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REVIEW

The Blood Platelet as a Model for Regulating Blood Coagulation on Cell Surfaces and Its Consequences

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Abstract—Platelets actively participate in regulating thrombin production following physical or chemical injury to blood vessels. Injury to blood vessels initiates activation of the large numbers of platelets that appear in the subendothelium where they become exposed to tissue factor and to molecules adhesive for platelets and normally found in the extracellular matrix. The complex of plasma factor VIIa with extravascular tissue factor both initiates and localizes thrombin production on platelets and on extravascular cells. Thrombin production at these sites in turn enhances platelet activation and the subsequent hemostatic plug formation to minimize bleeding. Thrombin production and platelet activation also initiate the process of wound healing requiring thrombin-dependent cell activation and platelet-dependent formation of new blood vessels (angiogenesis). Activated platelets release from their storage granules several proteins and other factors that regulate local thrombin formation and the responses of blood vessel cells to injury to assure hemostasis and effective wound healing. Failure to localize and adequately regulate thrombin production and/or platelet activation can have pathological consequences, including the development and propagation of atherosclerosis and enhancement of tumor development. The primary basis for the pathological consequences of the failure to adequately regulate thrombin production is that the multi-functional thrombin activates several types of cells to initiate their mitogenesis. Mitogenesis precedes many of the undesirable consequences of poorly regulated thrombin production and platelet activation. In addition, activated platelets release a variety of products which influence the functions of several cell types to the extent that inadequate regulation of platelet activation (by excessive thrombin production) could contribute to the pathogenesis of acute and chronic arterial thrombosis and to tumor development. Activated platelets participate in tumor development by releasing several factors that positively (and negatively) regulate blood vessel formation.

Key words: platelets, thrombin, blood coagulation, hemostasis, arterial thrombosis, angiogenesis

Blood coagulation is a series of coordinated and Ca^{2+}-dependent proenzyme to serine protease conversions likely to be localized on the surfaces of activated cells *in vivo*, and culminating in the conversion (activation) of prothrombin to thrombin by the coagulant enzyme complex known as prothrombinase. Blood coagulation is initiated after the serine protease, factor VIIa, in the blood is exposed to and binds to tissue factor, a transmembrane receptor for factor VII/VIIa. Tissue factor is constitutively expressed on subendothelial cells and also becomes expressed on the surface of activated monocytes and other inflammatory cells which accumulate at sites of injury [1-4]. The apparent endpoint of blood coagulation, namely the conversion of the soluble plasma protein fibrinogen into insoluble fibrin, is only one of several necessary reactions of hemostasis catalyzed by thrombin. As is true for most of the enzyme complexes that activate clotting factor zymogens into their respective serine proteases, prothrombinase consists of a serine protease (factor Xa) and its cofactor (factor Va) bound to (artificial or) activated cell surfaces in acidic phospholipids- and Ca^{2+}-dependent manner. The cofactor factor Va and the surfaces on which prothrombinase assembles jointly accelerate, by several orders of magnitude, the rate of prothrombin activation by factor Xa [5-7]. Cell surfaces such as activated platelets, monocytes, endothelial cells, and lymphocytes which also serve as assembly sites (i.e., coagulant surfaces) for coagulation enzyme complexes to essentially decrease the apparent K_m describing the proenzyme to enzyme conversions by increasing the local concentrations of the reactants involved in blood coagulation. Coagulant surfaces also increase the turnover numbers of the proenzyme to enzyme conversions. These combined effects of coagulant surfaces and cofactors optimize the rates of clotting factor zymogens activation by their respective proteolytic enzyme/cofactor complexes [8, 9]. Coagulant cell surfaces are also the sites where activated protein C, the enzyme which inactivates coagulation cofactors factor Va and factor VIIIa,

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as well as agents that inactivate many of the serine proteases of the coagulation assemble (see below).

