrete oval organelles, but recent studies have shown that mitochondrial fuse to form complex networks within the cell and that these networks are important for normal mitochondrial function. We observed that endothelial cells collected from patients with diabetes mellitus show a loss of normal networks and marked fragmentation of mitochondria. These changes were accompanied by increased levels of Fis1, a protein that controls mitochondrial fission. When commercially available endothelial cells were exposed to high glucose concentrations in tissue culture, we observed a similar loss of mitochondrial networks, increased Fis1, and impaired endothelial function. When we prevent mitochondrial fragmentation by blocking expression of Fis1, we maintain normal mitochondrial networks and prevent endothelial dysfunction. This study suggests a new target for therapy in diabetes mellitus and raises the possibility that a drug that prevents mitochondrial fission might protect against the development of vascular disease in diabetic patients. Such drugs are currently under development.

**Conclusion:** These findings implicate increased mitochondrial fission as a contributing mechanism for endothelial dysfunction in diabetic states.53

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