Neutrophil-Derived Matrix Metalloproteinase 9 Triggers Acute Aortic Dissection

Summary—Acute aortic dissection (AAD) is a potentially fatal vascular disease, and prompt diagnosis and treatment by timely surgery are required for survival of the patients. No efficient biomarkers are available for diagnosis of AAD prior to determination of the disease by computed tomography. Medial degeneration is known as an important risk factor for the development of AAD; however, the emergent nature of the disease and the paucity of animal models prevent us from studying the molecular mechanisms for triggering the disease. We found that matrix metalloproteinase 9 (MMP9) and angiotensin II were increased significantly in blood samples from AAD patients compared with those from normal subjects and the patients with nonruptured aortic aneurysm. This was accompanied by enhanced infiltrations of MMP9-producing neutrophils in the dissected aortas. Based on the data, we established a mouse model of AAD, which was induced by infusion of angiotensin II to mice pretreated with β-aminopropionitrile monofumarate (a lysyl oxidase inhibitor). All mice exhibited AAD within 24 hours after angiotensin II infusion. Aortic tissue from the AAD mice showed enhanced expression and activity of MMP9, and MMP9-immunoreactive neutrophils were infiltrated in both dissected media and intima of nondissected lesions. Genetic depletion or pharmaceutical inhibition of MMP9 and neutrophil ablation attenuated the AAD incidence. These data demonstrate that neutrophil-derived MMP9 is responsible for triggering AAD in this model. Taken together, MMP9 could serve as a potential biomarker for diagnostic screening of AAD, and administration of angiotensin II receptor blockers or MMP9 inhibitors could be effective therapeutic approaches to AAD.

Conclusions—These data suggest that AAD is initiated by neutrophils that have infiltrated the aortic intima and released MMP9 in response to angiotensin II.

Association Between 2 Angiographic Subtypes of Renal Artery Fibromuscular Dysplasia and Clinical Characteristics

Summary—Fibromuscular dysplasia is a heterogeneous group of idiopathic, noninflammatory, and nonatherosclerotic stenosing vascular diseases mostly involving renal and cervical arteries. It is the second most frequent cause of renovascular hypertension. Its historical classification based on histology is no longer relevant now that percutaneous revascularization has replaced surgery in most cases. In this study, we describe an angiographic classification of renal artery fibromuscular dysplasia lesions into a unifocal and a multifocal subtype. Fewer patients have unifocal lesions (18% of all patients with renal artery fibromuscular dysplasia), and their characteristics contrast with those of patients with multifocal lesions: They are younger at diagnosis (30 versus 49 years); the proportion of women is lower (69% versus 83%); they are more often current smokers (50% versus 26%); with higher blood pressure levels (157/97 versus 146/88 mm Hg); the disease is more often unilateral (79% versus 38%); they are more amenable to revascularization (90% versus 35%); and have a higher cure rate when revascularization is performed (54% versus 26%).

Conclusions—A binary angiographic classification into unifocal or multifocal renal artery fibromuscular dysplasia is straightforward and discriminates 2 groups of patients with different clinical phenotypes.2

GRK2-Mediated Inhibition of Adrenergic and Dopaminergic Signaling in Right Ventricular Hypertrophy: Therapeutic Implications in Pulmonary Hypertension

Summary—Right ventricular (RV) failure in pulmonary arterial hypertension is associated with adrenergic activation. Clinicians are often confronted with two questions: (1) Which is the optimal inotrope in RV failure? (2) Is there a long-term role for modulating the adrenergic system? In left ventricular failure, G protein–coupled receptor kinase-2 (GRK2) mediates adrenergic receptor downregulation/desensitization, and GRK2 inhibitors improve adrenergic signaling and function. We explored the molecular basis and therapeutic relevance of adrenergic abnormalities in RV failure and RV hypertrophy (RVH). Using human tissues and rodent models (of maladaptive and adaptive-RVH), we show that RVH results in downregulation of α1- and β1-adrenoreceptors and dopamine receptors. These changes are confined to the RV in adaptive RVH, but in the more clinically relevant maladaptive models, the receptor downregulation also involves the left ventricle. Receptor downregulation is functionally important, reducing inotropic reserve. The basis for the adrenergic changes in RVH is activation of GRK2, and disrupting the interaction between Gβγ–GRK2 in vivo (using gallein) is beneficial, improving cardiac function.

Conclusions—Receptor desensitization and G protein–coupled receptor (GPCR) alterations are adaptive mechanisms that provide inotropic support in early RV hypertrophy. However, when the adaptive response becomes maladaptive, there is widespread cardiac inactivation, which is associated with progressive RV failure. Inhibiting GRK2 kinase activity, with pharmacological and genetically encoded inhibitors, ameliorates inactivation and improves function. These data support the concept that receptor downregulation is a fundamental mechanism of RVH and a major determinant of clinical severity and outcome, and GRK2 is a molecular target that may provide a viable therapeutic strategy for RVH.
output and exercise tolerance. The comparison of dobutamine and dopamine showed better efficacy for dobutamine in all models. This largely reflects its superior coupling to adenyl cyclase. In addition, we discovered a new role for the D1-dopamine receptor in RV contractile reserve. In RVH, dopamine interacts with this receptor to augment contractility, and its loss contributes to the inferior performance of dopamine. We conclude that adrenergic remodeling in the RV is worse in maladaptive RVH, is mediated by GRK2, and contributes to RV failure. Adrenergic signaling and interactions between Gβγ–GRK2 are promising therapeutic targets.

Conclusions—GRK2-mediated desensitization-downregulation of adrenergic and dopaminergic receptors impairs inotropic reserve in PAH-RVH. Acute inotropic support in RVH is best accomplished by dobutamine, reflecting its better coupling to adenyl cyclase and the reliance of dopamine on dopamine-1-receptor signaling, which is impaired in RVH. Inhibiting Gβγ–GRK2 interactions has therapeutic benefit in RVH.

**Effects of Interleukin-1β Inhibition With Canakinumab on Hemoglobin A1c, Lipids, C-Reactive Protein, Interleukin-6, and Fibrinogen: A Phase IIb Randomized, Placebo-Controlled Trial**

Summary—Atherosclerosis in an inflammatory condition, and biomarkers of inflammation including CRP, IL-6, and fibrinogen associate with increased vascular risk. However, whether inhibiting inflammation will reduce vascular events is uncertain. One promising anti-inflammatory approach with potential relevance for cardiovascular disease is inhibition of the proinflammatory cytokine IL-1β, particularly the IL-1β isoform that is secreted and acts locally but that also induces systemic effects. In a phase IIb randomized trial conducted among high-risk diabetic patients comparing placebo with canakinumab, a monoclonal antibody targeting IL-1β, we observed statistically significant dose-dependent reductions in all 3 of these inflammatory biomarkers without major effect on LDL-C or HDL-C. There were no differences in clinical adverse events between active and placebo patients, although a small increase in triglycerides was observed at higher canakinumab doses. These phase II trial data support the use of canakinumab as a potential therapeutic method to test directly the inflammatory hypothesis of atherosclerosis.

Conclusions—Canakinumab, a human monoclonal antibody that neutralizes interleukin-1β, significantly reduces inflammation without major effect on low-density lipoprotein cholesterol or high-density lipoprotein cholesterol. These phase II trial data support the use of canakinumab as a potential therapeutic method to test directly the inflammatory hypothesis of atherosclerosis.

**Effective Treatment of Edema and Endothelial Barrier Dysfunction With Imatinib**

Summary—Endothelial barrier dysfunction is a major contributor to morbidity and mortality in the critically ill. Loss of the endothelial barrier follows exposure of the endothelium to inflammatory mediators and drives vascular leakage and edema formation. To date endothelial barrier function and vascular leakage still lack appropriate therapy. This study shows that imatinib—an US Food and Drug Administration–approved tyrosine kinase inhibitor—directly protects the endothelial barrier under inflammatory conditions. With the use of endothelial cells isolated from various vascular beds, it was shown that imatinib attenuates the loss of endothelial barrier on stimulation with inflammatory mediators. Imatinib protects against endothelial barrier dysfunction predominantly by inhibition of the tyrosine kinase Abl-related gene (Arg), a novel mediator of endothelial barrier disruption. The effect of imatinib on endothelial barrier was established in various mouse models of vascular leakage. Notably, imatinib attenuated vascular leakage in a murine model of sepsis, even when imatinib treatment was initiated considerable time after induction of sepsis. This study carries important clinical implications. First, imatinib may form a suitable therapy for treatment of diseases characterized by vascular leakage. The longstanding experience with imatinib, together with the fact that imatinib concentrations used in this study parallel plasma values in cancer patients, are apparent advantages in this case. Logical first steps in further development of imatinib involve Phase I and II trials to evaluate safety and efficacy of imatinib in patients with profound vascular leakage. Second, the identification of Arg as a novel and druggable target opens perspectives for more specific pharmaceutical interventions.

Conclusions—Thus, imatinib prevents endothelial barrier dysfunction and edema formation via inhibition of Arg. These findings identify imatinib as a promising approach to permeability edema and indicate Arg as novel target for edema treatment.

