

*Mechanisms of Disease*FRANKLIN H. EPSTEIN, M.D., *Editor***VASCULAR-BED-SPECIFIC  
HEMOSTASIS AND  
HYPERCOAGULABLE STATES**ROBERT D. ROSENBERG, M.D., PH.D.,  
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**H**EMOSTASIS is a physiologic mechanism that maintains blood in a fluid state within the circulation. The coagulation of blood is mediated by cellular components and soluble plasma proteins. In response to vascular injury, circulating platelets adhere, aggregate, and provide cell-surface phospholipid for the assembly of blood-clotting enzyme complexes. The extrinsic pathway of blood coagulation is initiated when blood is exposed to non-vascular-cell-bound tissue factor in the subendothelial space (Fig. 1). Tissue factor binds to activated factor VII, and the resulting enzyme complex activates factors IX and X of the intrinsic and common coagulation pathways, respectively. Factor IX activated by the tissue-factor pathway in turn activates additional factor X, in a reaction that is greatly accelerated by a cofactor, factor VIII. Once activated, factor X converts prothrombin to thrombin (factor IIa) in a reaction that is accelerated by factor V. In the final step of the coagulation pathway, thrombin cleaves fibrinogen to generate fibrin monomers, which then polymerize and link to one another to form a chemically stable clot. Thrombin also feeds back to activate cofactors VIII and V, thereby amplifying the coagulation mechanism.

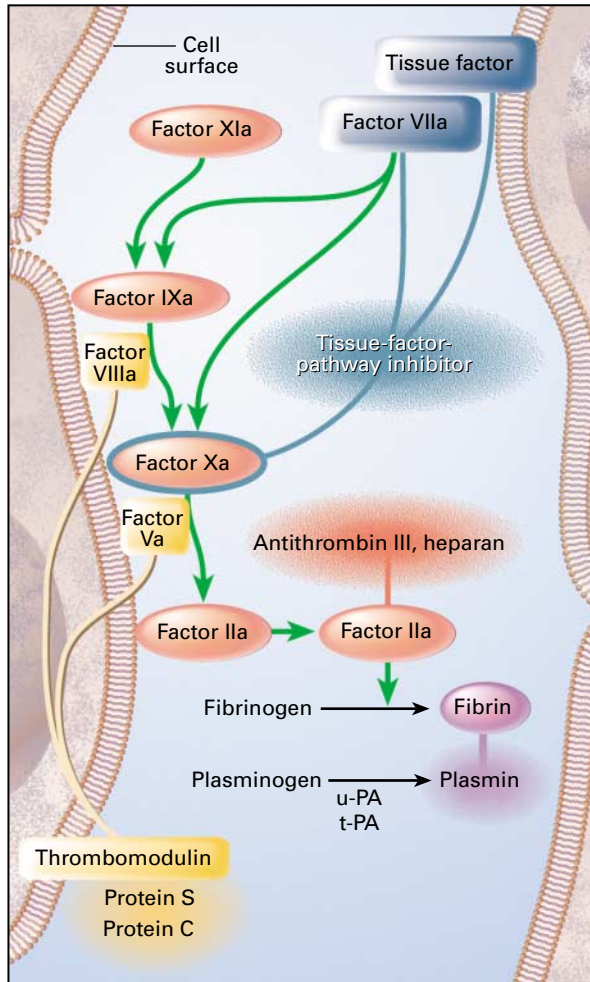
The blood-coagulation cascade has the ability to transduce a small initiating stimulus into a large fibrin clot. The potentially explosive nature of this

cascade is offset by natural anticoagulant mechanisms. The maintenance of adequate blood flow and the regulation of cell-surface activity limit the local accumulation of activated blood-clotting enzymes and complexes. Antithrombin III is a plasma protein that inhibits the activity of the serine proteases of the intrinsic and common coagulation pathways. In the presence of endogenous heparan sulfate, the rate of inactivation is increased by a factor of several thousand. In the presence of thrombomodulin bound to endothelial cells, thrombin activates protein C, which in turn cleaves activated factors VIII and V. Like other reactions in hemostasis, this one is accelerated by a cofactor, in this case protein S. The tissue-factor-pathway inhibitor is a lipoprotein-associated plasma protein that forms a quaternary complex with tissue factor and activated factors VII and X, thereby inhibiting the extrinsic coagulation pathway. Finally, a series of linked enzymatic reactions generates plasmin, a serine protease that acts on fibrin to dissolve preformed clots.