Both thrombin (generated during blood coagulation) and platelets (after their activation by thrombin) participate in the maintenance of hemostasis and formation of thrombi. In addition to its well known functions in blood coagulation, hemostasis, and thrombosis [10-15], thrombin has several direct and indirect roles in inflammation, wound healing, embryogenesis, angiogenesis, and morphogenesis [16-20], processes initiated after thrombin activates the several cell types that participate in the above cellular responses. Thrombin-mediated cell activation is initiated after this enzyme binds to and cleaves (activates) its receptors on several cells. Binding of thrombin to its receptor on a variety of cells and the subsequent thrombin receptor cleavage initiate several signaling cascades within these cells. Cells activated by thrombin include epithelial cells, endothelial cells, subendothelial cells, smooth muscle cells, neural cells, and hemopoietic cells [21-30]. After their activation, many of these cells also serve as surfaces on which prothrombinase and other coagulant enzyme/cofactor complexes assemble. Given the diversity of the enzymatic reactions that are catalyzed by thrombin, including activation of several types of cells [8, 21-32], it goes without saying that effective regulation of thrombin formation on cells, and equally effective regulation of the enzymatic activity of thrombin, are necessary for cellular homeostasis. In addition to thrombin, two enzymes of blood coagulation, namely factor Xa and factor VIIa–tissue factor complex, as well as tissue factor by itself, can initiate cell signaling [33-37]. Therefore, it is probably also necessary that cell signaling initiated by cell-bound tissue factor, factor Xa, and factor VIIa–tissue factor be effectively regulated to limit activation of these cells by the above three cell activators only to the levels necessary for hemostasis, wound healing, localized angiogenesis, and morphogenesis.

The choice of platelets as model cells for reviewing the reactions on cell surfaces that both accelerate and inhibit prothrombin arises from the following considerations. Thrombin is the most potent physiological activator of human platelets [38, 39], and thrombin-activated platelets release and/or recruit all the reactants necessary both for accelerating and for inhibiting prothrombin activation on platelets [40-46]. Furthermore, activated platelets interact with endothelial cells, subendothelial cells, and hemopoietic cells and in ways that, when well regulated, normally result in hemostasis, wound healing, limited inflammation, and other necessary morphogenic changes [6, 16, 18, 27, 29, 47, 48]. However, when adequate regulation of platelet activation by thrombin fails, increased interactions of thrombin-activated platelets with endothelial cells, hemopoietic cells, and subendothelial cells can have significant pathological consequences, including development and propagation of atherosclerosis and tumor growth [19, 49].

This review is divided into three sections. The first section will consider the contributions platelet-derived and plasma-derived reactants initially make to facilitate and subsequently to limit prothrombin activation on activated platelets. The second section will briefly review the mechanisms of platelet activation and the critical role of platelet activation in hemostasis. The third section will review how some of the pathological consequences that can arise from inadequate regulation of platelet-dependent activation of blood coagulation, and from subsequent activation of other cells by combinations of the excess thrombin and platelet-derived factors.

CONTRIBUTIONS OF PLATELET-DERIVED AND PLASMA-DERIVED REACTANTS TO PROTHROMBIN ACTIVATION ON PLATELETS

Exposure of blood to tissue factor subsequent to physical or chemical injury to vessel walls likely initiates blood coagulation *in vivo* [3, 4]. Tissue factor is constitutively expressed on subendothelial cells and smooth muscle cells [3, 4]; and tissue factor is also synthesized by, and becomes expressed, on the surfaces of endothelial cells and monocytes subsequent to bacterial and viral infections. Bacterial and viral infections lead to elaboration of cytokines, including tumor necrosis factor and interleukin-1 (IL-1), which stimulate tissue factor synthesis by, and its expression on, endothelial cells and monocytes [16, 50-52]. The inflammatory responses caused by physical damage to endothelial cells subsequent to balloon angioplasty or by sickle cell disease, for example, similarly induce tissue factor synthesis and its expression on endothelial cells. Tissue factor synthesis in these instances is invariably associated with increased factor VII activation and consumption, platelet activation, leukocyte activation, as well as prothrombin activation *in vivo* [53-57]. Tissue factor is a member of the type II cytokine family of glycoproteins. While platelets do not synthesize tissue factor, tissue factor can be readily demonstrated on platelets within 5 min after the initiation of vascular injury [50]. Exposure of blood to subendothelial tissue factor following endothelial injury also exposes increased numbers of platelets to the subendothelium where activated platelets and fibrin are ultimately incorporated into hemostatic plugs to minimize blood loss (see below). Fibrin and activated platelets constitute the major constituents of hemostatic plugs.