**Exosomes Mediate the Cytoprotective Action of Mesenchymal Stromal Cells on Hypoxia-Induced Pulmonary Hypertension**

Summary—Pulmonary arterial hypertension remains without cure despite significant progress in our understanding of its pathophysiology. Given the complex molecular and cellular pathways underlying the development of pulmonary arterial hypertension, therapies aimed at multiple pathways and cellular targets may prove to be more efficacious. Stem cell–based therapies hold such a promise because they can simultaneously target diverse signaling pathways and have long-lasting effects. Accumulating studies support an important cytoprotective, antifibrotic role for mesenchymal stem cells with demonstrated efficacy against pulmonary hypertension in animal models of disease. We previously reported both prevention and reversal of severe pulmonary hypertension and right heart failure in a mouse model of hypoxia-induced pulmonary hypertension and have postulated a paracrine mode of mesenchymal stem cell protective functions. In this report, we show that mesenchymal stem cells secrete microvesicles (exosomes), which are the vectors of their action, being both necessary and sufficient to confer cytoprotection on the lung vasculature. We show that, potentially through epigenetic mechanisms involving microRNA signaling, mesenchymal stromal cell exosome preparations can have long-lasting therapeutic effects on pulmonary hypertension. These findings may lead to the development of alternative strategies in the field of stem cell–based therapies, at least for certain lung diseases, in that delivery of in vitro purified exosomes could substitute for the delivery of intact cells. Exosome treatment, in addition to its enhanced practicality in terms of storage and administration, does not carry the danger of oncogenic potential of donor cells, an important consideration in all stem cell–based therapies.

Conclusions—This study indicates that mesenchymal stromal cell–derived exosomes exert a pleiotropic protective effect on the lung and inhibit pulmonary hypertension through suppression of hyperproliferative pathways, including signal transducer and activator of transcription 3-mediated signaling induced by hypoxia.
Oxysterol-Induced Soluble Endoglin Release and Its Involvement in Hypertension

Summary—Preeclampsia is one of the most severe complications of pregnancy. Characterized by systemic hypertension, proteinuria, and edema in the third trimester of pregnancy, preeclampsia is responsible for the highest rates of morbidity and mortality for both pregnant women and neonates in the developed world. Treatment of hypertension in preeclampsia is especially needed because of the absence of effective therapies except for the delivery of the baby and placenta. Hypoxia in the placenta is considered a key event in the pathogenesis of preeclampsia, whereas soluble endoglin (sEng) is a prognostic marker and plays a pathogenic role. In this article, we report that the hypoxia-dependent cholesterol derivatives oxysterols, via the liver X receptors, are able to increase sEng levels in vitro and in vivo by a mechanism involving activation of matrix metalloproteinase-14. Interestingly, mice treated with oxysterols or liver X receptor agonists underwent an increase in plasmatic sEng levels and an augmentation of arterial pressure. In addition, administration of an endoglin fragment containing the matrix metalloproteinase-14 cleavage site prevented the oxysterol-dependent increase in arterial pressure and sEng levels in mice. These data reveal for the first time the involvement of the liver X receptor pathway in sEng release and its contribution to vascular homeostasis. They also suggest that administration of endoglin peptides in preeclamptic women might serve to counteract the pathogenic effect of the elevated circulating sEng. Further studies are needed to confirm the beneficial effect of these peptides in experimental models of preeclampsia and in clinical trials.

Conclusions—These studies provide a clue to the involvement of the liver X receptor pathway in sEng release and its pathogenic role in vascular disorders such as preeclampsia.7

Low-Density Lipoprotein Cholesterol–Lowering Effects of AMG 145, a Monoclonal Antibody to Proprotein Convertase Subtilisin/Kexin Type 9 Serine Protease in Patients With Heterozygous Familial Hypercholesterolemia: The Reduction of LDL-C With PCSK9 Inhibition in Heterozygous Familial Hypercholesterolemia Disorder (RUTHERFORD) Randomized Trial

Summary—Heterozygous familial hypercholesterolemia, a dominant genetic disorder found in 0.2% of the population, results in early coronary artery disease. Current lipid-lowering therapy fails to achieve optimal low-density lipoprotein cholesterol (LDL-C) goals in many patients. We evaluated AMG 145, a fully human monoclonal antibody against PCSK9, to reduce LDL-C in heterozygous familial hypercholesterolemia patients already on maximally tolerated lipid-lowering therapy and LDL-C >2.6 mmol/L (100 mg/dL). The phase 2, double-blind, placebo-controlled trial randomized 168 patients (56 placebo, 56 AMG 145 350 mg, 56 AMG 145 420 mg) to every 4 weeks subcutaneous injections for 12 weeks. The primary endpoint was percentage change in LDL-C, measured by ultracentrifugation, from baseline at Week 12. Safety assessments included adverse events and laboratory tests. LDL-C reduction (least squares mean [standard error]) was 43 (3)% and 55 (3)% for AMG 145 350 mg and 420 mg, respectively, compared with 1 (3)% increase with placebo (P < 0.001 for both dose groups). AMG 145 420 mg every 4 weeks resulted in 89% of patients reaching LDL-C levels of <2.6 mmol/L (100 mg/dL) and 65% achieving <1.8 mmol/L (70 mg/dL), respectively, compared with 2% and 0% of placebo subjects, respectively. There was a significant dose-dependent reduction in lipoprotein (a) with AMG 145 therapy of 23% and 32% compared with placebo. Serious adverse events (not considered treatment-related) occurred in 2 patients on AMG 145. We conclude that AMG 145 administered every 4 weeks yields rapid and substantial reductions in LDL-C with minimal adverse events and good tolerability in patients with heterozygous familial hypercholesterolemia and elevated LDL-C despite intensive lipid-lowering therapy.

Conclusions—AMG 145 administered every 4 weeks yielded rapid and substantial reductions in LDL-C in heterozygous familial hypercholesterolemia patients despite intensive statin use, with or without ezetimibe, with minimal adverse events and good tolerability.8

Ribosomal Protein L17, RpL17, is an Inhibitor of Vascular Smooth Muscle Growth and Carotid Intima Formation

Summary—Carotid intima-media thickening is a highly predictive risk factor for cardiovascular events including myocardial infarction and stroke. Genetic models have been useful in determining causative genes that regulate carotid intima-media thickening and atherosclerosis, although much has yet to be discovered. Our genetic and bioinformatic study provides new insights into genetic pathways and a target gene, ribosomal protein L17 (RpL17), which regulates carotid intima formation. Ribosomal proteins can demonstrate extraribosomal roles outside the translational machinery to control cell growth. In diseases such as Diamond Blackfan anemia and colorectal and gastric cancers, as well, deficiency in specific ribosomal proteins leads to malignancy. Currently, ribosomal proteins are being considered for potential therapeutic strategies to limit uncontrolled cell growth and reduce tumor size. A major contributing factor to vascular remodeling associated with intima-media thickening is increased proliferation of vascular smooth muscle cells. Thus, targeting RpL17 expression in the vascular wall may have therapeutic implications for limiting intimal hyperplasia and potential applications in the study of angiogenesis and tumor biology.

Conclusions—RpL17 acts as a vascular smooth muscle cell growth inhibitor (akin to a tumor suppressor) and represents a potential therapeutic target to limit carotid intima-media thickening.9

Endogenous and Natural Complement Inhibitor Attenuates Myocardial Injury and Arterial Thrombogenesis

Summary—Reperfusion of ischemic tissues induces tissue injury that is mediated by complement activation. We have identified a novel, endogenous, natural complement inhibitor that displaces the 3 serine proteases (ie, mannose-binding lectin/ficolin-associated serine protease-1, -2, and -3) from the mannose-binding lectin complex in a dose-dependent manner. Furthermore, at pharmacologic concentrations, mannose-binding lectin-associated protein-1 prevents arterial thrombogenesis, as well as myocardial injury after ischemia and reperfusion in vivo. The mannose-binding lectin complex has been associated with several clinical diseases, and mannose-binding lectin-associated protein-1 may represent a novel molecular mechanism to modulate its activity in vivo.

Conclusions—Our results suggest that the natural, endogenous inhibitor MAP-1 effectively inhibits lectin pathway activation in vivo. MAP-1 at pharmacological doses represents a novel therapeutic approach for human diseases involving the lectin pathway and its associated MASPs.10

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

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

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

Rapid Estrogen Receptor Signaling Is Essential for the Protective Effects of Estrogen Against Vascular Injury

**Summary**—Clinical studies have shown that estrogen therapy in postmenopausal women has the potential to exert both beneficial and harmful cardiovascular effects. Therefore, better understanding of the molecular mechanisms by which estrogen exerts its cardiovascular effects could lead to the development of new therapeutic agents that provide the beneficial effects of estrogen on the cardiovascular system without concomitant harmful effects. Estrogen signals through estrogen receptors (ERs) via 2 major signaling pathways: (1) a classic genomic pathway in which ERs directly regulate gene expression (genomic signaling) and (2) a rapid, nonnuclear pathway in which ERs activate specific kinase cascades at the cell membrane. In this article, we demonstrate that this rapid, nonnuclear signaling pathway is required for estrogen-mediated inhibition of vascular smooth muscle cell proliferation, both in vitro and in vivo. We show further that estrogen-mediated protection against vascular injury also requires rapid ER signaling in an in vivo mouse model. These findings support the concept that development of therapeutic agents that selectively activate rapid, nonnuclear ER signaling, without activating genomic signaling, may represent a novel therapeutic approach to prevent cardiovascular disease.