Congenital and acquired hypercoagulable states arise when there is an imbalance between the anticoagulant and prothrombotic activities of plasma in which the prothrombotic activities predominate.<sup>1-6</sup> The mechanisms that underlie the thrombotic phenotype are defined by Virchow's triad: a decrease in blood flow, injury to the vessel wall, and a change in the systemic balance of procoagulant and anticoagulant factors. According to this scheme, one might predict that the loss of a circulating anticoagulant would cause a shift in the hemostatic balance and thereby promote a diffuse thrombotic diathesis. This prediction does not hold true. In fact, systemic alterations in the hemostatic mechanism typically give rise to local thrombotic lesions in discrete segments of the vascular tree. The pathophysiologic basis for this observation is poorly understood. The conventional wisdom is that the focal lesions are attributable to superimposed defects in the vascular wall or blood flow. In other words, the phenotypic fate of systemic hypercoagulable states rests on the ability of these two local mechanisms to compensate for a uniform change in the hemostatic balance. We now believe that the focal nature of thrombotic lesions is better understood in the context of signaling pathways specific to the vascular bed.

According to this model, the endothelium integrates different extracellular signals and cellular responses in different regions of the vascular tree. On the one hand, the endothelium is exposed to diverse environmental cues, including those from integrins and growth factors, hemodynamic forces, and cell-

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**Figure 1.** The Clotting Cascade.

Coagulation is initiated by the exposure of blood to tissue factor bound to cell membranes. Tissue factor interacts with factor VIIa to convert factor IX to factor IXa and factor X to factor Xa (only the activated forms are shown). Factor IXa converts factor X to factor Xa. Factor Xa generates factor IIa (thrombin) from factor II (prothrombin). Each of these reactions takes place on an activated cell surface. Once factor IIa is generated, it cleaves plasma fibrinogen to generate fibrin. The tissue-factor-pathway inhibitor forms a quaternary structure with tissue factor, factor VIIa, and factor Xa (shown in blue). The thrombomodulin-protein C-protein S pathway (shown in yellow) inactivates factors Va and VIIIa. Antithrombin III inactivates factors XIa, IXa, Xa, and IIa (shown in orange) in a reaction that is accelerated by the presence of heparan sulfate. In the fibrinolytic pathway, tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA) convert plasminogen to plasmin. Once generated, plasmin proteolytically degrades fibrin (shown in purple).

to-cell signaling. On the other hand, the ability of the endothelium to transduce a given signal is regulated in both space and time. As a result of these variables, endothelial-cell-derived procoagulant and anticoagulant activities are differentially expressed throughout the vascular tree (Fig. 2). The focal thrombotic phenotype that is associated with a systemic loss of anticoagulant function reflects the critical role of that particular mechanism in restricted sites of the vasculature.

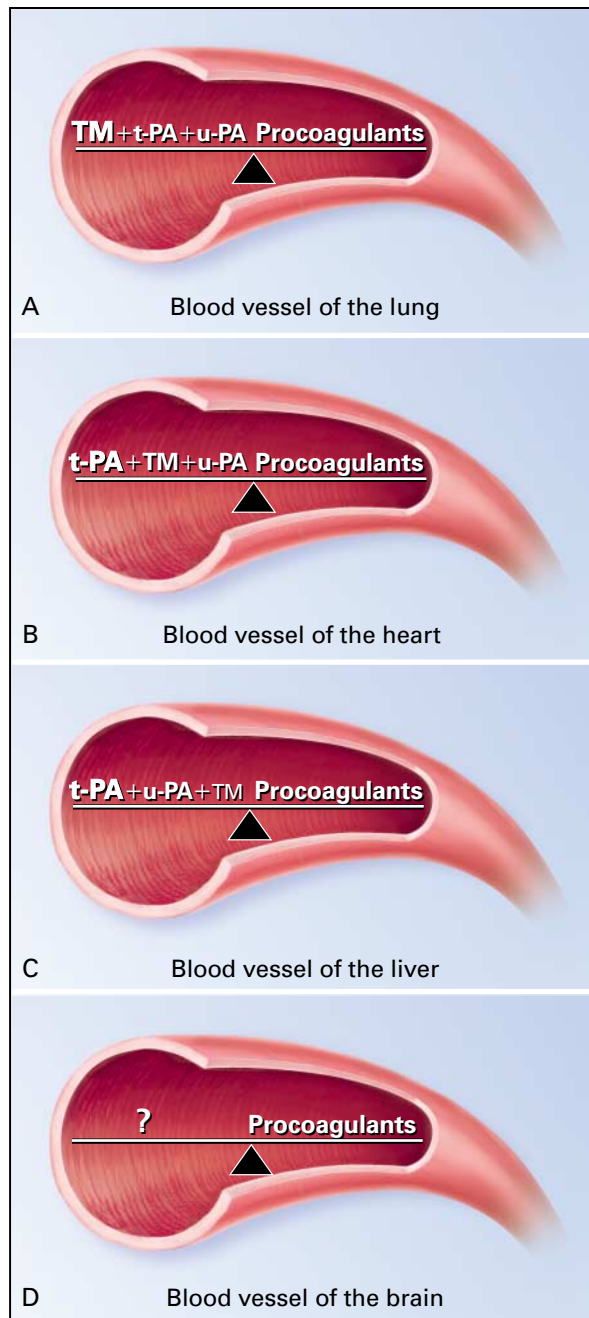
In the sections that follow, we will provide evidence that supports our model of vascular-bed-specific hemostasis. We will review the focal patterns of thrombosis in hypercoagulable states in both humans and animals. We will then discuss recent data suggesting that the components of hemostasis are differentially regulated in various vascular beds.