The complex of serine protease factor VIIa with its cofactor tissue factor (i.e., factor VIIa–tissue factor) is the enzyme complex that initiates blood coagulation by activating both factor X and factor IX by limited proteolysis [1-5]. As noted previously, thrombin is a potent cell activator. Factor VIIa–tissue factor also activates cells as it causes increases in intracellular [Ca^{2+}] and the phos-

phorylation of mitogen-activated protein kinases, which can ultimately result in transcription of the *Egr-1* (early growth response) gene, a gene also inducible by cytokines and growth factors [34, 58, 59]. The factor Xa thus generated probably produces sub-nanomolar concentrations of thrombin; and the thrombin thus generated will, in turn, accelerate prothrombinase formation. Specifically, the initially formed sub-nanomolar thrombin will activate platelets in the subendothelium to enhance adhesion of the platelets to the subendothelium [60, 61]. This initial thrombin will also activate factor V and factor VIII to provide two critical cofactors for prothrombinase and intrinsic tenase (factor IXa–factor VIII enzyme complex), respectively, to assemble [1, 2, 5, 14, 15] on the activated platelets. Increased thrombin production generates fibrin, which helps consolidate the hemostatic plug.

Thrombin-activated platelets rapidly release additional procoagulant moieties from their α-granules and many of these procoagulant entities rapidly become expressed on (i.e., bind to) the surface of activated platelets to help localize thrombin formation at sites of extravascular injury. The procoagulant moieties secreted by activated platelets (which would then become adherent to sites of vascular injury) include fibrinogen, factor XI, factor IX, factor V, fibrinogen, factor XIII, and von Willebrand factor [43]. Of special significance is that upon their release from activated platelets, each of the above platelet-derived coagulant proteins will bind to its specific sites on thrombin-activated platelets. Thus, these proteins would probably be in the orientations on platelets that favor their participation in thrombin production at sites of vascular injury. Like that of factor X, evidence for factor VIII storage by platelets or their release by activated platelets has not been reported. Since factor VIII is not known to be stored in platelet granules, release by, and the expression of von Willebrand factor on, thrombin-activated platelets could be particularly important for assuring the localization of plasma factor VIII on platelets for hemostasis. The carrier (binding) protein for factor VIII in plasma is von Willebrand factor. The factor VIII thus localized on its binding protein expressed on platelets would be activated by thrombin generated on the platelets. Activation of factor VIII by thrombin would dissociate factor VIIIa from von Willebrand factor for this cofactor to interact with factor IXa in intrinsic tenase [1, 5]. Factor VIII bound to von Willebrand factor can only be activated by thrombin, and not factor Xa [62]. The factor VIIIa thus made available on activated platelets would then participate in factor X activation by intrinsic tenase on the platelets.