**Conclusions**—Taken together, these results support the concept that rapid, nonnuclear ER signaling contributes to the transcriptional regulatory functions of ER and is essential for many of the vasoprotective effects of estrogen. These findings also identify the rapid ER signaling pathway as a potential target for the development of novel therapeutic agents.

Dietary Nitrate Supplementation Improves Revascularization in Chronic Ischemia

**Summary**—With the worldwide increase in cardiovascular diseases in recent decades, the need for novel preventive and noninvasive therapeutic strategies has grown tremendously. In this context, there is accumulating evidence that inorganic nitrate from dietary sources is able to influence the hallmarks of cardiovascular functions, including blood pressure regulation. The bioactivation of nitrate from dietary or endogenous sources is carried out mainly by commensal bacteria that express effective nitrate reductase enzymes and are located in the gastrointestinal tract and on body surfaces. Under conditions of low oxygen tensions, nitrate and nitrite are physiologically recycled in blood and tissues to form nitric oxide and other bioactive nitrogen oxides that mediate cytoprotective signaling in the setting of pathological ischemia. The present study provides the first evidence that dietary nitrate supplementation improves revascularization in chronic ischemia. This study identified that dietary nitrate supplementation increases mobilization and migration of regenerative cells, improves the regenerative capacities of chronically ischemic tissue, and decreases apoptosis at the site of ischemia. Eradicating the commensal bacteria in the oral cavity and thus interrupting the bioactivation of the ingested nitrate decreased circulating levels of bioactive nitrogen oxides and reversed all of these beneficial effects. These data underscore the potential therapeutic value of inorganic nitrate and suggest the possible application of a nutritional approach in the prevention and treatment of cardiovascular diseases.

**Conclusions**—Long-term dietary nitrate supplementation may represent a novel nutrition-based strategy to enhance ischemia-induced revascularization.

Effect of 9p21.3 Coronary Artery Disease Locus Neighboring Genes on Atherosclerosis in Mice

**Summary**—Atherosclerotic coronary artery disease (CAD) is the leading cause of death in the developed world. Epidemiological data show that environmental and genetic factors have roughly equal weight in determining the susceptibility to CAD and the rate of disease progression, although the genes that control this variability are not well characterized. Recently, large genome-wide association studies have identified >30 CAD risk loci. Some of the CAD risk loci work through known CAD risk factors such as lipids; however, this is not true for the majority of loci. This study systematically dissected the highly replicated 9p21.3 locus using different knock-out mice models of the neighboring genes, including CDKN2A, CDKN2B, and MTAP. We describe the complex molecular regulation within the region and show that MTAP affects the progression of atherosclerosis through several potential mechanisms including lymphocyte activation, and changes in metabolic and methylation profiles. This study is one of the first to show a change in atherosclerosis phenotype independent of lipids in a mice model generated as a follow-up of new locus identified from genome-wide association studies. This discovery suggests a novel pathway for atherosclerosis and hence opens the door to develop new markers of predicting the risk of atherosclerosis at the genomic, epigenomic, and metabolomic levels, and eventually new strategies for primary and secondary prevention of coronary artery disease.
Conclusions—Mtap plays a protective role against atherosclerosis, whereas Cdkn2a appears to be modestly proatherogenic. However, no relation was found between the 9p21 genotype and the transcription of 9p21 neighboring genes in primary human aortic vascular cells in vitro. There is extensive compensatory regulation in the highly conserved 9p21 orthologous region in mice.14

Role of BMPR2 Alternative Splicing in Heritable Pulmonary Arterial Hypertension Penetrance

Summary—One of the most perplexing features of heritable pulmonary arterial hypertension is its reduced penetrance. Nearly 80% of mutation carriers have no clinical symptoms, but they can produce offspring who are affected by heritable pulmonary arterial hypertension. Thus, disease development cannot be predicted in a mutation carrier, creating anxiety in the patient and uncertainty about treatment in the physician. The data presented in this article point to a novel explanation for the reduced penetrance seen in heritable pulmonary arterial hypertension. Our data show that BMPR2 alternative splicing plays a role in this reduced penetrance. BMPR2 mutation carriers were more likely to have pulmonary arterial hypertension if they had higher levels of an alternatively spliced BMPR2 transcript, isoform-B, relative to the full-length BMPR2 transcript. Thus, our data suggest that although a BMPR2 mutation creates baseline susceptibility, an important determinant of disease penetrance appears to be the higher relative expression of the alternative splicing BMPR2 isoform-B. These data emphasize the importance of BMPR2 alternative splicing in heritable pulmonary arterial hypertension and raise an intriguing question: Can a predictive model of disease based on expression levels of the BMPR2 alternative splicing be developed? This predictive model will have obvious clinical utility in predicting which mutation carriers may eventually develop disease and thus will require appropriate follow-up. Because splicing can be manipulated in vivo by drugs, our findings suggest that manipulation of BMPR2 alternative splicing should be explored as a potential new intervention in heritable pulmonary arterial hypertension.

Conclusions—Alternations in BMPR2 isoform ratios may provide an explanation of the reduced penetrance among BMPR2 mutation carriers. This ratio is controlled by an exonic splice enhancer in exon 12 and its associated splicing factor, SRSF2.15

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

Summary—Atherosclerosis is the major cause of death in Westernized societies, being the underlying cause of severe cardiovascular diseases such as heart attack and stroke. Toll-like receptor (TLR) signaling has been implicated as a critical pathogenic factor in atherosclerosis and has attracted increased interest as a potential therapeutic target for the treatment of atherosclerosis. Our in vivo studies showed that TLR signaling exerts surprisingly diverse functions in endothelial cells and macrophages differentially affecting the development of atherosclerosis. Endothelial cell–specific inhibition of TLR signaling reduced atherosclerosis severity in female mice by inhibiting nuclear factor-κB activation and the expression of proinflammatory mediators by the vascular endothelium in response to oxidized lipids, resulting in less efficient recruitment of monocytes into the developing plaques. In contrast, myeloid cell–specific inhibition of TLR signaling surprisingly resulted in more severe atherosclerosis by compromising the antiinflammatory properties, endoplasmic reticulum stress survival responses, and efferocytosis capacity of macrophages. Collectively, these results revealed a previously unappreciated functional diversity of TLR signaling in 2 cell types that are critical for the development of atherosclerosis. In response to modified lipids, TLR signaling exerts proatherogenic functions in endothelial cells in female mice by inducing the expression of proinflammatory mediators facilitating monocyte recruitment. In contrast, TLR signaling in macrophages is atheroprotective by inducing interleukin-10 expression, suppressing endoplasmic reticulum stress–induced macrophage death, and stimulating efferocytosis. These findings urge caution in the systemic application of TLR signaling inhibitors in atherosclerosis and suggest that strategies specifically targeting endothelial cells would have a greater therapeutic potential.

Conclusions—Toll-like receptor–mediated TRAF6 signaling acts in endothelial cells to promote atherosclerosis but displays atheroprotective, antiinflammatory and prosurvival functions in myeloid cells.16

Periprocedural Heparin Bridging in Patients Receiving Vitamin K Antagonists: Systematic Review and Meta-Analysis of Bleeding and Thromboembolic Rates

Summary—Periprocedural heparin bridging with low-molecular-weight heparin (LMWH) in patients on long-term warfarin aims to reduce the risk of thromboembolic events in the immediate periprocedural period. However, although periprocedural anticoagulation remains a common clinical problem, optimal methods have not been established. Recently published international guidelines on antithrombotic therapy recommend an individualized approach to determining the need for bridging anticoagulation based on a patient’s estimated thromboembolic risk and periprocedural bleed risk. Using established methods, we conducted a systematic review and meta-analysis of 34 studies (including 1 randomized trial) that used low-molecular-weight heparin as periprocedural bridging therapy. We used low-thromboembolic-risk groups who did not receive bridging therapy or patients who were not on warfarin for comparators to assess baseline periprocedural thromboembolic and bleed risks. We found a >5-fold increased risk of overall bleeding and >3-fold increased risk of major bleeding associated with the use of bridging therapy, with a similar risk of thromboembolism (including arterial thromboembolism) in bridged and nonbridged patients. There was also an increased risk of overall bleeding when full and prophylactic or intermediate doses of low-molecular-weight heparin bridging were compared. In studies that stratified procedural bleed risk, bleed rates were highest in mostly bridged patients undergoing high-bleed-risk procedures. We concluded that patients on long-term warfarin should avoid routine periprocedural bridging with low-molecular-weight heparin, especially patients not at high thromboembolic risk using therapeutic doses of bridging therapy and undergoing high-bleed-risk procedures. Randomized trials are urgently needed to define the role of periprocedural heparin bridging.