### THE SYSTEMIC-DEFECT- LOCAL-PHENOTYPE PARADOX

#### Hypercoagulability in Humans

Hypercoagulable states arise from an imbalance between procoagulant and anticoagulant forces. A striking feature of these conditions is the focal nature of the thrombotic diathesis (Table 1).<sup>1-29</sup> For example, congenital deficiencies of antithrombin III, protein C, and protein S are associated with an increased risk of deep-vein thrombosis of the lower but not upper limbs, and deficiency states involving these proteins do not confer a predisposition to arterial thrombosis.<sup>1-7</sup> One interesting exception is a mutation of the heparin-binding site of antithrombin III. In this case, the abnormal antithrombin III does not bind to heparin and is therefore much less efficient at inhibiting thrombin and other, more proximal enzymes in the coagulation cascade. The resulting thrombotic phenotype involves both arteries and veins.<sup>8-11</sup> Factor V Leiden is usually associated with an increased risk of deep venous thrombosis of the legs and brain<sup>12,13</sup> but may also confer an increased risk of acute myocardial infarction in young women who smoke.<sup>13,14</sup> The prothrombin G20210A mutation predisposes patients to deep venous thrombosis in the legs and brain<sup>12,15,16</sup> and may be a genetic risk factor for both stroke and ischemic heart disease.<sup>17-19</sup>

Several acquired hypercoagulable states are also associated with vascular-bed-specific thrombosis. Paroxysmal nocturnal hemoglobinuria and myeloproliferative diseases are characterized by an unusually high incidence of thrombosis of the hepatic, portal, and mesenteric veins.<sup>20-22</sup> Patients with the antiphospholipid-antibody syndrome have a propensity toward the formation of clots within particular venous and arterial segments of the vascular tree, including blood vessels of the retina and placenta.<sup>23-25</sup> Warfarin-induced necrosis of the skin is associated with extensive thrombosis of the postcapillary venules and small veins within subcutaneous adipose tissue.<sup>26</sup> The



**Figure 2.** Vascular-Bed-Specific Hemostasis.

The interaction of the various anticoagulant and procoagulant forces promotes overall hemostasis, but the actual components of this interaction differ from one vascular bed and one organ to another. Thrombomodulin (TM) is more important in maintaining the hemostatic balance of the lungs and heart (Panels A and B) than it is in the liver, whereas the fibrinolytic pathway (tissue-type plasminogen activator [t-PA] and urokinase-type plasminogen activator [u-PA]) is important in mediating blood fluidity in all three vascular beds (Panels A, B, and C). Neither thrombomodulin nor fibrinolysis is essential in maintaining balanced hemostasis in the blood vessels of the brain (Panel D). The physiologically relevant natural anticoagulant mechanisms that are operative in this vascular bed have not been identified.

microthrombotic lesions characteristic of thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome are detectable in all organs, with the notable exception of the liver and the lungs.<sup>27-29</sup>

On the one hand, each of these disorders is characterized by a systemic dysfunction of the hemostatic pathway, caused by a congenital or acquired defect resulting in the absence of a circulating natural anticoagulant or the presence of an activated cell-surface membrane. On the other hand, each condition is associated with a unique and restricted phenotype. How do we account for the diverse responses to what seem to be disruptions of the entire hemostatic system? In keeping with Virchow's triad, the phenotype must arise from local changes in blood flow, disruption of the vascular wall, or vascular-bed-restricted alterations in the balance of anticoagulant and procoagulant factors.

There is little question that blood flow limits the extent of the procoagulant response, and its relative deficiency may underlie the tendency for thrombi to develop in certain parts of the vascular tree. However, blood flow is not the principal determinant of hemostatic control in all vascular beds. Local disruption of the integrity of the blood-vessel wall may also have an important effect on certain thrombotic phenotypes. For example, surgical procedures put patients with congenital deficiencies of protein C, protein S, or antithrombin III at particularly high risk for perioperative deep-vein thrombosis.<sup>30</sup> However, similar procedures in patients with factor V Leiden do not confer an increased risk of thrombosis.<sup>31</sup> Moreover, the loss of integrity of the vessel wall caused by the rupture of a coronary atheromatous plaque does not lead to a higher rate of occlusive thrombus in patients with defects in the protein C, protein S, or antithrombin III pathways. Indeed, the very absence of an association between the loss of these natural anticoagulant mechanisms and the incidence of myocardial infarction suggests that these particular pathways are relatively unimportant contributors to the hemostatic balance of the coronary vasculature. Such epidemiologic observations provide strong support for the existence of regionally distinct hemostatic balances.