Localization of factor VIII on platelets by platelet-bound von Willebrand factor, if this occurs *in vivo*, would be important for the following reasons. Activated platelets also secrete tissue factor pathway inhibitor (TFPI) [63-65], a highly effective anticoagulant as TFPI effectively inactivates both factor VIIa–tissue factor (the initiator of coagulation *in vivo*) and factor Xa within prothrombinase [64-67]. Specifically, TFPI rapidly inactivates both free factor Xa and prothrombinase-bound factor Xa [64-67]. It appears that TFPI, and not antithrombin III, is the key inhibitor of prothrombinase *in vivo* [68]. Factor Xa–TFPI rapidly binds to factor VIIa–tissue factor to form the quaternary complex factor Xa–TFPI–factor VIIa–tissue factor containing equimolar concentrations of each of the four constituents. Neither factor Xa nor factor VIIa in this quaternary complex is enzymatically active [64-67]. Thus, TFPI inactivates both the key enzyme that initiates coagulation (i.e., factor VIIa–tissue factor) and factor Xa within prothrombinase.

Vascular endothelial cells may be primary sites of TFPI synthesis [69-71]; and like platelets activated by thrombin, endothelial cells activated by thrombin secrete TFPI [71]. Thrombin-induced release of TFPI by, and its expression on, endothelial cells may similarly limit thrombin production by both the injured and healthy endothelium. The Ca^{2+}-dependent and rapid inactivation of both factor Xa and factor VIIa–tissue factor by endothelial-derived and platelet-derived TFPI would also serve to rapidly abrogate factor IX and factor X activation by factor VIIa–tissue factor. Abrogation of factor X and factor IX by factor VIIa–tissue factor by TFPI necessitates propagation by other coagulation enzyme complexes of factor IX and factor X activation to the extent necessary to achieve hemostasis, especially after traumatic injury. The second (i.e., factor VIIa–tissue factor-independent) coagulation enzyme complex that activates factor X consists of equimolar factor IXa and factor VIIIa (intrinsic tenase) bound to coagulant (activated) cell surfaces, such as activated platelets, epithelial cells, monocytes, lymphocytes, neutrophils, macrophages, and endothelial cells [1, 6, 8, 72-76]. This requirement for both factor IXa and factor VIIIa (in intrinsic tenase) for propagating factor X activation explains why males with severe factor VIII or factor IX deficiency bleed, especially after traumatic injury. As noted above, factor VIII (which would be normally bound to von Willebrand factor in the circulation) probably becomes expressed on activated platelets after it binds to von Willebrand factor already expressed on platelets, which have been activated by sub-nanomolar thrombin also formed on the platelets. Expression of von Willebrand factor on thrombin-activated platelets may facilitate the translocation of factor VIII from plasma von Willebrand factor to the von Willebrand factor expressed on thrombin-activated platelets. Activation by thrombin of the factor VIII thus localized on activated platelet would provide the cofactor (factor VIIIa) for propagating factor X activation by factor IXa, once VIIa–tissue factor has been inactivated in the quaternary factor Xa–TFPI–factor VIIa–tissue factor complex [64-67]. The platelet-derived factor IX, which becomes

externalized on thrombin-activated platelets, appears to be all the factor IX required to optimally propagate activation of both factor X and prothrombin in platelet-rich plasmas in response to sub-nanomolar tissue factor addition to the plasmas [77].

Propagation of the activation of factor IX bound to activated platelets also expressing TFPI would be catalyzed by factor XIa since factor VIIa–tissue factor would no longer be available to activate factor IX (after this enzyme complex is localized in the quaternary TFPI-dependent complex). Both platelet-derived and plasma-derived factor XI would likely be activated by thrombin on platelets, as proposed in a revised model of blood coagulation [78-81]. It is noteworthy that activated platelets also release protease nexin-1 [82], a potent inhibitor of factor XIa and thrombin [83, 84]. Protease nexin-1 released by activated platelets may inactivate both platelet-bound factor XIa and thrombin to thereby limit activation of factor IX by factor XIa on platelets. Neural tissue is a rich source of protease nexin-1 and this antithrombin and anti-factor XIa probably regulates thrombin production by neural cells. Thrombin is also a potent activator of neural cells [84]. In the absence of any cofactors, thrombin activates factor XI at a rate 10-fold faster than factor XIIa. Factor XI activation is further accelerated 10^3-fold by negatively charged surfaces due to autoactivation of factor XI by factor XIa [79, 80]. The preferential activation of factor X by platelet-bound factor IXa [81] is consistent with platelet-derived TFPI inhibiting activation of factor X on platelets by factor VIIa–tissue factor.

