REVIEW ARTICLE

MECHANISMS OF DISEASE

Platelets, Petechiae, and Preservation of the Vascular Wall

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Platelets help to maintain blood circulation by controlling hemorrhage after an injury to the blood-vessel wall that causes physical or biochemical disruption of the endothelium. The development of an activated platelet plug at the site of trauma seals the vascular lesion and brings about hemostasis. The membrane-oriented processes of subendothelial adhesion of platelets, granule release, cohesion, aggregation, and plug stabilization involve several well-characterized ligand–receptor interactions[1] and will not be discussed further in this review.

Instead, we will focus on other functional contacts between platelets and endothelial cells. The most important of these is the maintenance of vascular integrity by the constitutive release of proangiogenic cytokines and growth factors (which we are calling trophogens) from platelets. These molecules, which platelets store within granules, bind to specific receptors on the surface of endothelial cells, thereby eliciting intracellular signaling that stabilizes the vascular-endothelium cadherin complex at intercellular adherens junctions (see Glossary).

One clinical aspect of the platelet–endothelial cell interaction can be seen in patients with thrombocytopenia. When platelets fall precipitously below critical levels (usually under 10,000 to 20,000 per cubic millimeter), molecular disassembly opens the zippers formed by adjacent intercellular endothelial junctions, causing extravasation of erythrocytes into the surrounding tissues. The characteristic microscopic feature of petechiae, the clinical hallmark of thrombocytopenia, is postcapillary venular extravasation of red cells at interendothelial junctions or gaps in the absence of overt trauma.[2] Marked thinning and attenuation of the endothelium with separation at the gap junctions has been noted in animals with thrombocytopenia.[3]

The Endothelial Cell–Megakaryocyte–Platelet Axis

Endothelial cells and megakaryocytes jointly participate in a number of physiological functions. They are the only cells that synthesize von Willebrand factor,[4,5] and they define a specific vascular niche in the bone marrow, where hematopoietic precursors and sinusoidal endothelium interact directly.[6] The process of platelet production through the formation of proplatelets and shedding of platelets occurs in megakaryocytes that have direct contacts with sinusoidal endothelial cells.[7] In vitro, megakaryocytes support the survival of bone marrow–derived sinusoidal endothelial cells, and marrow sinusoidal endothelial monolayers promote the ex vivo expansion of megakaryocytes.[8,9] Megakaryocytes produce an abundance of proangiogenic cytokines and synthesize or transport a number of key endothelial-cell trophogens, including vascular endothelial growth factor A (VEGF-A), stromal cell–derived factor 1, angiopoietin 1, epidermal growth factor, and brain-derived neurotrophic factor, all of which mediate cross-talk between megakaryocytes and endothelial cells. In a reciprocal manner, endothelial cells release an array of trophic cytokines that support megakaryocyte development and platelet production (Fig. 1).[10-13]

Glossary

α-Catenin: An intracellular catenin that binds to β-catenin and links the complex to the actin cytoskeleton.

Adherens junction: Complex of intercellular-adhesion transmembrane vascular-endothelium cadherin molecules coupled to members of a family of intracellular catenins linked to the cytoskeleton.

Angiopoietin 1 (ANG1): A protein expressed primarily by perivascular and mural cells for vessel stabilization.

β-Catenin: An intracellular catenin that binds to the cytoplasmic domain of vascular-endothelium cadherin; when not bound, acts as a transcription factor.

Brain-derived neurotrophic factor receptor (BDNF-R): Serves as a neurotrophin receptor on the endothelium.

CD148: A component of the adherens complex, also known as density-enhanced phosphatase; dephosphorylates vascular endothelial growth factor receptor 2 (VEGF-R2).

Endothelial differentiation gene 1 (EDG1): A sphingosine-1-phosphate receptor.

P120: An intracellular catenin that participates in stabilization of the intracellular complex.

Platelet-activating factor (PAF): A bioactive proinflammatory phospholipid.

Platelet-activating factor receptor (PAF-R): A G-coupled receptor induced by shear stress.

Sphingosine-1-phosphate (S1P): A bioactive lysophospholipid of which platelets are the major source.

Vascular-endothelium cadherin: A vascular endothelial intercellular-adhesion protein that forms calcium-dependent homodimers.

Platelets also influence the development and fate of endothelial-cell progenitors. In addition to promoting the migration and adherence of bone marrow–derived cells to sites of angiogenesis, platelets also induce differentiation of endothelial-cell progenitors into mature endothelial cells. Under the influence of platelets, endothelial-cell progenitors down-regulate expression of c-kit (a growth-factor receptor on immature cells) and increase the synthesis of CD31 (platelet–endothelial cell adhesion molecule, or PECAM1) and other markers of mature endothelial cells. These mature cells contain Weibel–Palade bodies, the organelles that store von Willebrand factor and P-selectin.[14] Aggregation and activation of platelets at sites of exposed subendothelium cause them to release stromal cell–derived factor 1, a potent angiogenic trophogen, which supports the recruitment and retention of bone marrow–derived endothelial-cell progenitors in focal areas of new blood-vessel formation (neoangiogenic niches). In this way, platelets contribute to the revascularization of ischemic tissue, tumor growth, and progression of atherosclerotic plaques.[10,15] Recent studies indicate that platelets participate not only in the recruitment of dendritic cells to such plaques but also in the differentiation of CD34+ hematopoietic stem cells into foam cells (lipid-laden macrophages) within these plaques.[16]

