Hemodynamic Modulation of Endocardial Thromboresistance

Navin K. Kapur, MD; Clayton B. Deming, MS; Sunil Kapur, BS; Ce Bian, MD; Hunter C. Champion, MD, PhD; J. Kevin Donahue, MD; David A. Kass, MD; Jeffrey J. Rade, MD

Background—Patients with heart failure are at increased risk for thromboembolic events, including stroke. Historically attributed to blood stasis, little is known about the adverse effects of elevated chamber filling pressure on endocardial function, which could predispose to intracardiac thrombus formation.

Methods and Results—We investigated changes in the expression of thrombomodulin, a key component of the anticoagulant protein C pathway, in rats subjected to acute atrial pressure overload caused by aortic banding. Acute elevation of left atrial filling pressure, without an associated decline in ventricular systolic function, caused a 70% inhibition of atrial endocardial thrombomodulin expression and resulted in increased local thrombin generation. Targeted restoration of atrial thrombomodulin expression with adenovirus-mediated gene transfer successfully reduced thrombin generation to baseline levels. In vitro co-culture studies revealed that thrombomodulin downregulation is caused by the paracrine release of transforming growth factor-β from cardiac connective tissue in response to mechanical stretch. This was confirmed in vivo by administration of a neutralizing transforming growth factor-β antibody, which effectively prevented thrombomodulin downregulation during acute pressure overload.

Conclusions—These findings suggest that increased hemodynamic load adversely affects endocardial function and is a potentially important contributor to thromboembolus formation in heart failure. (Circulation. 2007;115:67-75.)

Key Words: endocardium | heart failure | thrombosis | thrombomodulin

Congestive heart failure is a growing public health problem that affects more than 5 million people in the United States. Thromboembolic events, including stroke, are a major cause of morbidity and mortality in patients with heart failure.1 The annual risk of a thromboembolic event with mild to moderate heart failure and sinus rhythm ranges from 1.5% to 2.5% and exceeds 5% in severe disease.2,3 Thromboembolus formation in heart failure has historically been ascribed to blood stasis within the dilated cardiac chambers.4 Stasis alone, however, cannot fully explain intracardiac thrombus formation, as even patients with heart failure and preserved systolic function remain at increased risk for stroke compared with those without heart failure.5 Although recent evidence does suggest that heart failure may be associated with a mild degree of hypercoagulation,6 what has received relatively little attention and yet may be a significant contributor to intracardiac thrombus formation is the third arm of Virchow’s triad—dysfunction of the endocardial endothelium.

Thrombomodulin is a 100-kDa membrane glycoprotein that is a major regulator of vascular thromboresistance.7 It is expressed in abundance by vascular endothelial cells, including those that comprise the endocardium.8 Thrombomodulin binds thrombin and renders it incapable of enzymatically cleaving fibrinogen or activating cellular thrombin receptors, but enables the activation of circulating protein C. Activated protein C (APC) degrades factors Va and XIIIa of the coagulation cascade, potently inhibiting further thrombin generation. Deletion of the thrombomodulin gene causes lethal thrombosis in mice, and the acquired loss of thrombomodulin in humans is thought to contribute to the thrombotic manifestations of bacterial sepsis, radiation enteropathy, and coronary atherosclerosis.9–11

We recently identified pressure-induced vascular stretch as a novel and potent inhibitory stimulus for endothelial thrombomodulin expression.12 This was first observed in rabbit vein segments implanted into the arterial circulation; thrombomodulin protein expression decreased by 95% and resulted in increased local thrombin generation and microthrombus formation.13 On the basis of these studies, we hypothesize that pressure-induced chamber stretch might also negatively regulate endocardial thrombomodulin expression and contribute to intracardiac thrombus formation during heart failure. To explore this, we characterized endocardial thrombomodulin expression and function in a rat model of acute pressure.
overload. We then used adenovirus-mediated gene transfer to determine the relationship between thrombomodulin function and endocardial thromboresistance and identified an important role of paracrine effects of transforming growth factor-β (TGF-β) in regulation of endocardial thrombomodulin expression.