A third mechanism for inhibiting coagulation on platelets is inactivation of two of critical cofactors of coagulation, namely factor Va and factor VIIIa by activated protein C [85]. There is conflicting information as to whether platelets decrease the ability of activated protein C to inactivate factor Va and factor VIIIa to thereby limit their participation in platelet-dependent prothrombin activation [86, 87].

PLATELET ACTIVATION AND HEMOSTASIS

Platelet activation following vessel wall injury results in platelet shape change, platelet adhesion to the subendothelium, platelet aggregation, and secretion of the contents of platelet α-granules and dense granules [19, 88-90]. In addition to release of the clotting factors and inhibitors of coagulant enzymes reviewed above, activated platelets release from their granules several constituents critically important for hemostasis (reviewed in this section). Activated platelets also release granular contents important for platelet-dependent pathological processes, including development of atherosclerosis and thrombosis (reviewed in the next section). Platelets have crucial functions, which help maintain the physical and functional integrity of the vasculature necessary for preventing blood loss from wounds. Contact of platelet glycoprotein Ib (GPIb) with von Willebrand factor (present in deposited on the subendothelial matrix) after vessel injury initiates platelet adhesion to subendothelial side injured blood vessels. This initial adhesion is strengthened by interactions the platelet receptor $\alpha_{IIb}\beta_3$ (GPIIb/IIIa) with its many ligands, including von Willebrand factor, fibrinogen, fibronectin, thrombospondin, and vitronectin [60, 91]. All the adhesive proteins are released from the α-granules of activated platelets. Based on the synthesis in a review by Clementson [60] of the results of several studies describing the formation of hemostatic plugs, the following simplified picture describing the significant steps and interactions between activated platelets and the vessel wall constituents that define the hemostatic plug formation of sites of vascular injury emerge. 1) Following vessel wall injury (which leads to significant increases in the numbers of platelets in the subendothelial side of the blood vessel), the initial rolling contacts between platelet GPIb and the A1 domain of von Willebrand factor (which would be bound via its A3 domain to extravascular collagen) slow down the flow of platelets to allow their activation to begin. Thus, interactions between GPIb and von Willebrand factor initiate hemostatic plug formation. The initial source of von Willebrand factor could be the blood escaping into the extravascular space or the extracellular matrix. The latter contains von Willebrand factor as well as the adhesive proteins fibronectin, thrombospondins, laminin, collagens, and proteoglycans [92, 93]. 2) Two events likely initiate activation of platelets in the subendothelium, the second crucial step in the hemostatic plug formation. The first event is binding of collagen to its receptor on platelets (the integrin $\alpha_2\beta_1$ or GPIa-IIa). The second event is binding of sub-nanomolar thrombin (generated on the platelets) to its principal receptor on platelets, namely protease activated receptor-1 (PAR-1). Binding of thrombin to PAR-1 results in cleavage of PAR-1 at Arg41–Ser42 by thrombin [27, 95-98].

Important consequences of PAR-1 cleavage (after thrombin binds to the hirudin-like domain of PAR-1 in a region downstream from the site cleaved by thrombin) are the initiation of signaling cascades within platelets that ultimately result in platelet shape change and aggregation, and in the release from platelet α-granules and dense granules of agents that help propagate (i.e., reinforce) platelet activation [99-102]. Key events preceding the platelet release reaction and platelet activation include inhibition of platelet adenyl cyclase, activation of phospholipase A_2, and activation of phospholipase C [100-102]. The necessary activation of platelet $\alpha_{IIb}\beta_3$ (GPIIb/IIIa) occurs so that this platelet integrin alters its confirmation to the conformer that binds to the several ligands required for platelet adhesion and