Conclusions—Vitamin K antagonist–treated patients receiving periprocedural heparin bridging appear to be at increased risk of overall and major bleeding and at similar risk of thromboembolic events compared to nonbridged patients. Randomized trials are needed to define the role of periprocedural heparin bridging.17
**Cytokine Therapy With Interleukin-2/ Anti–Interleukin-2 Monoclonal Antibody Complexes Expands CD4+CD25+Foxp3+ Regulatory T Cells and Attenuates Development and Progression of Atherosclerosis**

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

Conclusions—IL-2/anti–IL-2 mAb treatment in vivo attenuates atherosclerosis via selective Treg expansion. The findings suggest that cytokine-based IL-2/anti–IL-2 mAb complex therapy could represent an attractive approach for treating atherosclerosis, because it markedly attenuates progression as well as development, by modulating its immunoinflammatory component.18

**Endothelium-Selective Activation of AMPK Activated Protein Kinase Prevents Diabetes Mellitus–Induced Impairment in Vascular Function and Reendothelialization via Induction of Heme Oxygenase-1 in Mice**

Summary—Diabetes mellitus is closely associated with accelerated atherosclerosis and an increased risk of cardiovascular disease. Endothelial dysfunction, which is caused in part by impaired endothelium-reparative capacity of endothelial progenitor cells (EPCs), is an early event in diabetes mellitus and in the pathogenesis of atherosclerosis. Therefore, therapeutic interventions targeting endothelial dysfunction hold great promise for the treatment of cardiovascular complications in diabetic patients. Emerging evidence from animal and clinical studies suggests that AMPK-activated protein kinase (AMPK), a master regulator of energy homeostasis, exhibits pleiotropic beneficial effects on both endothelial cells and EPCs. A number of drugs with antidiabetic or cardiovascular protective properties, including metformin, statins, thiazolidinediones, fenofibrate, and estradiol, have been shown to activate AMPK in endothelial cells or EPCs. However, because of the ubiquitous expression pattern of AMPK, it is currently unclear whether the protective effects of these agents against endothelial dysfunction are a direct consequence of AMPK activation in endothelial cells or EPCs or an indirect effect related to the improvement in insulin sensitivity and glycemic control. In the present study, we generated a transgenic mouse model with selective activation of AMPK in both endothelial cells and EPCs and demonstrated that these transgenic mice are resistant to diabetes mellitus–induced impairment in endothelial function and reparative neovascularization. Furthermore, we showed that endothelial AMPK promotes reendothelialization of injured blood vessels by activation of heme oxygenase-1, another potential therapeutic target for cardiovascular disease. These findings suggest that selective therapeutic activation of AMPK in endothelial cells or EPCs is sufficient to prevent the vascular complications of diabetes mellitus.

Conclusions—Endothelium-specific AMPK activation is sufficient to protect against diabetes mellitus–induced aggravation of vascular injury by promoting EPC function and reendothelialization via upregulation of heme oxygenase-1 and SDF-1α.19

**Bionic Baroreceptor Corrects Postural Hypotension in Rats With Impaired Baroreceptor**

Summary—Orthostatic hypotension is one of the most common medical problems. The prevalence of orthostatic hypotension increases with age and reaches 20% in those older than 65 years. Similarly, baroreceptor sensitivity degenerates with aging and atherosclerosis. Because arterial baroreflex dysfunction encompasses orthostatic intolerance, it is reasonable to consider that an impaired baroreceptor plays a pivotal role in orthostatic hypotension in most elderly patients. However, there is no available treatment for baroreceptor impairment. In the present study, we developed a bionic baroreceptor that consists of a pressure sensor, a regulator, and a neurostimulator. The bionic regulator translates the aortic pressure into electric stimulation of the aortic depressor nerve in real time as if it were the native baroreceptor. We demonstrated that a bionic baroreceptor system restores the pressure buffering function against head-up tilt–induced hypotension in rats without baroreflex function as well as the native baroreflex. This study proposes that application of a bionic baroreceptor could become a novel therapeutic tool for patients with orthostatic hypotension caused by baroreceptor impairment. In addition, our bionic baroreceptor theoretically enables bidirectional arterial pressure regulation. Therefore, this system will be specifically beneficial for patients with coexistent supine hypotension and orthostatic hypotension, which often causes a particularly difficult therapeutic dilemma.

Conclusions—The BBR restores the pressure buffering function. Although this research demonstrated feasibility of the BBR, further research is needed to verify its long-term effect and safety in larger animal models and humans.20

**Impaired Natural Killer Cell Phenotype and Function in Idiopathic and Heritable Pulmonary Arterial Hypertension**

Summary—Pulmonary arterial hypertension (PAH) is a disease of occlusive vascular remodeling that leads to elevated pulmonary arterial pressure and death from right heart failure. An association between PAH and immune dysfunction has been recognized for decades, although the cellular basis for this link has remained obscure. In the current study, we identify a defect in the circulating natural killer (NK) cells of PAH patients and 2 widely used animal models of disease. Beyond their traditional function as the cytotoxic effector cells of innate immunity, several studies have recently demonstrated
a role for NK cells in the regulation of spiral artery remodeling during pregnancy, tumor angiogenesis and the revascularization of ischemic limbs. By drawing upon recent literature characterizing the impairment of NK cells in HIV infection, we identify an altered NK cell phenotype in PAH patients that is exemplified by decreased surface expression of certain killer immunoglobulin-like receptors, diminished cytotoxicity, reduced cytokine secretion, and the increased production of matrix-degrading enzymes, including matrix metalloproteinase 9. We also propose a mechanism whereby this NK cell impairment is the result of excessive transforming growth factor-β signaling. Our work is the first to propose a direct role for NK cells in the regulation of pulmonary vascular remodeling. This discovery, which is based on emerging concepts from reproductive medicine and immunology, not only highlights the importance of cellular immunity in the pathobiology of PAH, but also provides a potential therapeutic target for the design of new PAH treatments.

Conclusions—Our work is the first to identify an impairment of NK cells in PAH and suggests a novel and substantive role for innate immunity in the pathobiology of this disease.

Activity of the Estrogen-Metabolizing Enzyme Cytochrome P450 1B1 Influences the Development of Pulmonary Arterial Hypertension

Summary—Pulmonary arterial hypertension (PAH) is a fatal condition with diverse origins that converge to promote pathological changes in the pulmonary vasculature. The nature of these origins is intriguing and stems from genetic and environmental factors to secondary risk factor–related disease. Estrogen is one such risk factor that has been causally related to PAH; however, the causation remains obscure. In this study, we have identified that the estrogen-metabolizing enzyme cytochrome P450 1B1 (CYP1B1) controls the formation of estrogen-derived mitogenic metabolites to drive vascular cell mitogenesis and PAH. Central to this, we report that CYP1B1 is robustly upregulated in 2 independent forms of clinical PAH, whereas the inhibition of this enzyme markedly inhibits the proliferative capacity of pulmonary artery smooth muscle cells isolated from PAH patients. In vivo, CYP1B1 is upregulated in 2 independent models of PAH, whereas the genetic ablation or pharmacological inhibition of CYP1B1 markedly attenuates the development of PAH, as reported in CYP1B1−/− and 2,3′,4,5′-tetramethoxystilbene–treated mice, respectively. Further investigation of estrogen metabolites reveals that 16α-hydroxyestrone is a key metabolite that robustly stimulates smooth muscle mitogenesis and promotes the development of PAH in mice in vivo. This study reinforces a pathogenic role of estrogen in human PAH and reveals for the first time the importance of estrogen metabolism in the genesis and progression of PAH.

Conclusions—Increased CYP1B1-mediated estrogen metabolism promotes the development of PAH, likely via the formation of mitogens, including 16α-hydroxyestrone. Collectively, this study reveals a possible novel therapeutic target in clinical PAH.

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

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

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

Aldosterone Inactivates the Endothelin-B Receptor via a Cysteiny1 Thiol Redox Switch to Decrease Pulmonary Endothelial Nitric Oxide Levels and Modulate Pulmonary Arterial Hypertension

Summary—Despite recent advances in diagnosis and treatment, pulmonary arterial hypertension (PAH) remains a devastating disease that is associated with a 10% mortality rate within the first year of diagnosis. Currently available pharmacotherapies based on known biological mediators of the disease are limited and, in certain cases, have waning long-term efficacy. In this study, we identify aldosterone as a novel contributor to the pathobiology of PAH and demonstrate that mineralocorticoid receptor antagonism is efficacious in the prevention and reversal of experimental PAH. We describe a novel mechanism for the increase in pulmonary aldosterone levels whereby elevated levels of endothelin-1, which have been observed in PAH, function as a potent stimulator of adrenal and extra-adrenal aldosterone synthesis to modulate pulmonary vascular dysfunction. Our findings demonstrate that aldosterone-induced oxidant stress impairs endothelin-B receptor signal transduction to diminish endothelin-B–dependent nitric oxide synthesis in pulmonary artery endothelial cells in vitro and promote negative remodeling of pulmonary arteries and pulmonary vascular dysfunction in 2 experimental rat models of PAH in vivo. Importantly, mineralocorticoid receptor antagonism with spironolactone or eplerenone prevented or reversed the adverse effects of hyperaldosteronism on pulmonary vascular remodeling and improved pulmonary vascular resistance, pulmonary artery pressure, and remodeling of the right ventricle. Moreover, our findings relating to the potential benefit of spironolactone or eplerenone in attenuating pulmonary vascular dysfunction in PAH may support future clinical
trials and/or repurposing of mineralocorticoid receptor antagonists, which are already an accepted medical therapy in patients with certain cardiovascular diseases, to those patients with PAH and other pulmonary vascular diseases with similar pathobiology.