#### Animal Models of Hypercoagulability

An important clue to the focal nature of hemostatic control is found in the uneven distribution of the various anticoagulant and procoagulant factors throughout the vascular tree. For example, the levels of von Willebrand factor messenger RNA (mRNA) and protein vary from one vascular bed to another.<sup>32-36</sup> Histochemical studies of the thrombomodulin receptor have revealed high levels within the endothelium of the lungs and heart, yet barely detectable levels within the blood-brain barrier.<sup>37</sup> Similarly, there is marked heterogeneity in the expression of the var-

**TABLE 1.** HYPERCOAGULABLE STATES AND ASSOCIATED SITES OF THROMBOSIS.

HYPERCOAGULABLE STATE	CHARACTERISTIC SITES OF THROMBOSIS	REFERENCE
<b>Congenital</b>		
Deficiency of protein C	Deep veins of legs	Rosenberg and Aird, <sup>1</sup> Greaves and Preston, <sup>2</sup> Nachman and Silverstein, <sup>3</sup> Macik and Ortel, <sup>4</sup> Thomas and Roberts, <sup>5</sup> De Stefano et al., <sup>6</sup> Martinelli et al. <sup>7</sup>
Deficiency of protein S	Deep veins of legs	Rosenberg and Aird, <sup>1</sup> Greaves and Preston, <sup>2</sup> Nachman and Silverstein, <sup>3</sup> Macik and Ortel, <sup>4</sup> Thomas and Roberts, <sup>5</sup> De Stefano et al., <sup>6</sup> Martinelli et al. <sup>7</sup>
Deficiency of antithrombin III Heterozygous	Deep veins of legs	Rosenberg and Aird, <sup>1</sup> Greaves and Preston, <sup>2</sup> Nachman and Silverstein, <sup>3</sup> Macik and Ortel, <sup>4</sup> Thomas and Roberts, <sup>5</sup> De Stefano et al., <sup>6</sup> Martinelli et al. <sup>7</sup>
Homozygous for mutation of heparin-binding domain	Deep veins and arteries	Boyer et al., <sup>8</sup> Finazzi et al., <sup>9</sup> Chowdhury et al., <sup>10</sup> Okajima et al. <sup>11</sup>
Presence of factor V Leiden	Deep veins of legs and brain, coronary arteries*	Martinelli et al., <sup>12</sup> Price and Ridker, <sup>13</sup> Rosendaal et al. <sup>14*</sup>
Presence of prothrombin G20210A mutation	Deep veins of legs and brain, coronary and cerebral arteries†	Martinelli et al., <sup>12</sup> Margaglione et al., <sup>15</sup> Reuner et al., <sup>16</sup> De Stefano et al., <sup>17</sup> Arruda et al., <sup>18</sup> Rosendaal et al. <sup>19</sup>
<b>Acquired</b>		
Paroxysmal nocturnal hemoglobinuria	Portal and hepatic veins	Dilawari et al., <sup>20</sup> Hillmen et al., <sup>21</sup> Socie et al. <sup>22</sup>
Myeloproliferative diseases	Portal and hepatic veins	Dilawari et al. <sup>20</sup>
Antiphospholipid-antibody syndrome	Arteries and veins	Asherson et al., <sup>23</sup> Khamashta et al., <sup>24</sup> Finazzi et al. <sup>25</sup>
Warfarin-induced skin necrosis	Subcutaneous microvessels	Comp et al. <sup>26</sup>
Thrombotic thrombocytopenic purpura	All microvessels, with the exception of those of the liver and lung	Ridolfi and Bell, <sup>27</sup> Asada et al., <sup>28</sup> Ruggenti and Remuzzi <sup>29</sup>

\*Most studies have not confirmed an association between factor V Leiden and arterial disease. The risk of ischemic heart disease may be limited to young women who smoke.

†The association between the prothrombin G20210A mutation and arterial disease requires confirmation in additional studies.

ious components of the fibrinolytic pathway, including urokinase-type plasminogen activator, tissue-type plasminogen activator, and plasminogen-activator inhibitor type I.<sup>38,39</sup> Taken together, these findings strongly suggest but do not prove that the coagulation system is regulated in a tissue-specific manner.