**Methods**

**Rat Model of Pressure Overload**

Animal protocols were approved by the Johns Hopkins Animal Care and Use Committee. A well-characterized rat model was used with modification. Six-week-old male Wistar rats that weighed ~100g (Charles River Laboratories, Wilmington, Mass) were anesthetized with 1% to 2% isoflurane and mechanically ventilated. Through a right thoracotomy, a 23-gauge needle was placed adjacent to the ascending aorta and a 4-0 silk suture ligature was applied around both. The needle was then removed to create an immediate >70% luminal stenosis. Sham control animals underwent thoracotomy without suture placement. To investigate the effects of TGF-β, rats were administered 1 mg/kg of either a neutralizing anti–TGF-β antibody (MAB240; R&D Systems, Minneapolis, Minn) or an IgG1 isotype control antibody (MAB005; R&D Systems) via peritoneal injection 24 hours before surgery, with a second dose of 0.5 mg/kg administered 48 hours after surgery. The antibody dose was based on previous studies in the rat that demonstrated effective neutralization of TGF-β.8

At euthanization, echocardiographic measurements were obtained with a 1.5-MHz probe (Acuson, Siemens, Malvern, Pa) with validated techniques in rodents.16 Ventricular pressures were measured directly via cardiac puncture with a 21-gauge needle attached to a pressure monitor (SpaceLabs, Redmond, Wash).

**Atrial Gene Transfer**

The construction of adenovirus vectors that express human thrombomodulin (AdTMh5) and no transgene (AdNull) have been previously described.13 The left atrium was exposed via a left thoracotomy and 400 μL of a solution of 20% Pluronic F-127 (Molecular Probes, Eugene, Ore), 0.5% trypsin, and 1010 plaque-forming units/mL of the indicated adenovirus vector were directly applied to the epicardial surface of the left atrium with a camel-hair paintbrush. The applied solution was allowed to gel for 10 minutes before the left atria with antibodies against rat thrombomodulin #3381 (American Diagnostica), human TGF-β latent associated peptide (EFO1; R&D), and rat prolyl 4-hydroxylase (6-9H6; Acris Antibodies, Hiddenhausen, Germany) as described.8

**Assessment of Neurohormonal Activation**

At the time of euthanization, levels of angiotensin II, atrial natriuretic peptide, and brain natriuretic peptide were determined in extracted tissue samples and cultured cells normalized to 18S ribosomal RNA (TaqMan Ribosomal RNA Reagents with VIC-labeled probe; Applied Biosystems).

**Western Blot and Immunohistochemical Analyses**

Western blot analysis was performed with anti-thrombomodulin antibodies (#3381 and #2380; American Diagnostica) and an anti-CD31 antibody (M0823; Dako, Carpinteria, Calif) as described.13 Bands were detected by autoradiography and quantified densitometrically with UN-SCAN-IT software (Silk Scientific, Orem, Utah). Immunohistochemical analysis was performed on formalin-fixed and paraffin-embedded sections of left atria with antibodies against rat thrombomodulin #3381 (American Diagnostica), human TGF-β latent associated peptide (EFO1; R&D), and rat prolyl 4-hydroxylase (6-9H6; Acris Antibodies, Hiddenhausen, Germany) as described.8

**In Situ Protein C and Thrombin Activity Assays**

Capacity to activate APC was measured on freshly-harvested whole left atria with a previously described protocol with modification.13 The atria were washed in Hank’s buffered salt solution and then incubated in 250 μL Hank’s buffered salt solution that contained 40 mmol/L human α-thrombin (Sigma-Aldrich, St Louis, Mo) and 1 μmol/L human protein C (American Diagnostica) at 37°C. After 60 minutes, thrombin was neutralized by excess lepirudin (Berk Laboratories, Montville, NJ). Aliquots measuring 100 μL were incubated at room temperature with a 3-mmol/L solution of the chromogenic substrate S-2366 (DiaPharma, West Chester, Ohio). The rate of substrate conversion was determined spectrophotometrically with a Vmax Kinetic Microplate Reader (Molecular Devices, Sunnyvale, Calif) and the amount of protein C activation calculated by comparison with a human APC (American Diagnostica) standard curve. Bound thrombin activity on the atrial endocardial surface was measured as previously described with some modification.13 Freshly excised whole atria were washed in Hank’s buffered salt solution, then incubated in 250 μL of substrate buffer (50 mmol/L Tris-HCl, 175 mmol/L NaCl, and 2 mmol/L CaCl2 [pH 7.8]) that contained 333 μmol/L of the chromogenic substrate, S-2238 (DiaPharma) at 37°C for 30 minutes. The change in absorbance at 405 nm before and after lepirudin treatment represented thrombin-specific substrate conversion. Bound thrombin activity was then calculated by comparison with a human α-thrombin standard curve.