Conclusions—Our findings demonstrate that aldosterone modulates an ET₄-cysteinyl thiol redox switch to decrease pulmonary endothelium-derived NO and promote PAH.¹⁷

Elevated Vascular Endothelial Growth Factor Receptor-2 Abundance Contributes to Increased Angiogenesis in Vascular Endothelial Growth Factor Receptor-1–Deficient Mice

Summary—Vascular endothelial growth factor receptor-1 (VEGFR-1) is a pleiotropic factor with important roles in biological processes such as vasculogenesis, angiogenesis, arteriogenesis, vascular maturation, and vascular permeability. Clinically, VEGFR-1 has been implicated in a variety of diseases. Elevated levels of VEGFR-1 are a hallmark of preeclampsia, and VEGFR-1 is also thought to mediate atherosclerotic plaque thrombosis through macrophage recruitment. Additionally, VEGFR-1 may play a role in promoting proliferation of tumor vasculature and may aid in exacerbating pathological angiogenesis in other ischemic conditions from retinopathy of prematurity in the retina to myocardial infarction in the heart. VEGFR-1 is expressed mainly on the surface of endothelial cells as well as monocytes and macrophages. Current literature supports conflicting roles for VEGFR-1, depicting it as both a positive and a negative modulator of angiogenesis, thus creating confusion when one attempts to define its role in development and disease. Thus far, the exact role of VEGFR-1 is confusing because of its heterogeneous interactions with other molecules such as its specific ligands VEGF-B and placental growth factor as well as with VEGF-A and VEGFR-2. This is further complicated by the scarcity of in vivo studies performed at the level of the receptor itself. Given the direct involvement of VEGFR-1 in disease and the ambiguous nature of its action in different biological contexts, this study aims to shed light on this enigmatic molecule. Using a VEGFR-1 conditional knockout system, we have found that VEGFR-1 may be an attractive target for proangiogenic therapies.

Conclusions—Upregulation of VEGFR-2 abundance at the protein level contributes in part to increased angiogenesis in VEGFR-1–deficient mice.¹⁷

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

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

Conclusions—CDK5-mediated hyperphosphorylation of SIRT1 facilitates the development of endothelial senescence and atherosclerosis.²⁶

Hyperbilirubinemia, Augmentation of Endothelial Function, and Decrease in Oxidative Stress in Gilbert Syndrome

Summary—A balance between ambient levels of superoxide and nitric oxide release plays a critical role in the maintenance of normal endothelial function. Patients with Gilbert syndrome have mild unconjugated nonhemolytic hyperbilirubinemia, and the incidence of Gilbert syndrome in the general population is 5% to 10%. Gilbert syndrome is an ideal model for determining how endothelium-dependent vasodilation is affected by bilirubin-induced decrease in oxidative stress. In the present study, we found that serum concentrations of malondialdehyde-modified low-density lipoprotein and urinary excretion of 8-hydroxy-2′-deoxyguanosine, as indices of oxidative stress, were lower in 108 patients with Gilbert syndrome than in 108 control subjects and that flow-mediated vasodilation was greater in patients with Gilbert syndrome than in normal control subjects. Flow-mediated vasodilation correlated with serum concentration of bilirubin and with urinary excretion of 8-hydroxy-2′-deoxyguanosine in patients with Gilbert syndrome but not in control subjects. In addition, serum concentration of bilirubin correlated with malondialdehyde-modified low-density lipoprotein and 8-hydroxy-2′-deoxyguanosine in patients with Gilbert syndrome. Patients with Gilbert syndrome had low levels of oxidative stress associated with hyperbilirubinemia and enhancement of endothelium-dependent vasodilation. The mechanism by which endothelial function was augmented in patients with Gilbert syndrome may be due to the bilirubin-induced decrease in oxidative stress. These beneficial effects on the vasculature may contribute to reduced prevalence of vascular complications in atherosclerotic patients with Gilbert syndrome compared with that in atherosclerotic patients without Gilbert syndrome. Bilirubin is a potent antioxidant and a mediator of endothelial function through inhibition of nitric oxide inactivity by its antioxidative effect.

Conclusions—Patients with Gilbert syndrome had low levels of oxidative stress associated with hyperbilirubinemia and enhancement of endothelium-dependent vasodilation.²⁷

Nucleotide Excision DNA Repair Is Associated With Age-Related Vascular Dysfunction

Summary—Aging strongly contributes to cardiovascular disease. It prolongs exposure to classic cardiovascular risk factors such as hypertension and diabetes mellitus but also acts as an independent risk factor. Recent evidence suggests that gradually accumulating DNA damage, leading to genomic instability, is a main cause of aging. This study is the first to show that mice with a defective DNA repair system not only age fast but also display accelerated development of vascular problems mimicking those in aging humans: increased blood pressure, increased vascular stiffness, decreased vascular relaxation, and cellular aging. Of interest, phosphodiesterase inhibition acutely improved the diminished relaxation in vitro, suggesting that enhanced breakdown of cGMP may underlie this phenomenon. Furthermore, in humans, variations in DNA repair genes were associated with markers for vascular aging. Taken together, these results indicate that genomic instability plays a central role in vascular aging. Genomic instability may also explain the high prevalence of cardiovascular death in Hutchinson-Gilford progeria and Werner progeroid syndrome, both of which feature genomic instability. Because
oxidative stress is an important inductor of DNA damage, future aging-suppressor agents may involve drugs that improve genomic integrity (eg, statins and rapamycin) and drugs that prevent oxidative stress (eg, renin-angiotensin system blockers and antioxidants). In addition, drugs facilitating the nitric oxide–soluble guanylate cyclase–cGMP–phosphodiesterase pathway might be of value. The successful application of such treatments requires proper risk stratification, preferably at younger ages. This might include analyses of genetic variations in DNA repair genes and the identification of all possible sources of cardiovascular DNA damage.

Conclusions—Mice with genomic instability recapitulate age-dependent vascular dysfunction as observed in animal models and in humans but with an accelerated progression compared with wild-type mice. In addition, we found associations between variations in human DNA repair genes and markers for vascular stiffness, which is associated with aging. Our study supports the concept that genomic instability contributes importantly to the development of cardiovascular disease.28

Histone Deacetylation Inhibition in Pulmonary Hypertension: Therapeutic Potential of Valproic Acid and Suberoylanilide Hydroxamic Acid

Summary—Histone deacetylases (HDACs) have emerged as key targets to reverse aberrant epigenetic changes associated with cancer and autoimmune disease, and HDAC inhibitors show promise as anticancer and antiinflammatory agents. We examined the pattern of HDAC expression in lungs from patients with pulmonary arterial hypertension and investigated the effect of HDAC inhibition on the reversal of pulmonary hypertension in a rat model. Coupled to this, we explored the effects on mechanisms (proliferation, apoptosis, and inflammation) relevant to the pathology of pulmonary arterial hypertension in human and animal cell model systems. Our results demonstrate that increased HDAC activity contributes to the vascular pathology of pulmonary hypertension. The effectiveness of the HDAC inhibitors valproic acid and suberoylanilide hydroxamic acid in models of pulmonary arterial hypertension supports a therapeutic strategy based on HDAC inhibition in pulmonary arterial hypertension.39

Conclusions—Increased HDAC activity contributes to the vascular pathology of pulmonary hypertension. The effectiveness of HDAC inhibitors, valproic acid, and suberoylanilide hydroxamic acid in models of pulmonary arterial hypertension supports a therapeutic strategy based on HDAC inhibition in pulmonary arterial hypertension.39

Development of a Clinical Prediction Rule for Risk Stratification of Recurrent Venous Thromboembolism in Patients With Cancer-Associated Venous Thromboembolism

Summary—Cancer patients who experience a venous thromboembolic event are at much higher risk of recurrent events while undergoing anticoagulation than any other patient group with similar events. As such, it can be argued that treatment is frequently ineffective, and new treatment strategies are warranted. However, given the heterogeneity of the cancer population, it is probable that not all cancer patients have this similar high risk. We have developed a prediction tool that enables us to identify a high-risk group with a risk of recurrence on the order of 20% and a low-risk group with a risk of recurrence on the order of 5%. This tool will allow us to identify patients in whom a closer vigilance is required and in whom new therapeutic strategies should be tested. The parameters used are very simple and include sex, primary tumor site and stage, and a history of prior venous thromboembolism. These are clinical parameters that are usually collected in all patients, thus ensuring the ease of use and applicability of this model.