More recently, gene-targeting studies have provided proof of this concept in animals. Mice have been bred with a thrombomodulin gene containing a point mutation that specifically deletes the anticoagulant activity of the protein.<sup>40</sup> Mice homozygous for the point mutation produce thrombomodulin that binds very poorly to thrombin and thus produce little activated protein C. Most important, these mice have 10 to 30 times as much fibrin deposition in the lungs, heart, and spleen as normal mice, and they have fibrin deposition and clots within the capillar-

ies, veins, and arteries of these organs. Under hypoxic conditions, there is an additional 10-fold increase in fibrin deposition in the lungs, suggesting that the environment interacts with the genotype to modulate phenotypic expression.

Fibrin deposition is increased in the heart, spleen, and lungs of mice that lack tissue-type plasminogen activator and urokinase-type plasminogen activator,<sup>40</sup> but the brain and kidneys are completely spared. However, in contrast to thrombomodulin-deficient mice, these animals have increased fibrin deposition in the liver. Moreover, the absolute levels of fibrin in the heart and lungs differ between mice with thrombomodulin deficiency and those with deficiencies of tissue-type plasminogen activator and urokinase-type plasminogen activator.<sup>40</sup> These results suggest that the contribution of a given anticoagulant mech-

**TABLE 2.** EXTRACELLULAR SIGNALS THAT REGULATE PROCOAGULANT OR ANTICOAGULANT mRNA EXPRESSION IN ENDOTHELIAL CELLS.

SOURCE OF SIGNAL	EFFECT	REFERENCE
Tumor necrosis factor $\alpha$	Decreases expression of thrombomodulin Increases expression of plasminogen-activator inhibitor type 1 and tissue factor	Conway and Rosenberg, <sup>43</sup> Hirokawa and Aoki <sup>44</sup> Sawdey et al., <sup>45</sup> Conway et al. <sup>46</sup>
Interleukin-1	Decreases expression of thrombomodulin	Hirokawa and Aoki <sup>44</sup>
Transforming growth factor $\beta$	Decreases expression of thrombomodulin Increases expression of plasminogen-activator inhibitor type 1	Ohji et al. <sup>47</sup> Sawdey et al. <sup>45</sup>
Vascular endothelial growth factor	Increases expression of thrombomodulin, plasminogen-activator inhibitor type 1, and tissue-type plasminogen activator	Calnek and Grinnell, <sup>48</sup> Pepper et al. <sup>49</sup>
Platelet-derived growth factor	Increases expression of von Willebrand factor	Edelberg et al. <sup>50</sup>
Shear stress	Increases expression of thrombomodulin, tissue-type plasminogen activator, tissue factor, and nitric oxide synthase	Malek et al., <sup>51</sup> Takada et al., <sup>52</sup> Kawai et al., <sup>53</sup> Lin et al., <sup>54</sup> Uematsu et al., <sup>55</sup> Ziegler et al. <sup>56</sup>
Hypoxia	Increases expression of plasminogen-activator inhibitor type 1 Decreases expression of tissue-type plasminogen activator	Pinsky et al. <sup>57</sup> Pinsky et al. <sup>57</sup>

anism to the hemostatic balance varies from one vascular bed to another. In organs such as the heart and lungs, thrombomodulin, tissue-type plasminogen activator, and urokinase-type plasminogen activator all have roles in tempering the procoagulant forces, whereas in organs such as the brain and kidneys, other anticoagulant mechanisms presumably counterbalance the thrombotic tendency (Fig. 2). Taken together, the data from these studies suggest that thrombosis occurs in an organ-specific fashion through the critical interplay of genetic and environmental factors.

#### VASCULAR-BED-SPECIFIC SIGNALING PATHWAYS

The endothelium is involved in a wide range of homeostatic processes, including the maintenance of blood fluidity, the control of vasomotor tone, and the transfer of nutrients and cells between blood and underlying tissue.<sup>41</sup> As discussed earlier, hemostasis is mediated by a balance of inducible anticoagulant and procoagulant forces. On the anticoagulant side, the endothelium releases heparan sulfate and prostacyclin; expresses thrombomodulin, tissue-type plasminogen activator, tissue-factor-pathway inhibitor, and endothelial nitric oxide synthase; and provides a nonthrombogenic cell-surface membrane.<sup>42</sup> On the procoagulant side, endothelial cells release von Willebrand factor and plasminogen-activator in-

hibitor type 1, express receptors for cell-surface tissue factor and thrombin, expose critical binding sites for coagulation-factor complexes, and attract platelets and monocytes to sites of activation. Under normal conditions, a delicate balance between the anticoagulant and procoagulant activities of the endothelium is achieved by a series of regulatory linking mechanisms. These mechanisms are capable of integrating multiple signals to generate a response that varies both in space and time, so that within any given segment of the vascular tree, the endothelium is capable of shifting the hemostatic balance from moment to moment. The temporal and spatial nature of these regulatory mechanisms endows the hemostatic system with tremendous flexibility. At the same time, these very properties make the endothelium vulnerable to focal dysfunction and pathophysiologic disorders.