PLATELETS AND THE VEGF SYSTEM

The language of platelet–endothelial cell cross-talk is written in glyphs of specific trophogens that the cells release in particular microenvironments. Site-specific deployment by platelets of angiogenic or antiangiogenic factors packaged in separate sets of alpha granules influences multiple pathologic states, from wound healing to diabetic retinopathy.[17-19] The angiogenic family of VEGFs is an important mediator of platelet–endothelial cell interactions in both steady and nonsteady states and accounts for much of the role of platelets in angiogenesis and maintenance of vascular integrity.[20]

A global understanding of vascular homeostasis must take into account how the platelets and endothelial cells orchestrate the expression of multiple members and isoforms of the VEGF family and other trophogens during the life cycle of the blood vessel, from vasculogenesis in the early embryo to vascular regression in adult tissues (Fig. 2). Stabilization of blood vessels and preservation of platelet-supported vascular integrity in the steady state are important features of this cycle.

VEGF, discovered 25 years ago, was initially referred to as vascular permeability factor.[21] In mammals, there are at least four members of the VEGF family: VEGF-A, VEGF-B, and the VEGF-C–VEGF-D pair, which has a common receptor, VEGF receptor 3 (VEGF-R3).[22]

VEGF-A, the most studied of the group, interacts with two tyrosine kinase receptors, VEGF receptor 1 (VEGF-R1) and VEGF receptor 2 (VEGF-R2), to promote angiogenesis. VEGF-A also contributes to vascular integrity: selective knockout of VEGF-A in endothelial cells increases cell death (apoptosis), which compromises the integrity of the junctions between endothelial cells.[23] VEGF-A is a proangiogenic cytokine during embryogenesis. In mice, deletion of a single *VEGF* allele leads to hemorrhage and death of the embryo, thus emphasizing the importance of *VEGF* gene dosage during development.[24]

VEGF-B, which can form heterodimers with VEGF-A, occurs predominantly in brown fat, myocardium, and skeletal muscle.[25] VEGF-C and

VEGF-D seem to regulate lymphangiogenesis, primarily through interaction with their tyrosine kinase receptor VEGF-R3.

The expression of VEGF-R3 in adults is restricted to the lymphatics and fenestrated endothelium.[26,27] Neuropilin 1 and neuropilin 2 are receptors that bind specific VEGF family members and are important in neuronal development and embryonic vasculogenesis.[28]

The multiplicity of VEGF ligands and receptors probably reflects the heterogeneity of the numerous vascular beds in embryos and adults. It is clear that the VEGF system operates under different regulatory constraints in the nonsteady state of the developing embryo and the physiologic steady state of the adult. Pregnancy, the female reproductive cycle,[29,30] inflammatory reactions,[31] and tumor development and growth recapitulate the proangiogenic signaling pathways of the embryo.[18]

Megakaryocytes and platelets contain the three major isoforms of VEGF-A — VEGF121, VEGF165, and VEGF189 — and after exposure to thrombin in vitro, they release VEGF-A.[8] VEGF-A alters the endothelial-cell phenotype by markedly increasing vascular permeability, up-regulating expression of urokinase, tissue plasminogen activator, connexin, osteopontin, and the vascular-cell adhesion molecule.[32,33] Cell-death pathways in endothelial cells respond to VEGF-receptor signaling with increases in the prosurvival factor Bcl-2 and modulation of the mitogen-activated protein kinase pathway, which regulates a variety of cellular functions.[34,35]