**In Vitro Stretch Experiments**

Human atrial endocardial cells isolated in our laboratory (see Data Supplement) and human cardiac fibroblasts purchased from Cell Applications (San Diego, Calif) of passage 2 to 3 were plated onto type I collagen-coated 6-well Bioflex plates (Flexcell International, Hillsborough, NC) and grown in either EGM-2 (endoocardial cells) or FGM-2 (fibroblasts) culture media (BioWhittaker). When nearly confluent, cells were refreshed in basal media and subjected to 0% to 10% cyclic strain delivered at 1 Hz for 24 hours at 37°C and 5% CO2 with a FX-4000T Tension Plus System (Flexcell International). For co-culture experiments, atrial endocardial cells were grown on 24-mm diameter Transwell inserts with a 0.4-μm pore size (Corning, Corning, NY) in EGM-2 medium. When confluent, the inserts were placed into the individual wells of the Bioflex plates with cardiac fibroblasts in basal medium (BioWhittaker). The cardiac fibroblasts were then subjected to 0% to 10% cyclic strain delivered at 1 Hz for 24 hours. To investigate the effects of TGF-β, 0.5 μg/mL of either a neutralizing anti–TGF-β antibody (MAB240; R&D) or IgG1 isotype control antibody (MAB005; R&D) were added to the medium before stretching the fibroblasts at 10% cyclic strain delivered at 1 Hz for 24 hours.

**Statistical Analysis**

All data are presented as mean±SEM. Comparison between 2 groups is by 2-tailed t-test and between multiple groups is by 1-way ANOVA with a Bonferroni correction for intergroup comparisons.
Sham controls (left atrium of banded rats declined by 60% compared with unchanged. Western blot analysis of left atrial tissue showed no significant changes in hemodynamic and echocardiographic measurements were obtained 96 hours after surgery. Compared with sham-operated controls, left ventricular systolic pressure increased 40% in banded animals, whereas left ventricular diastolic pressure, equivalent to mean left atrial pressure, increased by nearly 400% (P<0.0001 for both; Figure 1A). Ventricular wall thickness and diastolic diameter were unchanged, whereas left atrial diameter increased slightly (Figure 1B). Systolic function was not depressed in this degree of acute increase in afterload results in pulmonary edema and death within 7 days of surgery, all hemodynamic and echocardiographic measurements were obtained 96 hours after surgery. Compared with sham-operated controls, left ventricular systolic pressure increased 40% in banded animals, whereas left ventricular diastolic pressure, equivalent to mean left atrial pressure, increased by nearly 400% (P<0.0001 for both; Figure 1A). Ventricular wall thickness and diastolic diameter were unchanged, whereas left atrial diameter increased slightly (Figure 1B). Systolic function was not depressed in this acute period, with slight increases in ejection fractions (77±9% banded versus 65±5% sham controls, P<0.01), which likely reflect acute compensation to high afterload. Serum atrial natriuretic peptide and brain natriuretic peptide levels were 3-fold higher in banded rats, consistent with the induction of heart failure, whereas angiotensin II and tumor necrosis factor-α levels did not differ from controls (Figure 1C).

To determine the impact of acute hemodynamic changes on endocardial thrombomodulin expression, tissue from the left atrium and ventricle of banded rats was subjected to quantitative PCR analysis and compared with sham controls (Figure 2A). Aortic tissue, distal to the suture ligature, was analyzed as an additional control. Data were normalized to CD31, an endothelial-specific adhesion molecule whose expression does not change with pressure-induced cell stretch. Thrombomodulin expression in the left atrium of banded rats declined by 50% compared with sham controls (P=0.01), whereas thrombomodulin expression in the left ventricle or in the distal aorta was unchanged. Western blot analysis of left atrial tissue confirmed a 70% decline in thrombomodulin expression (P<0.002; Figure 2B). Left atrial tissue stained with an anti-mouse thrombomodulin antibody verified decreased thrombomodulin expression by the atrial endocardial endothelium (Figure 2C). These data suggest that endocardial thrombomodulin expression is directly modulated by changes in chamber loading rather than by systemic factors induced by acute heart failure.