#### Extracellular Signals

What are the mechanisms responsible for generating and maintaining vascular-bed-specific phenotypes? One is the array of signals residing in the microenvironment that regulate the procoagulant and anticoagulant properties of the endothelium. These signals include growth factors, cytokines, mechanical forces, circulating lipoproteins, coagulation factors, components of the extracellular matrix, and neighboring cells (Table 2).<sup>43-57</sup> The extracellular signals

**TABLE 3.** RESPONSES OF ENDOTHELIAL CELLS TO EXTRACELLULAR SIGNALS.

SIGNAL	RESPONSE	REFERENCE
<b>In vitro</b>		
Thrombin	Increases expression of G protein in endothelial cells derived from cerebral microvessels and the temporal artery but not in endothelial cells derived from omental arteries; mediates endothelial-dependent relaxation of the internal thoracic artery and contraction of the saphenous vein	Shreeve et al. <sup>64</sup> Yang et al. <sup>65</sup>
Interleukin-1 $\alpha$	Specifically represses gene expression in dermal endothelial cells but not in other endothelial cells	Gille et al. <sup>66</sup>
Cyclic strain and tumor necrosis factor $\alpha$	Confer differential effects on tissue-factor activity in endothelial cells derived from the aorta, the umbilical vein, and dermal microvessels	Silverman et al. <sup>67</sup>
Plasma from patients with thrombotic thrombocytopenic purpura	Mediates differential effects on production of prostacyclin and apoptosis in endothelial cells derived from various vascular beds	Mitra et al. <sup>68</sup>
Platelet-derived growth factor	Induces expression of von Willebrand factor in a subgroup of microvascular endothelial cells of the heart	Edelberg et al. <sup>50</sup>
<b>In vivo</b>		
<i>Escherichia coli</i> sepsis	Selectively up-regulates tissue factor in a subgroup of endothelial cells in the marginal zone of splenic follicles	Drake et al. <sup>69</sup>
Traumatic shock	Preferentially increases expression of P-selectin in the postcapillary venules of the lung	Armstead et al. <sup>70</sup>
Blood flow	Induces expression of endothelial nitric oxide synthase in the aorta but not in the pulmonary artery	Everett et al. <sup>71</sup>

are transduced by endothelial cell-signaling networks, resulting in alterations in procoagulant and anticoagulant mRNA, protein, and function.

The extracellular milieu is dynamically regulated in both time and space. For example, the degree of shear stress varies in different regions of the vascular tree. The hemodynamic force is higher in arteries than in veins and is accentuated at sites just distal to bifurcations in blood vessels.<sup>58,59</sup>

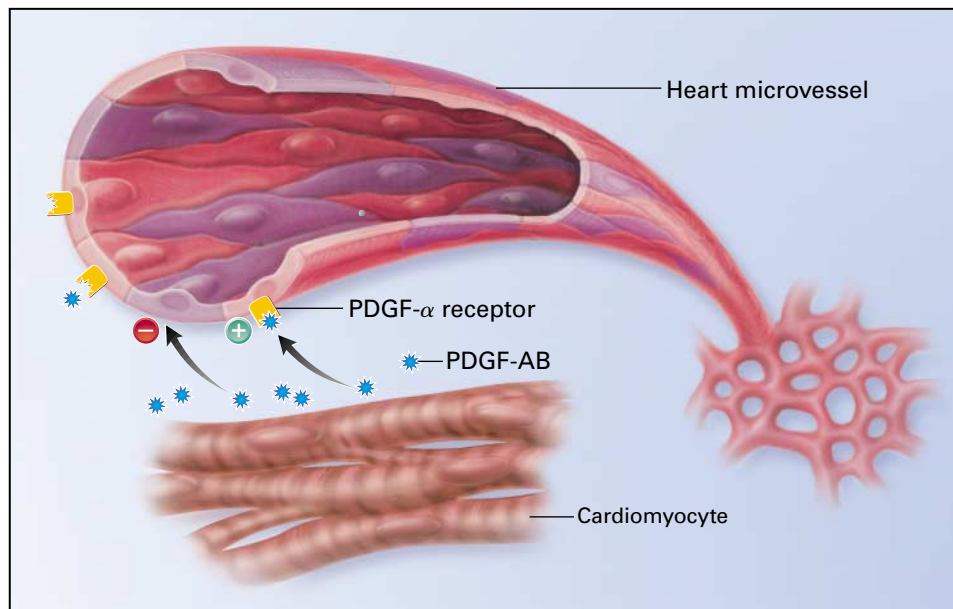
Levels of shear stress also vary from one moment to the next. The frictional drag on the endothelium is increased during the systolic phase of the cardiac cycle and during exercise.<sup>60</sup> Shear stress induces the expression of a network of endothelial-cell genes that includes thrombomodulin, tissue-type plasminogen activator, tissue factor, and nitric oxide synthase.<sup>51-56</sup> The spatial and temporal changes in the force of shear stress probably contribute to the different patterns of expression of these procoagulant and anticoagulant factors.