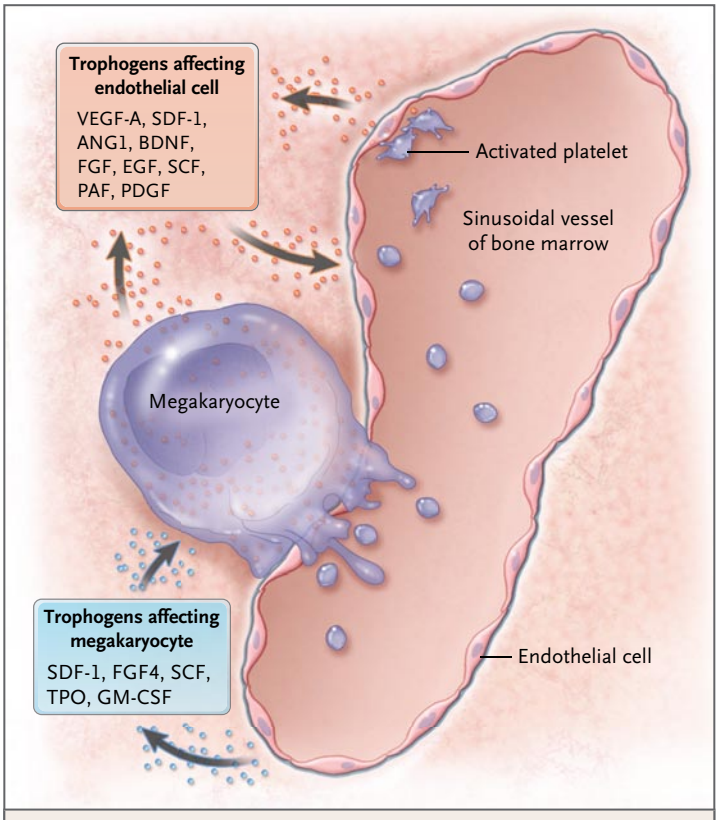

Figure 1. Megakaryocyte–Endothelial Cell Cross-Talk.

The megakaryocyte, through platelet production and release of key endothelial trophogens, directly determines the integrity and viability of the microvascular bed of bone marrow. In a reciprocal manner, in the "vascular niche" microenvironment, the endothelium directly influences megakaryocyte integrity by releasing a number of megakaryocyte trophogens. ANG1 denotes angiopoietin 1, BDNF brain-derived neurotrophic factor, EGF epidermal growth factor, FGF fibroblast growth factor, FGF4 fibroblast growth factor 4, GM-CSF granulocyte–macrophage colony-stimulating factor, PAF platelet-activating factor, PDGF platelet-derived growth factor, SCF stem-cell factor–c-kit ligand, SDF-1 stromal cell–derived factor 1, TPO thrombopoietin, and VEGF-A vascular endothelial growth factor A.

BIOLOGIC CHARACTERISTICS OF JUNCTIONS

The major anatomical sites of bleeding in patients with thrombocytopenia are the intercellular gaps in the postcapillary venular bed.[3,36] Key molecules in junctions in this region include transmembrane adhesion proteins and associated intracellular binding components.[37,38] The diverse vascular beds, each with specific functional requirements, require different types of junctions, each with specialized protein constituents and relations among macromolecules. The regulatory controls of postcapillary venular permeability in the skin and mucosal surfaces, for example, are completely different from those of the microvasculature in the brain.[39] Capillaries in the brain contain intercellular tight junctions composed of several specialized transmembrane proteins associated with specific intracellular kinases and phosphatases. Tight regulation of capillary permeability in the brain is essential, and intercellular leakage must be held to an absolute minimum. Thus, even in patients with severe acute thrombocytopenia, nontraumatic intracerebral bleeding is relatively rare.[40]

Adherens junctions are abundant in the postcapillary venular bed. They consist of clusters of the highly specialized membrane-spanning vascular-endothelium cadherin molecules that form the bridge across adjacent cell membranes (Fig. 3). Vascular-endothelium cadherins are coupled in-

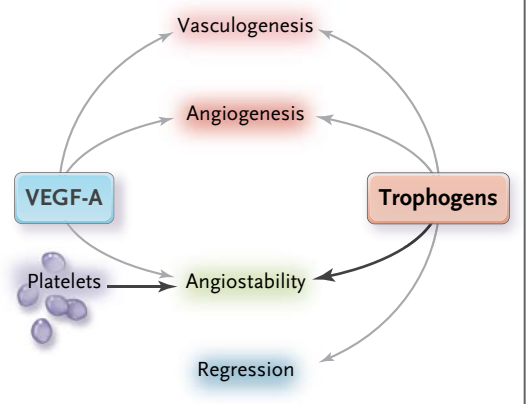

Figure 2. Vascular Homeostasis.

Stages in the life cycle of the vasculature are shown, from developmental vasculogenesis to non–steady-state angiogenesis in adult tissues to physiologic regression. Angiostability in the physiologic steady state is dependent on constitutive endogenous production of vascular endothelial growth factor A (VEGF-A), stimulated by platelets. Multiple angiogenic trophogens also participate in these stages. The black arrows indicate the major influences on maintenance of vascular integrity.

tracellularly to catenins, a family of proteins that form signaling complexes with downstream kinases and phosphatases.[41-43] The typical tight junctions of brain capillaries are primitive or absent in postcapillary venular beds.[44]

VEGF-A–induced signaling directly controls the interactions of the junctional machinery. The cytoplasmic domain of VEGF-R2 is a part of a macromolecular cytoplasmic vascular-endothelium cadherin–β-catenin complex.[45,46] Shear stress appears to enhance the formation of the complex, thereby converting the adherens junction into a mechanical transducer that initiates "outside–inside" signaling.[47] The binding of intracellular β-catenin to vascular-endothelium cadherin is increased by as much as a factor of 700 after phosphorylation of specific cadherin-serine residues.[48,49] By contrast, phosphorylation of β-catenin at specific tyrosine residues destabilizes the adherens junction.[50,51] Thus, the architectural integrity of the junctional intracellular and extracellular machinery is controlled by the state of phosphorylation of key components of the cytoplasmic assembly.