We also determined whether acute pressure overload altered the atrial expression of other molecules known to modulate endocardial thromboresistance (Figure 3). Aortic banding did not alter the expression of endothelial nitric oxide synthase but was associated with increased tissue factor pathway inhibitor and decreased tissue factor expression. The net effect of these changes would be expected to reduce local thrombin generation at the endocardial surface. Interestingly, banding also increased the expressions of both tissue plasminogen activator and plasminogen activator inhibitor-1.

### Results

#### Effects of Acute Pressure Overload on Endocardial Thrombomodulin Expression

The ascending aortae of 6-week-old rats were suture-banded to induce a >70% luminal constriction. Because this degree of acute increase in afterload results in pulmonary edema and death within 7 days of surgery, all hemodynamic and echocardiographic measurements were obtained 96 hours after surgery. Compared with sham-operated controls, left ventricular systolic pressure increased 40% in banded animals, whereas left ventricular diastolic pressure, equivalent to mean left atrial pressure, increased by nearly 400% (P<0.0001 for both; Figure 1A). Ventricular wall thickness and diastolic diameter were unchanged, whereas left atrial diameter increased slightly (Figure 1B). Systolic function was not depressed in this acute period, with slight increases in ejection fractions (77±9% banded versus 65±5% sham controls, P<0.01), which likely reflect acute compensation to high afterload. Serum atrial natriuretic peptide and brain natriuretic peptide levels were >3-fold higher in banded rats, consistent with the induction of heart failure, whereas angiotensin II and tumor necrosis factor-α levels did not differ from controls (Figure 1C).

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#### Consequences of Thrombomodulin Downregulation on Endocardial Thromboresistance

Thrombomodulin exerts its anticoagulant effect via activation of circulating protein C. Endocardial capacity to generate APC was measured in resected whole left atria 96 hours after surgery. Aortic banding reduced the capacity to generate APC in the left atria of banded rats by >35% compared with sham controls (P=0.01; Figure 4A). To determine the effect on in situ thrombin generation, the activity of thrombin bound to the atrial endocardial surface was then quantified. Thrombin generated at sites of vascular injury binds to fibrin strands within a developing clot and is protected from inactivation by circulating inhibitors. Bound thrombin activity is therefore proportional to the degree of thrombus that is present and is capable of detecting the presence of microscopic amounts of fibrin clot. Bound thrombin activity in the atria of banded rats was significantly higher than in atria from sham-operated controls and approached that observed in atria subjected to direct mechanical forcep injury (Figure 4B).
We then determined the effects of thrombomodulin expression restoration on capacity to generate APC and bound thrombin activity. This was accomplished with a "gene painting" technique, in which solutions that contain adenovirus vectors that express either human thrombomodulin (AdTM5) or no transgene (AdNull) were applied to the epicardial surfaces of the rat left atria. Previous studies in the pig revealed that this technique results in efficient transmural transduction of atrial cells, including the endocardial endothelium.19 Figure 1 illustrates the degree of gene transfer that can be attained with this method in the rat. Three days after transduction, protein lysates of left atrial tissue subjected to ELISA that detects only the human form of thrombomodulin13 revealed an average of 76.5±24.9 ng of human thrombomodulin per milligram of atrial tissue in AdTMh5-transduced atria (n=4), whereas no human thrombomodulin was detected in AdNull-transduced atria (n=3; P<0.004). These results were confirmed by Western blot analysis (Figure 4C). To verify that acute pressure overload does not attenuate transgene expression, transduced atria from suture-banded rats were assayed for the expression of both human and native rat thrombomodulin by quantitative PCR 96 hours after surgery. Figure 4D demonstrates that human thrombomodulin gene expression remains robust throughout the experimental period in AdTMh5-transduced atria, whereas no human thrombomodulin gene expression could be detected in AdNull-transduced atria. Restoration of thrombomodulin expression with adenovirus-mediated gene transfer effectively prevented the loss of capacity to generate APC induced by aortic banding (Figure 4A) and reduced the levels of bound thrombin activity to baseline values (Figure 4B). Taken together, these data support the concept that downregulation of endocardial thrombomodulin contributes to increased local thrombin generation.