cohesion. Activation of GPIIb/IIIa precedes the rearrangement of the platelet cytoskeleton that occurs during platelet activation. 3) The activated platelet $\alpha_{IIb}\beta_3$ conformer is the receptor for fibrinogen, von Willebrand factor, thrombospondin, fibronectin, and vitronectin [91, 94, 102]. Binding of fibrinogen to activated platelet integrin $\alpha_{IIb}\beta_3$ is necessary for platelet–platelet cohesion and subsequent aggregation of the platelets during hemostatic plug formation. The platelet-integrin $\alpha_2\beta_1$ may similarly become activated after platelets bind to the subendothelium for this receptor to effectively bind collagen [60].

As noted above, a necessary step for propagating platelet activation, even in response to a strong platelet agonist such as thrombin, is the release of several platelet granular constituents. Platelet granular constituents important for propagating and consolidating platelet responses to external agonists include ADP, thromboxane A_2 (synthesized from the arachidonate released from membrane phospholipids by phospholipase A_2 during platelet activation), 5-hydroxy tryptamine (serotonin), and fibrinogen. Binding of platelet-derived ADP, thromboxane A_2, and serotonin to their receptors on platelets provide additional signaling to optimize propagation of platelet activation in response to ≤1 nM thrombin and collagen [99-103]. The clinical effectiveness of aspirin (which inhibits platelet thromboxane A_2 synthesis) and ADP receptor antagonists in arterial thrombosis attest to the importance of these two platelet-derived agonists in helping optimize the propagation of platelet responses to physiological concentrations (i.e., <1 nM) of thrombin. In addition to decreasing platelet activation *in vivo* during arterial thrombosis, aspirin decreases prothrombin activation *in vivo* [104]. This apparent anti-prothrombinase action of aspirin *in vivo* provides strong evidence for a direct role of platelets in supporting prothrombin activation *in vivo*. As noted above, binding of fibrinogen to activate the platelet integrin $\alpha_{IIb}\beta_3$ is necessary for platelet–platelet cohesion and the subsequent platelet adhesion.

PATHOLOGICAL CONSEQUENCES OF INADEQUATE REGULATION OF PLATELET ACTIVATION

Interactions of activated platelets with endothelial cells and with hemopoietic cells in the setting of atherosclerosis and inflammation have been the subject of several recent reviews [16, 19, 90, 105-108]. Interactions between the hemostatic and inflammatory pathways contribute to the pathogenesis of acute and chronic coronary syndromes [16, 19, 90]. As noted above, activated endothelial cells and hemopoietic cells also support thrombin production during inflammation as inflammatory stimuli induce tissue factor synthesis by, and its expression on, hemopoietic and endothelial cells. A major difficulty in unraveling the interactions between platelets and inflammatory cells, or interactions between platelets and atherosclerotic cells, therefore, is that the thrombin produced by all the various cells involved in atherosclerosis and inflammation directly activates all of these cells. Specifically, even in the absence of activated platelets, thrombin directly activates endothelial cells, neutrophils, monocytes, T-lymphocytes, macrophages, smooth muscle cells, and connective tissue mast cells during inflammation [10-16, 21-30]. The source of the thrombin for activating these cells *in vivo* could be the inflammatory cells themselves and not necessarily activated platelets. Thrombin also directly activates platelets and subendothelial cells. Nonetheless, it is worth attempting to decipher how platelets activated by thrombin interact with hemopoietic cells and perivascular cells to induce the pathologic phenotypes of these cells.