Interactions involving VEGF receptors have been assumed to entail a multicellular signaling pathway in which the ligand (VEGF) secreted by one or more cells activates the receptor (VEGF-R2) on an adjacent target cell. In this paracrine arrangement, platelet-derived VEGF can induce the phosphorylation and activation of endothelial VEGF-R2. Recent data strongly suggest that endothelial VEGF-A activates the cell's own VEGF-R2.[23] This autocrine pathway was discovered in mice, in which the *VEGFA* gene was deleted (knocked out) only in endothelial cells. Such mice had a marked hemorrhagic phenotype, with microinfarctions and a diffusely abnormal, disrupted vasculature.[23] Phosphorylation of VEGF-R2, present in wild-type endothelium in the absence of exogenous (paracrine) VEGF, was undetectable in endothelial cells that lacked the *VEGFA* gene. Thus, this autocrine pathway of VEGF-triggered signaling is obligatory for normal steady-state endothelial function.[23] We suggest that the phosphorylation of VEGF-R2 by endogenous VEGF, and subsequent "inside-out" signaling of the catenin–vascular-endothelium cadherin complex, stabilizes the ectodomain zipper structure of the endothelial lining of blood vessels (Fig. 3). This autocrine loop appears to constrain vascular permeability in the steady state, whereas the paracrine pathway seems paramount in promoting vascular permeability in the nonsteady states of embryogenesis, inflammation, and pregnancy.

The paracrine and autocrine activation pathways that VEGF-A triggers have different end points (Fig. 4). The two pathways also have different effects on the vascular-endothelium cadherin–β-catenin macromolecular complex. In nonsteady states, the paracrine pathway causes disassembly of the complex, whereas in the steady state, autocrine activation stabilizes the complex.[23] Paracrine stimulation, which increases vascular permeability, is an absolute requirement for vasculogenesis in the embryo and in the angiogenesis of cancer, inflammation, pregnancy, and the female reproductive cycle. Autocrine stimulation, which decreases vascular permeability, does not support angiogenesis but is essential for maintaining the integrity of endothelial-cell junctions in the steady state.[23] Hematopoiesis depends on endogenous VEGF-A; the generation of committed stem cells requires little or no exogenous VEGF-A.[52]

How does the platelet influence these two pathways? Multiple platelet-driven signals acting synchronously or sequentially appear to activate the autocrine pathway (Fig. 3). In nonsteady