**Effects of Stretch on Thrombomodulin Expression in Isolated Endocardial Endothelial Cells**

We have previously shown that thrombomodulin down-regulation in vein segments exposed to arterial pressure results from pressure-induced vascular stretch and not from the direct effects of pressure per se.12 To determine whether stretch can directly inhibit thrombomodulin gene expression, endocardial endothelial cells isolated from human right atrial appendages were grown on collagen-coated silastic membranes and subjected to increasing...
amounts of cyclic stretch for 24 hours. Surprisingly, cyclic stretch resulted in a trend toward increased thrombomodulin gene expression (Figure 5A). Similar results were observed in stretched endothelial cells isolated from both rabbit jugular veins and human umbilical veins (data not shown). Because endocardial endothelial cells in situ lie adjacent to cardiac connective tissue, we next tested whether this interaction might influence thrombomodulin expression by stretch. Human atrial endocardial cells were cultured on stationary filters suspended in the media of wells that contain human cardiac fibroblast subjected to increasing amounts of cyclic stretch. Stretching of cardiac fibroblasts resulted in a dose-dependent decrease in thrombomodulin expression in the stationary endocardial cells (Figure 5B). Experiments that substituted human aortic smooth cells for cardiac fibroblasts yielded identical results (data not shown). These results indicate that endocardial thrombomodulin expression is modulated in paracrine fashion by a soluble factor released by cardiac connective tissue in response to stretch.

Modulation of Thrombomodulin Expression by Paracrine Release of TGF-β

TGF-β is a multifunctional stretch-induced growth factor known to be intimately involved in cardiac remodeling induced by heart failure and has been reported to inhibit thrombomodulin protein expression in human umbilical vein endothelial cells. TGF-β gene expression in cardiac fibroblasts exhibited a dose-dependent increase in response to cyclic stretch (Figure 6A). In addition, thrombomodulin gene expression in human atrial endocardial cells was inhibited in a dose-dependent manner after 24-hour exposure to recombinant TGF-β (Figure 6B). To determine whether TGF-β is the paracrine factor responsible for thrombomodulin downregulation, human endocardial cells were plated on stationary filters submerged in the media of cardiac fibroblasts subjected to 10% cyclic stretch for 24 hours in the presence of a neutralizing anti–TGF-β antibody or an isotype control antibody. Figure 6C shows that neutralization of TGF-β effectively

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Figure 4. Effects of thrombomodulin expression on endocardial thromboresistance. A, Capacity to generate APC was assessed 96 hours after surgery in the left atrium of sham-operated and banded rats (n=10 per group) as well as in banded rats whose left atria were transduced with either AdNull or AdTMh5 5 days before surgical banding (n=5 per group). B, Bound thrombin activity, proportional to microthrombus burden, was measured 96 hours after surgery in the left atria of sham-operated and banded rats (n=10 per group) as well as in banded rats whose left atria were transduced with either AdNull or AdTMh5 5 days before surgical banding (n=5 per group). C, Rat LA were transduced with either AdNull (n=5) or AdTMh5 (n=8) 5 days before surgical banding; 96 hours after banding, thrombomodulin gene expression was determined in atrial tissue by quantitative PCR with species-specific probes and primers. D, Rat left atria were transduced with either AdNull or AdTMh5, an adenovirus vector expressing human thrombomodulin. Three days after transduction, atrial tissue was subjected to Western blot analysis with antibodies that recognize the human isoform of thrombomodulin and rat CD31. The depicted blot is representative of 3 experiments.

Figure 5. Effect of cyclic stretch on endocardial thrombomodulin expression. A, Human atrial endothelial cells were plated on collagen-coated Bioflex plates and subjected to increasing amounts of cyclic stretch at 1 Hz for 24 hours. Thrombomodulin gene expression, normalized to rRNA, was determined by quantitative PCR (n=5 per group). B, Human atrial endocardial cells were plated on stationary filters, submerged in the media of human cardiac fibroblasts, and subjected to increasing amounts of cyclic stretch at 1 Hz for 24 hours. Thrombomodulin gene expression, normalized to rRNA, was determined by quantitative PCR (n=4 per group).
The downregulation of endocardial thrombomodulin gene expression was prevented by TGF-β inhibition. To determine whether paracrine release of TGF-β is responsible for in vivo modulation of thrombomodulin, we first assessed TGF-β expression in the cardiovascular tissue of rats subjected to aortic banding. Compared with sham-operated controls, TGF-β gene expression in the left atrium of banded rats increased by 200% (P < 0.001), whereas there was no significant change in TGF-β expression in the left ventricle or in the distal aorta (Figure 7A). Banding was also associated with increased TGF-β activation, as evidenced by positive immunostaining for latency-associated peptide, the cleaved propeptide of the TGF-β precursor molecule that remains noncovalently attached to active TGF-β. Latency-associated peptide staining localized predominantly to fibroblast-appearing cells in the subendocardial space that also expressed an abundance of the prolyl 4-hydroxylase, an enzyme involved in collagen synthesis (Figure 7B). To determine whether TGF-β inhibition could prevent thrombomodulin downregulation, rats were administered either a neutralizing anti-TGF-β antibody or an isotype control antibody in the perioperative period. Neutralization of TGF-β effectively prevented thrombomodulin downregulation in the left atrium 96 hours after aortic banding (Figure 7C). Importantly, there was no meaningful difference in the hemodynamic response to banding between rats administered the anti-TGF-β antibody versus the isotype control antibody (left ventricular end-diastolic pressure 17.7 ± 0.6 versus 15.8 ± 0.2 mm Hg, respectively; P = 0.02). These data confirm that the effects of pressure overload on endocardial thrombomodulin expression and thromboreistance are mediated via paracrine release of TGF-β.