Cell-to-cell communication also has an important role in setting up local gradients of hemostatic factors. For example, expression of the fibrinolytic protein plasminogen-activator inhibitor type 1 in human endothelial cells is down-regulated by conditioned medium obtained from smooth-muscle cells of the

pulmonary artery but is up-regulated by smooth-muscle cells isolated from the aorta, the umbilical vein, and arteries.<sup>61,62</sup> In another study, the expression of von Willebrand factor in endothelial cells of the heart was shown to be induced by a cardiomyocyte-dependent pathway.<sup>63</sup> The local blend of micro-environmental cues provides the endothelium with an overall stimulus, thereby modulating the pattern of expression of anticoagulant and procoagulant genes. The diversity of these cues has a key role in generating and maintaining vascular-bed-specific phenotypes.

#### Cell-Subtype-Specific Signaling Pathways

A second mechanism that contributes to the generation of vascular-bed-specific phenotypes is signaling pathways specific to cell subtype. Endothelial cells from various vascular beds have different responses to the same signals (Table 3).<sup>64-71</sup> For example, increases in blood flow lead to an up-regulation of nitric oxide synthase mRNA in the endothelium of the aorta, but not in the endothelium of the pulmonary artery.<sup>71</sup> In the heart, only some of the microvascular endothelial cells in the myocardium express the gene for von Willebrand factor.<sup>50</sup> The restricted distribution of this protein is governed



**Figure 3.** Endothelial-Cell-Specific Signaling Pathways.

Vascular-bed-specific hemostatic balances are controlled at two levels. First, endothelial cells from various regions of the vascular tree are subject to the influence of the local environment. For example, microvascular endothelial cells in the heart are exposed to platelet-derived growth factor AB heterodimer (PDGF-AB) by virtue of their proximity to underlying cardiomyocytes. In contrast, PDGF-AB is not detectable in other microvascular beds, such as that of the lung. Second, endothelial cells from a single region vary in their capacity to transduce a given signal. In this example, only some of the microvascular endothelial cells in the heart have PDGF- $\alpha$  receptors. Endothelial cells that have these receptors (green circles) respond to the PDGF-AB signal, whereas those without the receptors (red circles) do not. The restricted nature of these linking mechanisms both between and within vascular beds contributes to the diverse nature of the hemostatic balance.

by a signaling pathway mediated by cardiomyocyte-dependent, platelet-derived growth factor AB heterodimer. Endothelial cells that have receptors for platelet-derived growth factor  $\alpha$  can transduce the signal, whereas neighboring endothelial cells without receptors for platelet-derived growth factor  $\alpha$  lack this ability (Fig. 3).<sup>50</sup> Finally, plasma from patients with thrombotic thrombocytopenic purpura has different effects on endothelial cells from different organs.<sup>68</sup> The production of prostacyclin is decreased and apoptosis is increased in endothelial cells from renal and cerebral vessels but not in endothelial cells from the lungs or the liver.<sup>68</sup> These findings are closely correlated with the distribution of microthrombi in patients with thrombotic thrombocytopenic purpura and suggest that the phenotype is governed by the various responses of endothelial cells to an overall stimulus.

The activity of regulatory linking mechanisms probably sets critical limits on the range of responses by endothelial cells. In other words, the responses are shaped by the extracellular milieu only to the extent that endothelial cells have the ability to transduce the local signals. In many cases, the extracellular and intracellular components of the signaling pathway

are closely related. For example, flk type 1, a vascular endothelial growth factor receptor, is up-regulated in cardiac microvascular endothelial cells by a myocyte-dependent signaling pathway, suggesting that any differences in the response to vascular endothelial growth factor are ultimately determined by the microenvironment.<sup>50</sup> In addition, a large number of growth factors, cytokines, selectins, and integrins are released by endothelial cells into the local environment, where they exert paracrine control over intracellular signaling pathways.

A final point is that signaling pathways for endothelial cells are typically involved in regulating networks of genes. For example, the pathway for platelet-derived growth factor  $\alpha$  receptor controls the expression of components within the angiogenic pathway (e.g., vascular endothelial growth factor and flk type 1) and the coagulation pathway (e.g., von Willebrand factor and tissue-type plasminogen activator).<sup>50</sup> Coregulation of these two systems is biologically plausible. In the case of a hemostatic imbalance in the coronary microcirculation, with an increase in the generation of fibrin, a parallel activation of angiogenesis would represent a compensatory mechanism. Indeed, one might speculate

that certain genetic or environmental conditions interfere with a critical control point, leading to arterial thrombosis in the absence of an angiogenic response.