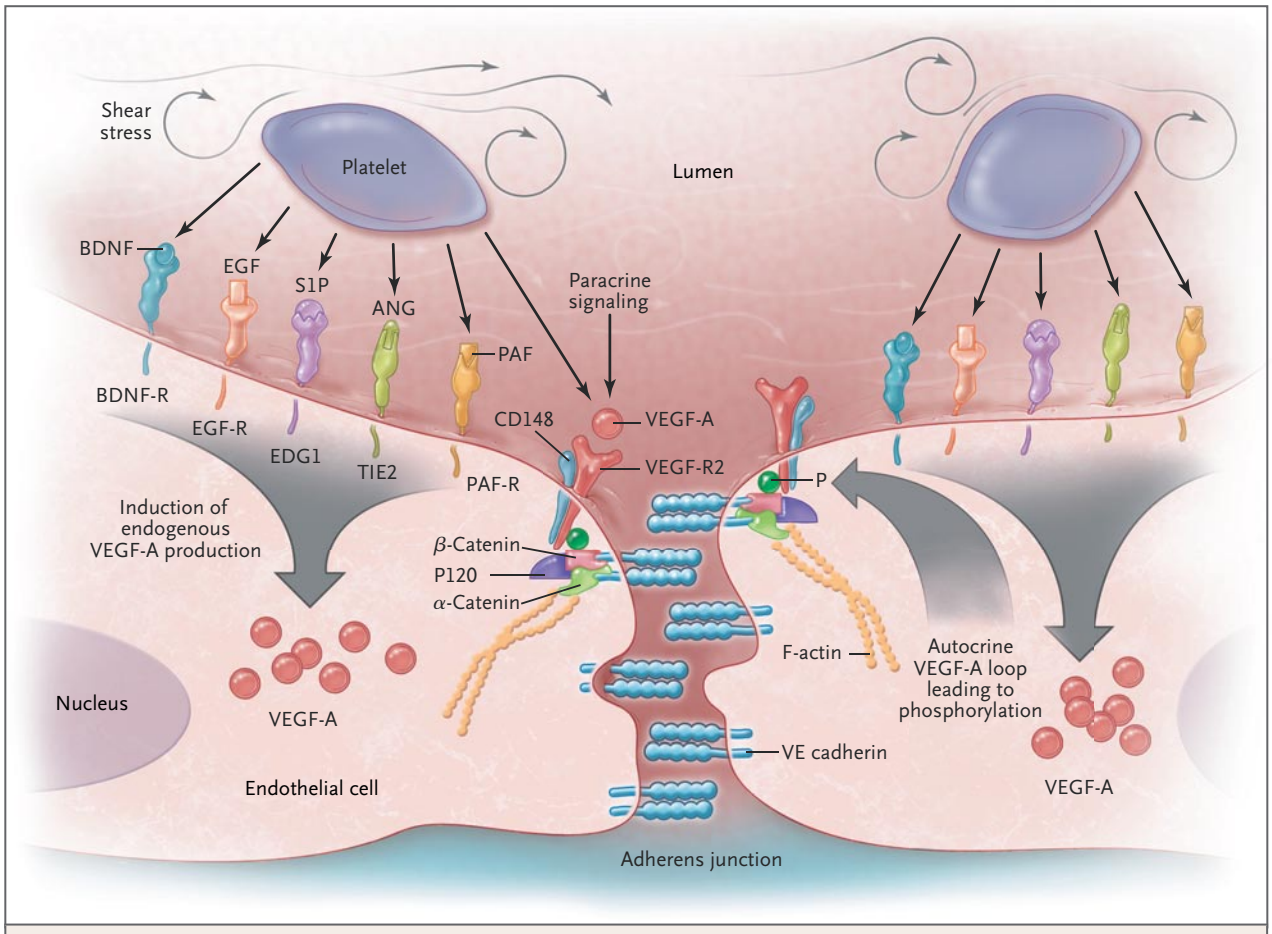

Figure 3. Adherens Junction at the Postcapillary Venular Bed.

In the steady state, transmembrane vascular-endothelium cadherin molecules form a calcium-dependent zipperlike structure across adjacent cell membranes, through homophilic interactions. The cytoplasmic tails of the cadherin are part of an intracellular macromolecular complex including β-catenin, P120, α-catenin, vascular endothelial growth factor (VEGF) receptor 2 (VEGF-R2), and CD148 phosphatase. The platelet in the steady state maintains the molecular integrity of the adherens junction by constitutively releasing a panoply of endothelial trophogens including brain-derived neurotrophic factor (BDNF), epidermal growth factor (EGF), platelet-activating factor (PAF), sphingosine-1-phosphate (S1P), angiopoietin (ANG), and VEGF A (VEGF-A), among others. These trophogens signal through their respective receptors (BDNF-R, which binds BDNF; EGF-R, which binds EGF; endothelial differentiation gene 1 [EDG1], which binds S1P; angiopoietin receptor specific to endothelial cells [TIE2], which binds ANG; and PAF-R, which binds PAF), induce endogenous VEGF-A production, and activate the autocrine VEGF-A loop, which in turn induces VEGF-R2 phosphorylation, which is required for maintenance of the stability of the zipper. Specific phosphorylation sites of the catenin and cadherin constituents in the complex control stability and integrity of the intercellular junction and facilitate cytoskeletal attachment through F-actin. The paracrine pathway of exogenous VEGF-A signaling promotes survival in the steady state but proliferation in the nonsteady state. Shear stress at the luminal surface of the vessel wall and the platelet membrane may participate in the constitutive triggering of these platelet responses. PAF denotes platelet activating factor, and VE vascular endothelial.

states, VEGF-A released by platelets activates the paracrine pathway and contributes to increased permeability and proliferation. There are other contributors to microvascular stability. Among these is Bcl-xL, an antiapoptotic cell-survival factor. In endothelial cells, Bcl-xL up-regulates VEGF-A production,[53] and in platelets, Bcl-xL is a major determinant of the life span.[54] Moreover, platelet-derived epidermal growth factor increases production of Bcl-xL by endothelial cells.[53] Paracrine VEGF-A, delivered to the vascular lining by platelets and other cells, increases endothelial-cell production of Bcl-2, another inhibitor of the cell-death pathway,[34,55] and platelet-activating factor, a phospholipid proinflammatory mediator, induces expression of VEGF-A by endothelial cells.[56] It is likely that these complex platelet–endothelial cell interactions go on continuously under physi-