Conclusions—By identifying VTE recurrence risk in cancer patients with VTE, we may be able to tailor treatment, improving clinical outcomes while minimizing costs.40

Periprocedural Bleeding and Thromboembolic Events With Dabigatran Compared With Warfarin: Results From the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) Randomized Trial

Summary—The Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) trial demonstrated that dabigatran is well-tolerated and that, compared with warfarin, dabigatran 150 mg BID is more effective at preventing stroke and systemic embolism, whereas dabigatran 110 mg BID is associated with a lower risk of major bleeding. However, because dabigatran does not yet have a specific antidote, and its anticoagulant effect is currently difficult to precisely measure, there is concern that dabigatran may increase the risk of bleeding in patients undergoing invasive procedures, particularly if performed on an emergency basis. The RE-LY trial highlights the importance of this scenario, because 25% of patients underwent at least 1 surgery or invasive procedure within 2 years. This analysis of periprocedural outcomes from RE-LY includes data on >7500 surgeries and procedures, making it the largest evaluation of any anticoagulant strategy in the periprocedural setting. The open-label design of RE-LY allowed a real-world evaluation of the periprocedural management or anticoagulation and demonstrated that dabigatran-treated patients were 4 times more likely to have their procedure completed within 48 hours of the discontinuation of anticoagulation than patients treated with warfarin. Overall, there was no detectable difference in the rate of minor, major, or fatal bleeding between patients treated with warfarin in comparison with either dose of dabigatran, nor was there any difference in the risk of thromboembolic events. In comparison with patients receiving warfarin, the rates of major bleeding with both doses of dabigatran were also similar in the subgroups of patients having major surgery and those having surgery on an emergency basis.

Conclusions—Dabigatran and warfarin were associated with similar rates of periprocedural bleeding, including patients having urgent surgery. Dabigatran facilitated a shorter interruption of oral anticoagulation.

Validation of 6-Minute Walk Distance as a Surrogate End Point in Pulmonary Arterial Hypertension Trials

Summary—This study shows that the change in 6-minute walk distance (6MWD) satisfies the statistical criteria as a mediator between drug therapy and clinical outcomes in randomized clinical trials. Thresholds in 6MWD change were identified such that if future drugs produced such changes, it could be inferred that these drugs would produce clinical effects as well. Higher thresholds in 6MWD may need to be used when testing new agents in the presence of background pulmonary arterial hypertension therapies; however, our
results indicate that 6MWD is likely not adequate for use as a surrogate end point in pulmonary arterial hypertension clinical trials, because only modest proportions of the effects of drugs on true clinical outcomes are explained by changes in 6MWD. Further research is needed to identify more robust surrogate end points or combinations.

Conclusions—Our results suggest that Δ6MWD does not explain a large proportion of the treatment effect, has only modest validity as a surrogate end point for clinical events, and may not be a sufficient surrogate end point. Further research is necessary to determine whether the threshold value of 41.8 m is valid for long-term outcomes or whether it differs among trials using background therapy or lacking placebo controls entirely.32

Immediate Antioxidant and Antiplatelet Effect of Atorvastatin via Inhibition of Nox2

Summary—There is evidence that statins exert an antithrombotic effect in patients at risk of or with acute thrombosis, but the underlying mechanism is still to be clarified. Because platelets play a key role in artery thrombosis, the present study addressed the question of whether statins possess a direct antiplatelet property. Patients with hypercholesterolemia were randomly allocated to a Mediterranean diet with low cholesterol intake (n=15) or atorvastatin (40 mg/d; n=15). Laboratory variables of platelet activation such as platelet recruitment, platelet isoprostanes, and thromboxane A2 were determined at baseline and after 2, 24, and 72 hours and 7 days of follow-up. A significant reduction of platelet recruitment and platelet isoprostanes was observed as early as 2 hours after atorvastatin administration. This change was coincident with downregulation of platelet and systemic oxidative stress, including Nox2 activation and urinary isoprostanes, suggesting that atorvastatin exerts a direct antiplatelet effect that is mediated by Nox2 downregulation and eventually platelet isoprostane lowering. Platelet thromboxane A2 was inhibited after 24 hours with a further decline up to 7 days of follow-up; this late change was associated with cholesterol lowering. An in vitro study supported a direct effect of atorvastatin on intraplatelet signaling, showing that it dose-dependently inhibited platelet Nox2 and phospholipase A2 activation, along with inhibition of platelet recruitment, platelet isoprostanes, and thromboxane A2, and increased vasodilator-stimulated phosphoprotein and nitric oxide. Together, these data show that atorvastatin exerts a direct antiplatelet effect and suggest its potential usefulness as an antiplatelet drug in patients at risk of thrombosis.

Conclusions—The study provides the first evidence that atorvastatin acutely and simultaneously decreases oxidative stress and platelet activation by directly inhibiting platelet Nox2 and ultimately platelet isoprostanes and thromboxane A2. These findings provide a rationale for the use of statins to prevent or modulate coronary thrombosis.33

Controlled Exposure of Healthy Young Volunteers to Ozone Causes Cardiovascular Effects

Summary—Many epidemiology studies report an association between airborne particulate matter and increased cardiovascular morbidity and mortality in susceptible populations. More recent epidemiology studies have also reported a similar association with ozone. However, the pathophysiological pathways that underlie adverse clinical events after ozone exposure are not well understood. Ozone is a highly reactive oxidant, and in contrast to inhaled particulate matter, which may leave the lung and enter the bloodstream where it could directly attack vascular or cardiac tissue, ozone is not believed to leave the lung. Using a randomized crossover design, we exposed 23 young healthy individuals to clean air and to 0.3-ppm ozone for 2 hours. Ozone exposure resulted in extrapulmonary effects, including a decrease in cardiac parasympathetic tone and plasminogen activator inhibitor-1 and an increase in measures of systemic inflammation and cardiac repolarization. The study confirmed previous reports of ozone-induced decreases in lung function and increased pulmonary inflammation. The ozone-induced changes reported here are similar to changes reported in humans exposed to particulate matter, suggesting that both pollutants may cause effects by the same pathways. This report provides some insight into potential mechanisms that might contribute to the risk for adverse clinical cardiovascular effects in susceptible individuals. It also supports improving environmental health literacy among physicians and counseling patients at highest risk from air pollutants to take measures to protect themselves such as use of the local Air Quality Index.

Conclusions—This controlled-human-exposure study shows that ozone can cause an increase in vascular markers of inflammation and changes in markers of fibrinolysis and markers that affect autonomic control of heart rate and repolarization. We believe that these findings provide biological plausibility for the epidemiology studies that associate ozone exposure with mortality.34

Suppression of Arterial Thrombosis Without Affecting Hemostatic Parameters With a Cell-Penetrating PAR1 Pepducin

Summary—Antiplatelet therapy is of paramount importance in the effective treatment of patients with acute coronary syndrome and those undergoing percutaneous coronary intervention. Thrombin is the most potent platelet activator. The thrombin receptor PAR1 has emerged as an important new therapeutic target to inhibit platelet function in patients with acute coronary syndrome undergoing percutaneous coronary intervention. We describe the development of PZ-128, a first-in-class PAR1 inhibitor that targets the cytoplasmic loops of the receptor. PZ-128 rapidly suppressed PAR1-induced platelet aggregation and arterial thrombosis in guinea pigs and baboons and was synergistic to oral clopidogrel. PZ-128 did not affect bleeding or coagulation in nonhuman primates or in blood from patients undergoing percutaneous coronary intervention. Platelet function returned to baseline 24 hours after PZ-128 infusion. PAR1 inhibition by PZ-128 appears to be a novel promising therapy for patients with acute coronary syndrome. Planned clinical trials will establish where this novel class of medication fits into our therapeutic armamentarium.

Conclusions—Based on the efficacy data in nonhuman primates with no noted adverse effects on hemostasis, we anticipate that the rapid onset of platelet inhibition and reversible properties of PZ-128 are well suited to the acute interventional setting of percutaneous coronary intervention and may provide an alternative to long-acting small-molecule inhibitors of PAR1.35

Antiplatelet Drug Response Status Does Not Predict Recurrent Ischemic Events in Stable Cardiovascular Patients: Results of the Antiplatelet Drug Resistances and Ischemic Events Study

Summary—Poor biological response to antiplatelet drugs has repeatedly been associated with recurrence of ischemic events
cardiovascular patients. However, most studies involved coronary artery disease patients with recent vessel injury shortly after the initiation of antiplatelet therapy. The present study provides outcome data on 771 stable cardiovascular patients who were treated with aspirin and/or clopidogrel and were followed up for 3 years and in whom antiplatelet drug responsiveness was characterized twice with various platelet function assays at least 1 month after their last acute ischemic event. Major adverse cardiovascular events (MACEs) occurred in 16% of the patients. Hypertension, smoking, older age, and elevated low-density lipoprotein cholesterol were predictive of MACE recurrence, but neither the results of the specific assays (serum thromboxane B2 for the response to aspirin and vasodilator phosphoprotein platelet reactivity index for the response to clopidogrel) nor those of the aggregation-based platelet function tests added any incremental predictive value for the recurrence of MACEs. MACE-free survival was not significantly different for patients with only 1 good response (to either aspirin or clopidogrel; hazard ratio, 0.94; 95% confidence interval, 0.51–1.76) and for patients with a good response to both aspirin and clopidogrel (hazard ratio, 0.87; 95% confidence interval, 0.44–1.69) compared with patients with poor responses to both drugs. These results contrast with studies of other populations, including acute patients and/or patients assessed for platelet reactivity <1 month after treatment initiation. The results of the Antiplatelet Drug Resistances and Ischemic Events (ADRIE) study do not support routine platelet function testing for MACE risk evaluation in stable cardiovascular outpatients.