Discussion

The major findings of this study are (1) acute elevations in filling pressure adversely affect atrial endocardial thrombomodulin expression; (2) downregulation of endocardial thrombomodulin expression impairs local protein C activation and contributes to local thrombin generation; and
endocardial thrombomodulin expression is negatively regulated during acute pressure overload by the paracrine effects of TGF-β secreted by cardiac connective tissue in response to pressure-induced chamber stretch.

It has long been recognized that patients with heart failure develop intracardiac thrombi that cause cerebral, pulmonary, and peripheral arterial thromboembolization. Thrombus formation has traditionally been viewed as a consequence of blood stasis that results from impaired ventricular function. If the presence of atrial fibrillation is excluded, however, the degree of left ventricular dysfunction only weakly correlates with thromboembolic risk. This suggests that factors other than stasis contribute to intracardiac thrombus formation in patients with heart failure, of which the overwhelming majority remain in sinus rhythm. We believe that ours is the first study to convincingly demonstrate that elevated cardiac filling pressures can adversely affect endocardial function and predispose to intracardiac thrombus formation independent of blood stasis.

Thrombomodulin is but 1 of several anticoagulant molecules expressed by endothelial cells that protect against pathological thrombosis. The level of thrombomodulin expression varies among endothelial cell types and therefore its relative contribution to vascular thromboresistance may differ between vascular beds. Several elements of the present study suggest that thrombomodulin is a critical contributor to endocardial thromboresistance. The first is that thrombomodulin is expressed in abundance by the endocardial endothelium. In addition to the rat, we have also found that in situ endocardial thrombomodulin expression is also robust in the rabbit and in humans (Figure II). Second, increased local thrombin generation in our model was proportional to the downregulation of thrombomodulin and occurred despite changes in the expression of other anticoagulant molecules that would be expected to inhibit thrombus formation. Third, and most important, targeted restoration of thrombomodulin expression with adenovirus-mediated gene transfer effectively reduced local thrombin generation.

In prior studies with rabbit vein grafts, we identified pressure-induced vascular stretch as a novel and potent negative regulator of in vivo thrombomodulin expression. The present study extends these findings by providing evidence of a second, and uniquely important, vascular bed in which stretch modulates thrombomodulin expression and by identifying paracrine release of TGF-β as the critical molecular mediator. TGF-β is a multifunctional dimeric polypeptide growth factor involved in a diverse array of biological processes that include embryogenesis, tumor growth, wound healing, and tissue remodeling. TGF-β is intimately involved in the adaptive response of cardiac and vascular tissue to pressure overload. In systolic heart failure, for example, TGF-β expression is known to be markedly increased in cardiac myocytes and fibroblasts, where it is a recognized autocrine and paracrine mediator of hypertrophy and fibrosis.