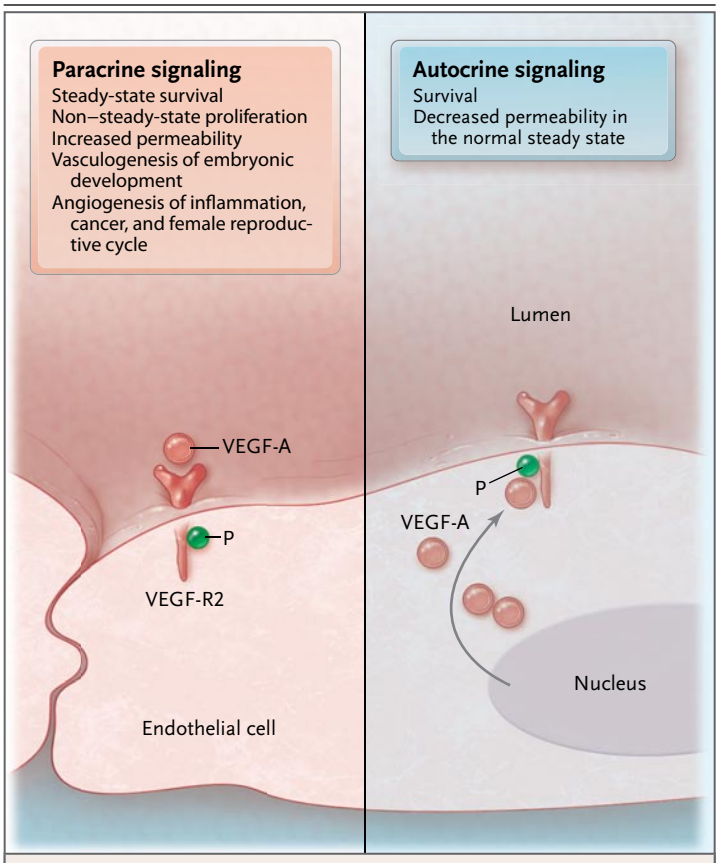

Figure 4. Paracrine and Autocrine Vascular Endothelial Growth Factor A (VEGF-A)–Induced Endothelial-Cell Interactions.

Exogenous VEGF-A in the physiologic steady state initiates signaling by phosphorylation of VEGF receptor 2 (VEGF-R2) and promotes cell survival. In nonsteady states, permeability is increased and proliferation is supported. Paracrine signaling is obligatory for the vasculogenesis of embryonic development and the angiogenesis of inflammation, cancer, pregnancy, and the female reproductive cycle. Autocrine endogenous VEGF-A also signals through phosphorylation of VEGF-R2 and in the steady state decreases permeability by stabilizing the intercellular junction. Autocrine stimulation is supportive of vasculogenesis and is not required for angiogenesis. The phosphorylation sites on VEGF-R2 may involve different residues after paracrine exogenous stimulation and autocrine endogenous stimulation.

ologic conditions. They are interrupted by marked thrombocytopenia or impaired platelet granule release caused by aspirin or clopidogrel.

ENDOTHELIAL-CELL STABILITY

Still other molecules influence the stability of the microvasculature. Ceramide and sphingosine are important regulatory components in apoptotic pathways.[57,58] Sphingosine released from dying cells is rapidly incorporated into platelets and phosphorylated.[59] Phosphorylated sphingosine-1-phosphate released from platelets supports endothelial-cell integrity and survival, inhibits apoptosis, and stabilizes endothelial-cell junctions by remodeling the actin cytoskeleton.[59,60] Fluid shear stress induces the release of sphingosine-1-phosphate from platelets,[61] and thus rheologic events affecting platelets in the postcapillary venular bed can contribute to the stability of endothelial cells. The cytoprotective effect of activated protein C also involves sphingosine-1-phosphate through the activation of the endothelial-cell receptor for sphingosine-1-phosphate and actin cytoskeletal reorganization.[62] The antiapoptotic effect of sphingosine-1-phosphate on the microvasculature may constitute a mechanism for radiation resistance, since radiation has been shown to activate sphingomyelinase in brain, intestine, and lung vasculature.[63]

Platelet sphingosine-1-phosphate regulates the N-cadherin contacts between endothelial cells and pericytes. Pericytes derived from smooth-muscle cell precursors surround the microvasculature by extending long cytoplasmic processes around the abluminal endothelial surface. Membrane-spanning N-cadherin molecules form contacts between adjacent pericytes and endothelial cells. Pericytes support vessel stability by forming a matrix and releasing growth factors that bind to endothelial cells.[64,65] A single pericyte interacts with several endothelial cells and can thus modulate the integrity of a capillary and venular bed.

CLINICAL IMPLICATIONS

Platelets and endothelial cells are intimately related and participate in cross-talk that has direct clinical implications. In the physiologic steady state, normal numbers of functioning platelets establish a platelet mass that is necessary to maintain the stability of the vasculature. This process probably takes place through a number of different mechanisms, including constitutive expression of trophogens on the surface of platelets or tonic release through mechanisms not yet fully understood. Regulated constitutive low-grade activation of platelets may be a normal rheologic event in the postcapillary bed.[66] It is of interest that the bleeding seen in patients who are receiving antiplatelet agents such as aspirin and clopidogrel is rarely associated with petechiae, such as occurs in thrombocytopenia, but instead manifests as easy bruising, gastrointestinal mucosal bleeding, and, rarely,

hemorrhagic strokes, all of which presumably occur in focal regions with preexisting, quiescent lesions. These antiplatelet drugs interfere with platelet granule release and aggregation and thus modify the primary hemostatic response to injury. Physiologic low-grade activation of platelets, with its concomitant endothelial-nurturing effect, may reflect a quantitative difference in granule release — with a platelet "whisper" rather than a platelet "shout" required in a full-fledged hemostatic challenge.