Conclusions—Biological antiplatelet drug responsiveness, measured with specific or aggregation-based assays, has no incremental predictive value over common cardiovascular risk factors for MACE recurrence in stable cardiovascular outpatients. These results do not support platelet function testing for MACE risk evaluation in stable cardiovascular patients.

Dietary Nitrate Ameliorates Pulmonary Hypertension: Cytoprotective Role for Endothelial Nitric Oxide Synthase and Xanthine Oxidoreductase

Summary—Loss of nitric oxide (NO) bioactivity underpins many of the hemodynamic and morphological changes in the cardiovascular circulation that characterize pulmonary hypertension, particularly the pulmonary arterial hypertension subclass. Recent evidence suggests that the NO metabolites nitrite (NO$_2^-$) and nitrate (NO$_3^-$) can be chemically reduced in vivo to biologically active NO, a phenomenon that occurs optimally under conditions of hypoxia and acidosis. This nitrate-nitrite-NO pathway has been shown to exert a number of beneficial effects, including lowering of systemic blood pressure and protection against ischemia-reperfusion injury. Herein, we have used 2 eitologically distinct experimental models of pulmonary hypertension to demonstrate that dietary nitrate (and, to a lesser extent, nitrite) can prevent and reverse the pathogenesis of this debilitating disease. In addition, we have identified the principal enzymatic routes (ie, endothelial NO synthase and xanthine oxidoreductase) by which endogenous NO generation occurs. These data provide convincing evidence that supplementation of dietary nitrate is likely to represent a novel means by which to slow, halt, or actually reverse the development of pulmonary hypertension. Exploitation of this mechanism represents a viable, orally active therapy for pulmonary hypertension that warrants evaluation in this patient population and, if efficacious, could be implemented rapidly and cost-effectively.

Conclusions—These data demonstrate that dietary nitrate, and to a lesser extent dietary nitrite, elicit pulmonary dilatation, prevent pulmonary vascular remodeling, and reduce the right ventricular hypertrophy characteristic of PH. This favorable pharmacodynamic profile depends on endothelial NO synthase and xanthine oxidoreductase-catalyzed reduction of nitrite to NO. Exploitation of this mechanism (ie, dietary nitrate/nitrite supplementation) represents a viable, orally active therapy for PH.

Lipids and Lipoproteins and Risk of Different Vascular Events in the MRC/BHF Heart Protection Study

Summary—Low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol are established risk factors for vascular disease, but there is uncertainty as to whether measures of the numbers of LDL and HDL particles, rather than their cholesterol content, might be better predictors of risk. Small LDL particles are widely believed to be particularly hazardous, but the independent predictive value of lipoprotein subclasses is also unresolved. The present investigation considers the associations between baseline concentrations of cholesterol fractions, apolipoproteins B and A$_1$, and lipoprotein particles assessed by nuclear magnetic resonance with vascular events during 5.3 years of follow-up among 20000 high-risk individuals (2% average coronary event risk per year). Cholesterol and particle measures of LDL were strongly correlated and had similar predictive value for incident major occlusive coronary events and for arterial revascularization procedures. Given the total LDL particle number, the distribution between small and large particles did not add predictive value. Associations of these different LDL-related measures were much weaker or nonexistent with ischemic stroke and other cardiac events (mainly heart failure). After adjustment for LDL particle number, the predictive values of the cholesterol and particle measures of HDL for incident major occlusive coronary events were similar. In contrast, other cardiac events were strongly associated with fewer total and small HDL particles and larger mean HDL size but only very weakly associated with HDL cholesterol. The present results indicate that LDL lipoprotein particle measurements provide little additional predictive value over traditional measures for occlusive vascular events in a high-risk population. It is unclear whether the associations between HDL particle numbers and other cardiac events represent a causal or reverse-causal effect.

Conclusions—In a population at 2% average coronary event risk per year, cholesterol, apolipoprotein, and particle measures of LDL were strongly correlated and had similar predictive values for incident major occlusive vascular events. It is unclear whether the associations between HDL particle numbers and other cardiac events represent a causal or reverse-causal effect.

Gap Junctions and Connexin Hemichannels Underpin Hemostasis and Thrombosis

Summary—On blood vessel injury, platelets adhere to exposed subendothelial collagens and are activated, triggering thrombus formation to prevent bleeding. Inappropriate activation of platelets under pathological conditions such as atherosclerosis results in thrombosis, which may lead to heart attack or stroke. Connexins are membrane proteins that assemble into channels (hemichannels or connexons) in the plasma membrane of selected cells, facilitating communication between the cytoplasm and external environments. Docking of connexons on neighboring cells also results in gap junctions, which mediate direct intercellular communication. Hence, hemichannels...
and gap junctions coordinate and synchronize the functions of various tissues and organs, eg, the heart. In the present study, we report the presence of multiple connexins in platelets. The results of this study indicate that gap junctions form between platelets in thrombi and convey intercellular signals that promote retraction of blood clots, an important step in wound repair. Connexins were also found to regulate the activation of isolated platelets, pointing to the importance of hemichannels on platelets before thrombus formation. Consistent with this, the deletion of the Cx37 gene in mice resulted in reduced clot retraction and platelet activation. Inhibition of platelet connexins with pharmacological agents diminished thrombus formation in vitro in human blood and thrombotic responses in mice. Together, this study provides evidence for a fundamental role of connexin hemichannels and gap junctions in the activation of platelets and the regulation of thrombus function and suggests that connexins may represent potential avenues for the development of novel antithrombotic agents.

Conclusions—Together, these data demonstrate that platelet gap junctions and hemichannels underpin the control of hemostasis and thrombosis and represent potential therapeutic targets.39

Vascular Klotho Deficiency Potentiates the Development of Human Artery Calcification and Mediates Resistance to Fibroblast Growth Factor 23

Summary—Loss of arterial tree elasticity because of calcification of the media of arteries is a major clinical challenge. Such changes with the potential consequence of heart failure are a particular problem for patients with chronic kidney disease, diabetes mellitus, and hypertension and also for older individuals experiencing premature vascular aging. Treatment modalities to date are limited to blood pressure control. This study investigated whether Klotho, an antiaging and a stress-protective protein, is expressed and protects the vascular wall from chronic, damaging stress conditions. Here, we show evidence that human arteries and, in particular, vascular smooth muscle cells express the Klotho protein. Metabolic conditions experienced by arteries from patients with chronic kidney disease suppressed Klotho. This masked protective effects of vascular-produced Klotho, resulting in smooth muscle cell transformation and increased vascular calcium deposition. Activation of the vascular vitamin D receptor reversed Klotho suppression and inhibited the deleterious process of vascular calcification. We have also shown that human arteries express fibroblast growth factor receptors. Expression of fibroblast growth factor receptors together with Klotho renders vascular smooth muscle cells a target tissue for fibroblast growth factor 23. Metabolic stress suppressed the expression of this receptor complex leading to fibroblast growth factor 23 resistance. However, fibroblast growth factor 23 resistance could be reversed with vitamin D receptor activator therapy, again resulting in the inhibition of vascular calcium deposition. We therefore propose vascular Klotho as a treatment target to inhibit calcification of the vascular wall.

Conclusions—Chronic metabolic stress factors found in CKD promote vascular Klotho deficiency. Mechanistic studies revealed a bifunctional role for local vascular Klotho, first, as an endogenous inhibitor of vascular calcification and, second, as a cofactor required for vascular FGF-23 signaling. Furthermore, vitamin D receptor activators can restore Klotho expression and unmask FGF-23 anticalcific effects.40

Pulmonary Arterial Hypertension in Patients Treated by Dasatinib

Summary—The prognosis of chronic myelogenous leukemia has been transformed by tyrosine kinase inhibitors that inhibit BCR/ABL kinase, such as imatinib, dasatinib, and nilotinib. The present report summarizes the clinical characteristics and outcomes of 9 incident cases of severe precapillary pulmonary hypertension (PH) fulfilling the criteria of pulmonary arterial hypertension induced by dasatinib use identified from the French PH registry. PH occurred after 8 to 48 months of dasatinib exposure. Most patients had severe symptoms and marked hemodynamic compromise at time of PH diagnosis. Based on our registry and data from the national pharmacovigilance agency, the lowest estimate of incident PH occurring in patients exposed to dasatinib in France was 0.45%. Of note, clinical, functional, and hemodynamic improvements were generally observed after dasatinib withdrawal. Nevertheless, all subjects remained at least mildly symptomatic and had persistent abnormal hemodynamics. Even if PH is a rare complication in patients treated with dasatinib, the increased use of this agent in the treatment of chronic myelogenous leukemia may result in higher numbers of patients at risk of developing drug-induced pulmonary arterial hypertension. Physicians need to be aware of this complication to appropriately monitor and manage these patients. We therefore recommend screening routinely for PH by echocardiography before commencing dasatinib and performing additional diagnostic testing in patients developing dyspnea or other symptoms suggestive of PH. Where these investigations indicate possible PH, a right heart catheterization is mandatory to confirm the diagnosis and mechanisms of PH.