#### Transcriptional Regulation

A final mechanism underlying the generation of vascular-bed-specific phenotypes is found at the level of transcription. Transcriptional control is best viewed as a regulatory linking mechanism. It is generally believed that tissue-specific genes are regulated by common mechanisms in different cells of the same lineage. For example, there is no evidence that factor VIII is regulated by distinct DNA-protein interactions in various regions of the liver. In at least some cases, however, endothelial-cell genes are regulated by different pathways in different regions of the vascular tree.

The most compelling support for this model of gene regulation is derived from studies in transgenic mice. A short 733-bp region of the von Willebrand factor promoter directed expression exclusively in endothelial cells of the brain, whereas a promoter that contained additional DNA elements upstream and downstream directed expression in endothelial cells of the heart and skeletal muscle,<sup>63,72</sup> and it seems likely that more distal promoter regions of the von Willebrand factor gene are necessary for expression in endothelial cells in other vascular beds such as those of the lung and the spleen. Similar findings have been reported for the *Tie-2* gene, which codes for a tyrosine protein kinase specific to endothelial cells and has a critical role in the development of the cardiovascular system.<sup>73</sup> A construct containing the immediate 5' upstream region of *Tie-2* mediated expression in a limited subgroup of endothelial cells in embryonic mice, whereas the inclusion of enhancer elements within the first intron resulted in expression in many endothelial cells in adult mice.<sup>74,75</sup> In the case of the von Willebrand factor gene, further studies showed that the elements of vascular-bed-specific transcriptional control were ultimately responsive to microenvironmental cues.<sup>50,63</sup> Thus, the overall expression of a single gene, such as that for von Willebrand factor, may be mediated by distinct vascular-bed-specific signaling pathways that begin in the extracellular milieu and end at separate sites on the promoter region of the gene. This expanded repertoire of interactions between DNA and protein provides the endothelium with an even greater capacity for integrating multiple extracellular signals.

#### CORONARY-ARTERY THROMBOSIS

The acute coronary artery syndrome, by definition, represents a hypercoagulable state. Traditionally, focal disruption of an atheromatous plaque triggers a generic coagulation response with subsequent

generation of thrombin and occlusion of the coronary artery. For many years, studies of the syndrome focused on the role of multiple environmental and genetic risk factors, cell types, and signaling pathways in mediating the generation and progression of the plaque itself. More recently, there has been a growing recognition of the primary role of the hemostatic mechanism in this process. In large clinical studies, subjects with higher plasma levels of certain procoagulant proteins and activation peptides had an increased risk of acute myocardial infarction.<sup>76-79</sup> In view of these findings, it is remarkable that congenital deficiencies of protein C, protein S, or antithrombin III do not confer an increased risk of infarction. This apparent paradox suggests that other anticoagulant mechanisms must be responsible for maintaining patent coronary vessels. Indeed, the gene-targeting studies discussed earlier have uncovered the critical role of fibrinolytic enzymes, such as tissue-type plasminogen activator and urokinase-type plasminogen activator, in preventing fibrin deposition within the vascular bed of the heart. Taken together, these findings suggest that the hemostatic balance within the coronary vessels is controlled by a vascular-bed-specific circuit. We speculate that thrombosis of the coronary artery arises through the interplay of plaque rupture and an alteration of this local hemostatic circuit.

#### CONCLUSIONS

We now recognize the role of the extrinsic coagulation pathway in initiating coagulation and the importance of the intrinsic coagulation pathway in amplifying the response through "cross-talk" and feedback mechanisms. We understand that natural anticoagulants, such as protein C, protein S, antithrombin III, and fibrinolytic enzymes, play an important part in dampening the coagulation response and ensuring that thrombin formation and fibrin deposition occur only when necessary. Only recently have we begun to appreciate the qualitatively unique nature of the hemostatic balance of each vascular bed. The molecular mechanisms that underlie these vascular-bed-specific differences are found in complex signaling networks that have evolved in the endothelial-cell lining of the vascular tree. As a barometer of its environment, the endothelium integrates and transduces multiple signals that vary in both time and space. Normally, regional networks of procoagulant and anticoagulant mechanisms yield a net balance in hemostasis.

In patients with congenital or acquired hypercoagulable states, signaling pathways are differentially affected in different segments of the vascular tree, leading to characteristic thrombotic phenotypes. The theory that the hypercoagulable state represents a systemic disorder is no longer tenable. Indeed, the emerging concept of multiple tissue-specific networks

of procoagulants and anticoagulants will probably serve as an important model for understanding other homeostatic processes.

*This article is dedicated to the memory of Judith Rosenberg, who contributed many of the ideas and wrote the first draft of this article.*

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