The interruption of the normal interaction between platelets and endothelial cells is clinically visible in patients with severe thrombocytopenia, in whom platelet-membrane exposure and release of endothelial-cell trophogens is reduced to the point at which the multimolecular vascular-endothelium cadherin complex disassembles, resulting in the loss of the intercellular barrier and red-cell extravasation into the tissues (Fig. 5). Rapid falls in the platelet count, such as those seen in some patients with drug-induced thrombocytopenia, are associated with more severe bleeding, with showers of petechiae and extensive mucosal hemorrhages. In patients with complex clinical disorders, clinically significant thrombocytopenic bleeding may well reflect the compounding effect of low platelet numbers in addition to the underlying systemic effects of sepsis, cancer, inflammation, or associated immunologic processes that can directly injure the microvasculature, compromising interjunctional endothelial integrity.

Bevacizumab (Avastin, Genentech), a humanized monoclonal antibody against VEGF, as well as VEGF-R2 tyrosine kinase inhibitors such as sorafenib and sunitinib, are antiangiogenic agents currently used in cancer therapy, with some success. These drugs are potential inhibitors of platelet–endothelial cell interactions and thus may be associated with hemorrhagic side effects. Major safety concerns with anti-VEGF agents to date include a small number of treatment-related deaths from bowel perforations, arterial thromboembolic events, and hemorrhage.[67,68] In most of these trials, clinically significant toxic effects occurred with the use of both bevacizumab and chemotherapy. The incidence of nonfatal bleeding in most bevacizumab trials ranged from 2 to 3%.[69] This relatively low incidence may reflect the inaccessibility of the intracellular VEGF pool to the extracellular antibody, which results in preservation of the stabilizing intracellular autocrine VEGF pathway. Combination therapy consisting of bevacizumab and a low-molecular-weight tyrosine kinase inhibitor may well increase the potential for bleeding, owing to the ability of the tyrosine kinase inhibitor to interfere with intracellular signaling. Approximately 40% of patients with metastatic renal-cell cancer had minor bleeding in a clinical trial involving both bevacizumab and erlotinib, a tyrosine kinase inhibitor of epidermal growth factor.[70]

New low-molecular-weight inhibitors of specific intracellular signaling pathways being developed as targeting agents may interfere with the complex molecular machinery that the endothelium uses to stabilize the adherens junction. This is particularly true of kinase inhibitors that result in alterations in the post-translational modification of vascular-endothelium cadherin–catenin complexes. In view of the marked heterogeneity of the microvascular bed in different organs and in different anatomical areas, it is likely that the specific signaling pathways generated in endothelium by platelets may vary as a consequence of different microenvironmental influences. Thus, the potential side effect of bleeding induced by various targeting agents may occur in some organs and not in others. It is reasonable to expect that direct delivery of some or a mixture of these trophogens with the use of liposomes or viral vectors may be useful in treating severe thrombocytopenia or alveolar or brain hemorrhage induced by the targeted molecules.

CONCLUSIONS

Platelets and endothelial cells are intimately related. It is evident that in patients with pathologic events or trauma in which vessel damage and disruption occurs, platelets rapidly come to the rescue by initiating a sequence of potentially lifesaving hemostatic steps that culminate in the arrest of bleeding and the subsequent repair of the blood-vessel wall. When these hemostatic defense and reparative mechanisms occur at the wrong time or in the wrong place, the consequences include a thrombotic event.

In the physiologic steady state, platelets maintain the stability of the vasculature by means of several mechanisms, including constitutive expression of trophogens on the surface of platelets or tonic release of cytokine and growth factors that preserve the structural and functional integrity of