In the model of acute pressure overload used for this study, a marked increase in local TGF-β expression was observed in the left atrium but not in the left ventricle and appeared to originate predominantly from cardiac fibroblasts. This was likely the result of the relatively greater hemodynamic load imposed on the atrium and explains why endocardial thrombomodulin expression in the ventricle did not change. A recognized limitation of this model is the absence of ventricular remodeling and systolic dysfunction common to most chronic heart failure models. Although the model mostly replicates features of diastolic heart failure, it was precisely the absence of systolic dysfunction that obviated potential confounding effects of blood stasis on intracardiac thrombin generation. In models of chronic systolic dysfunction, TGF-β expression is increased in ventricular tissue, and it is reasonable to predict that thrombomodulin expression in the overlying endocardium would also be reduced. Adaptive responses, however, may complicate this issue, as there is evidence that myocardial TGF-β expression may vary according to the cause of heart failure and may change over time as heart failure progresses. This raises the possibility that the risk and chamber origin of thrombus formation may change over time, and the risk may be greater for different types of heart failure. With the observed association between TGF-β expression and endocardial dysfunction as a conceptual framework, future studies that use chronic models of systolic heart failure can be used to investigate these possibilities.

Extremely little is known about the molecular mechanism by which TGF-β regulates thrombomodulin expression in endothelial cells. TGF-β signal transduction is initiated via binding to specific serine/threonine type I and type II receptor complexes located on the endothelial cell surface. Receptor engagement causes the phosphorylation of several intracellular effector molecules known as Smads. Whereas most of the Smad proteins positively regulate TGF-β signal transduction, Smad6 and Smad7 are known to exert inhibitory effects. In the only published study to date that investigates the mechanism of thrombomodulin modulation by TGF-β, antisense inhibition of Smad7 potentiated the effects of TGF-β, whereas antisense inhibition of a splice variant of Smad6, known as Smad6s, unexpectedly blunted the downregulation of thrombomodulin in human umbilical endothelial cells by TGF-β. As Smad6 and Smad7 are differentially expressed in the endothelium of normal and atherosclerotic arteries, they may be able to differentially modulate the paracrine effects of TGF-β on thrombomodulin expression in various vascular beds and disease states. What remains completely unknown at the present time is the nature of the signaling pathway distal to Smad activation that may regulate thrombomodulin gene transcription.

In addition to heart failure, our findings may have implications for understanding the thrombogenic potential of other forms of cardiovascular disease, such as atrial fibrillation. Thrombus formation in atrial fibrillation is also primarily ascribed to blood stasis in a left atrial appendage that is not contracting. However, it is well known that atrial fibrillation in patients with structurally normal hearts, ie, “lone atrial fibrillation,” is associated
with a relatively small risk (<0.5% per year) of thromboembolic stroke compared with atrial fibrillation that occurs in the setting of hypertension, heart failure, or valvular heart disease (5% to 12% per year). As these latter conditions are all characterized by pressure-induced atrial distension, it is possible that endocardial dysfunction and altered thrombomodulin expression may contribute substantially to the thromboembolic risk in these patients. This concept is supported by data from a rat model of atrial fibrillation in which atrial thrombomodulin expression decreased by 35% after 8 hours of rapid atrial pacing. Interestingly, the left atrial pressure in this model triples during pacing, which raises the possibility that the effects on thrombomodulin expression were primarily the result of hemodynamic rather than electrical influences.

Sources of Funding
This work was supported in part by an American Heart Association Grant-in-Aid (0555515U) to Dr Rade and by National Heart, Lung and Blood Institute Grant HL-080142 to Dr Rade. The Flow Cytometry Cell Sorting Core Laboratory of the Bloomberg School of Public Health is supported by National Institute of Allergy and Infectious Disease Grant 5P30AI042855.

Disclosures
None.

References
Patients with congestive heart failure, atrial fibrillation, and valvular heart disease are at risk for thromboembolic events, including stroke. Thromboembolus formation in these patients has historically been attributable to blood stasis within the cardiac chambers. The present study reveals that elevated cardiac chamber filling pressure, which is common to each of these conditions, can adversely affect endocardial function and contribute to intracardiac thrombus formation. This may help explain why patients with heart failure and preserved ventricular function remain at risk for thromboembolic stroke, whereas patients with atrial fibrillation and structurally normal hearts are at relatively low risk. The present study also identifies transforming growth factor-β, released in paracrine fashion by pressure-induced chamber stretch, as a potent negative regulator of endocardial thromboresistance. Targeted inhibition of the transforming growth factor-β signaling pathway could represent a novel therapeutic alternative for prevention of thromboembolus formation in selected cardiac patients currently being treated with systemic anticoagulation.
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Circulation. 2007;115:67-75; originally published online December 26, 2006;
doi: 10.1161/CIRCULATIONAHA.106.640698
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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