Conclusions—Dasatinib may induce severe precapillary PH fulfilling the criteria of pulmonary arterial hypertension, thus suggesting a direct and specific effect of dasatinib on pulmonary vessels. Improvement is usually observed after withdrawal of dasatinib.41

Systemic and Pulmonary Vascular Dysfunction in Children Conceived by Assisted Reproductive Technologies

Summary—Assisted reproductive technology (ART) has been used for 3 decades, and the children born after ART now make up for 1% to 4% of the births in developed countries. ART involves the manipulation of early embryos at a time when they may be particularly vulnerable to external disturbances. Environmental influences during the embryonic and fetal development influence the individual’s susceptibility to cardiovascular disease, raising concerns about the potential consequences of ART on the long-term health of the offspring. Here, we show for the very first time that apparently healthy children born after ART show systemic and pulmonary vascular dysfunction, as evidenced by endothelial dysfunction, increased arterial stiffness, and carotid intima-media thickness in the systemic circulation and exaggerated hypoxic pulmonary hypertension during short-term high-altitude exposure. This vascular dysfunction does not appear to be related to parental factors or hormonal stimulation of the ovulation in the mother but to the ART procedure itself. For the practicing physician, this study indicates that ART children who live at high altitude or suffer from diseases associated with chronic hypoxia are at risk for exaggerated pulmonary hypertension and need to be monitored for this problem. In the systemic circulation, it is not known yet how this vascular dysfunction, which is similar in magnitude to that in children suffering from type 1 diabetes mellitus, will evolve. Although future mechanistic studies in ART mice may reveal possibilities for targeted intervention to improve or prevent ART-induced vascular dysfunction
Body Mass Index, Surgery, and Risk of Venous Thromboembolism in Middle-Aged Women: A Cohort Study

Summary—Obesity and surgery are established risk factors for venous thromboembolism, but there is limited information about the independent effects of excess body weight on the incidence of postoperative venous thromboembolism. We linked questionnaire data from a large cohort study (the Million Women Study) with hospital admission and death records to examine the risk of venous thromboembolism in relation to body mass index, both in the absence of surgery and in the first 12 weeks following surgery. In the absence of surgery, there was a clear trend of increasing risk of venous thromboembolism with increasing body mass index. We also found that the risk of being admitted to hospital for surgery increased with increasing adiposity. Following surgery, both overweight and obese women had higher risks of venous thromboembolism, when compared with women of a healthy weight. This relationship between adiposity and postoperative venous thromboembolism risk was seen for both day and inpatient surgery, although the excess incidence was much greater following inpatient surgery. Clinical guidelines identify obesity as an additional risk factor for postoperative venous thromboembolism. Our findings suggest that venous thromboembolism prophylaxis is important for overweight, as well as obese, women undergoing either day or inpatient surgery. The observed progressive increase in venous thromboembolism incidence with increasing body mass index also suggests that the avoidance of further weight gain and even small reductions in body size are likely to be beneficial for those who are overweight and obese.

Conclusions—VTE risk increases with increasing BMI and the associated excess risk is much greater after surgery than without surgery.

Auto-Antigenic Protein-DNA Complexes Stimulate Plasmacytoid Dendritic Cells to Promote Atherosclerosis

Summary—Atherosclerosis remains the number 1 cause of death in the Western world, and the therapeutic options currently available are limited. Chronic inflammation of the vessel wall has been closely linked to autoimmune processes in atherosclerosis. The role of the specialized subset of plasmacytoid dendritic cells present in human atherosclerotic plaques, however, has not been addressed previously. We provide evidence for a hitherto unrecognized plasmacytoid dendritic cell–driven pathway of autoimmune activation in atherosclerosis that critically amplifies early atherosclerotic lesion formation. Self-DNA (eg, released from dying cells or in neutrophil extracellular traps) and an increased expression of the antimicrobial peptide Cramp/LL37 in atherosclerotic lesions has shown to stimulate the development of tolerance to self-DNA and promote interferon-α production by plasmacytoid dendritic cells, aggravating early atherosclerosis and the formation of anti–double-stranded DNA antibodies in apolipoprotein E–deficient mice. Notably, anti–double-stranded DNA antibodies were also found to be elevated in patients with symptomatic versus asymptomatic carotid artery stenosis. Although possibly of limited clinical relevance in a rheumatological context, moderately increased levels of circulating anti–double-stranded DNA antibodies may predispose to atherothrombosis, in line with findings showing elevated anti-nuclear antibody titers to be associated with decreased carotid elasticity and early atherosclerosis. More importantly, chronically increased interferon-α levels and circulating anti–double-stranded DNA antibody titers may provide an explanation for the increased risk of atherosclerosis well recognized in patients with psoriasis and systemic lupus erythematosus.

Conclusions—Self-DNA (eg, released from dying cells or in neutrophil extracellular traps) and an increased expression of the antimicrobial peptide Cramp/LL37 in atherosclerotic lesions may thus stimulate a pDC-driven pathway of autoimmune activation and the generation of anti–double-stranded-DNA antibodies, critically aggravating atherosclerosis lesion formation. These key factors may thus represent novel therapeutic targets.

Developmental Endothelial Locus-1 (Del-1) Mediates Clearance of Platelet Microparticles by the Endothelium

Summary—Microparticles are submicron-sized, membrane-enclosed fragments released from cells in response to activation or during apoptosis. Platelet-derived microparticles, released on platelet activation, constitute a major fraction of microparticles in the circulating blood. Platelet microparticles have a procoagulant function in normal hemostasis because of the expression of phosphatidylserine on their surface. In addition, platelet microparticles have been shown to stimulate hematopoietic cells, transfer platelet-specific receptors to the surface of other cells, and elicit cytokine responses from synovial fibroblasts. Microparticles are rapidly cleared from the circulation, and their fate is largely unknown. In flowing blood, microparticles are pushed toward the plasma-endothelial interface because of their size, and they are poised to interact with the endothelial cell surface. Here, we studied the clearance of microparticles by endothelium and the role of developmental endothelial locus–1 (Del-1), a 52-kDa glycoprotein (also termed Edil3) secreted by endothelial cells. Del-1 binds to platelet microparticles (via phosphatidylserine) and anchors them to integrins on cultured endothelial cells (via the RGD motif) for efficient endocytosis. After infusion of fluorescent microparticles in vivo, Del-1–deficient mice have an impaired uptake by the endothelium. In addition, Del-1–deficient mice also have increased microparticles after endotoxin administration. These results suggest that microparticles are taken by the endothelium and that Del-1 functions as a physiological mediator of microparticle clearance by the endothelium.

Conclusions—These studies show a physiological role for Del-1 in the clearance of phosphatidylserine-expressing microparticles by endothelium.

Epoxyeicosatrienoic Acids Contribute With Altered Nitric Oxide and Endothelin-1 Pathways to Conduit Artery Endothelial Dysfunction in Essential Hypertension

Summary—Patients with essential hypertension are at increased cardiovascular risk even when their blood pressure levels are well controlled, and restoration of the endothelial function of conduit arteries has emerged as a therapeutic target that may help to prevent the development of cardiovascular complications. In the present study, we demonstrated that several mechanisms contribute to conduit artery
endothelial dysfunction in hypertensive patients. In addition to nitric oxide/reactive oxygen species imbalance and alteration in the endothelin-1 pathway, a decrease in epoxideisotrisatrienioic acid availability is involved. This is particularly important because epoxideisotrisatrienioic acids are not only endothelium-derived relaxing factors, but they also play a major role in maintaining cardiovascular homeostasis by contributing to the regulation of vascular inflammation, cell proliferation, angiogenesis, and hemostasis. Consequently, a new class of pharmacological agents referred to as soluble epoxide hydrolase inhibitors is under development. Therefore, to increase epoxideisotrisatrienioic acid availability by reducing their degradation may be particularly useful during essential hypertension. Thus, in addition to decreasing blood pressure, epoxideisotrisatrienioic acids could restore the protective action of the endothelium in conduit arteries and prevent the development of atherosclerosis and cardiovascular complications.

Conclusions—These results show that an impaired role of epoxideisotrisatrienioic acids contributes, together with an alteration in NO/reactive oxygen species balance and endothelin-1 pathway, to conduit artery endothelial dysfunction in essential hypertension.

Gremlin Plays a Key Role in the Pathogenesis of Pulmonary Hypertension

Summary—Pulmonary hypertension is a disease characterized by pulmonary vascular remodeling and increased pulmonary vascular resistance. It occurs commonly in chronic hypoxic lung diseases and leads to increased morbidity and mortality. A major breakthrough in our understanding of pulmonary hypertension was achieved with the identification of heterozygous mutations in the bone morphogenetic receptor type 2 as the cause of the rare heritable form of pulmonary arterial hypertension. It was subsequently found that bone morphogenetic receptor type 2 is the cause of the rare heritable form of pulmonary arterial hypertension.47

Conclusions—These findings demonstrate a central role for increased gremlin in hypoxia-induced pulmonary vascular remodeling and the increased pulmonary vascular resistance in hypoxic pulmonary hypertension. High levels of basal gremlin expression in the lung may account for the unique vulnerability of the pulmonary circulation to heterozygous mutations of BMP type 2 receptor in pulmonary arterial hypertension.

References