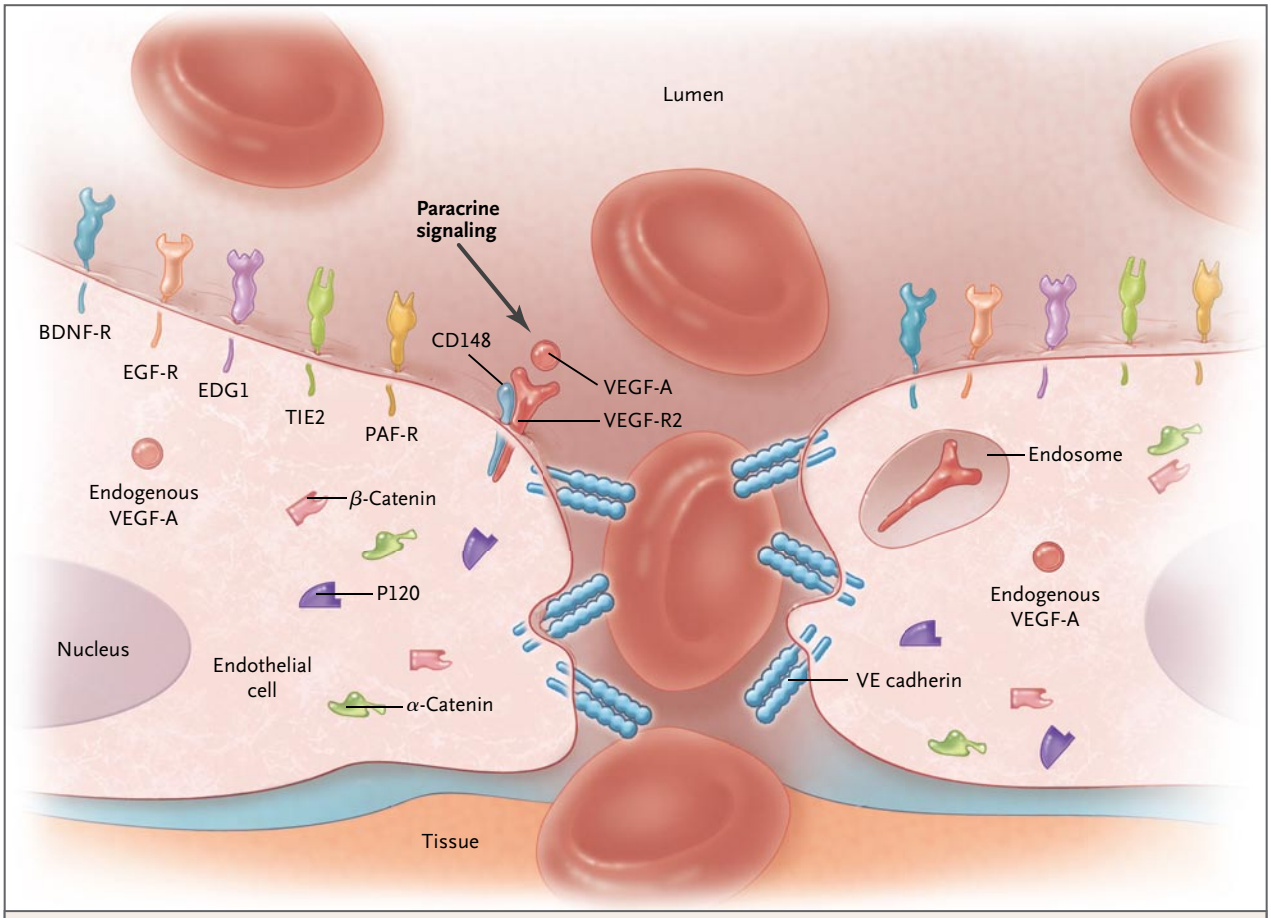

Figure 5. Bleeding in Patients with Thrombocytopenia through Disassembly of the Adherens Junction.

Below a critical number of platelets, the steady-state trophic effects on the endothelium are impaired and the multimolecular vascular-endothelium cadherin complex breaks down, with subsequent loss of the intercellular barrier, permitting extravasation of red cells into the surrounding tissues. The autocrine vascular endothelial growth factor A (VEGF-A) loop is interrupted, with resultant downstream alterations in the phosphorylated status of the constituents in the complex. VEGF receptor 2 (VEGF-R2) becomes internalized by the cell in endosomes. In most patients, disassembly of the vascular-endothelium cadherin complex manifests in the skin as petechiae and in mucosal surfaces as local hemorrhagic blisters. These trophogens signal through their respective receptors. BDNF-R denotes brain-derived neurotrophic factor receptor, EDG1 endothelial differentiation gene 1, EGF-R epidermal growth factor receptor, PAF-R platelet-activating factor receptor, TIE2 angiopoietin receptor specific to endothelial cells, and VE vascular endothelial.

the vascular-endothelium cadherin zipperlike machinery at the intercellular gaps. Regulated, constitutive low-grade activation of platelets may be the result of a normal rheologic event in the postcapillary bed.[66] Intact junctional assemblies also preserve the steady state and homeostasis by actively participating in bidirectional signaling associated with trophogen-receptor–induced complex formation as well as engagement of transcription factors such as β-catenin at the inner face of the membrane.[51] Post-translational modifications of the vascular-endothelium cadherin–β-catenin complexes, such as phosphorylation and dephosphorylation, are important for stabilization of the functioning adherens junction, and cytoskeletal attachment is a critical feature. With severe thrombocytopenia, platelet-membrane exposure and release of endothelial-cell trophogens are reduced to the point at which the vascular-endothelium cadherin multimolecular complex is disassembled, resulting in the loss of the zipperlike intercellular barrier, with red-cell extravasation into the tissues (Fig. 5). In view of the marked heterogeneity of the microvascular bed in different organs and in different anatomical areas, it is not unexpected that a number of different endothe-

lial-cell trophogens, stored in platelets, take part in the physiologic process. It remains to be determined how much the induced signaling pathways overlap and whether the autocrine VEGF-A–VEGF-R2 receptor loop is the final common pathway.

We view the platelet in steady-state physiologic circumstances as a biochemical reservoir that nourishes and stabilizes intact endothelium. The specific signaling pathways generated by platelets may vary in specialized vascular beds among various organs as a consequence of different microenvironmental influences. Direct delivery of some or a mixture of these trophogens with the use of liposomes or viral vectors may be useful in patients with severe thrombocytopenia.

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