The following specific examples will serve to illustrate these points. Regardless of its source of production, thrombin induces endothelium-dependent relaxation of the underlying arteries. This endothelium-dependent relaxation of arteries can be decreased by the nitric oxide synthase inhibitor NG-nitro-L-arginine methyl ester and by indomethacin. These observations suggest that endothelial-derived nitric oxide and the prostacyclin generated after thrombin stimulates the endothelial cells cause the relaxation of the underlying arteries seen. Furthermore, thrombin-activated platelets marginally influence relaxation of arteries caused by thrombin-mediated activation of arterial endothelial cells. Once the endothelium is removed from the underlying arteries, however, thrombin-activated platelets induce marked contractions of the arteries, which had been denuded of their endothelial cells [109]. This contraction of the denuded arteries by thrombin-activated platelets is reversible by ridogel, a thromboxane A_2 synthase/receptor antagonist [109]. These results suggest that thrombin-activated platelets release thromboxane A_2, which induces vasoconstriction of arteries, and that the vasoconstriction is largely reduced by the prostacyclin and nitric oxide normally synthesized by thrombin-stimulated endothelial cells. Further, in patients with coronary artery disease (whose arteries likely have areas of endothelial cells with impaired function), therapeutic elimination of platelet-thromboxane A_2 production by aspirin (and thus, loss of the vasoconstrictive action of thromboxane A_2 on the arteries) may provide a means for decreasing thrombin-induced platelet–vessel wall interactions [109].

Activation of a variety of cells subsequent to thrombin-mediated receptor cleavage elicits the events that cause the cell division necessary for inflammation, tissue remodeling, and wound repair [16, 27] as thrombin is an important mitogen for these cells. Beyond these physio-

logical actions, however, the combined actions of thrombin and activated platelets contribute to the migration and proliferation of, and the deposition of extracellular matrix by vascular smooth muscle cells seen in atherosclerosis and after angioplasty [19, 91, 109-113]. Aggregating platelets generated after balloon angioplasty also release several growth factors, including transforming growth factor (TGF) β_1, platelet-derived and growth factor$_{AB}$ ($PDGF_{AB}$), each of which enhances thrombin receptor expression by vascular smooth muscle cells [109-113]. Increased thrombin receptor expression is also found in atherosclerotic arteries, especially in areas of smooth muscle cell proliferation. As noted previously, activated platelets also release the vasoactive serotonin, and ADP and thromboxane A_2. Serotonin increases thrombin receptor mRNA expression by vascular smooth muscle cells to a similar level as $PDGF_{AB}$, but to a lesser extent than TGF-β_1 [109]. Thus, the combined actions of thrombin and the products released by activated platelets enhance thrombin receptor expression preceding the excessive smooth muscle proliferation that can occur after balloon angioplasty and is also seen in atherosclerotic arteries.

Activated platelets secrete several proteins that facilitate the interactions of activated platelets with other platelets, endothelial cells, and white cells. These include P-selectin (for adherence of neutrophils, monocytes, and endothelial cells to activated platelets [114-117]); thrombospondin, fibrinogen, and fibronectin (necessary for platelet–monocyte adherence, platelet–platelet cohesion, and subsequent aggregation through the platelet integrin $\alpha_{IIb}\beta_3$ [118-123]); and vitronectin (required for fibrinogen-dependent and fibronectin-dependent inflammatory cell interactions with activated platelets [99, 120-122]). Other proteins secreted by activated platelets that also influence the functions of other cells include platelet-derived growth factor, β-thromboglobulin, and platelet factor 4. β-Thromboglobulin binds to and inhibits prostacyclin synthesis by endothelial cells [123] and is a potent fibroblast chemotactic agonist [124]. Thus, this protein facilitates fibroblast migration to sites of vascular injury as a necessary step in wound healing. The potent anti-heparin platelet factor 4 is chemotactic for monocytes and neutrophils [40]. Platelet-derived growth factor stimulates the proliferation of fibroblasts and arterial smooth muscle cells [125]. If poorly controlled, proliferation of subendothelial fibroblasts and smooth muscle cells may lead to development and propagation of atherosclerosis [19, 91]. Hence, the need to tightly regulate prothrombin activation on platelets since the thrombin made on platelets and products released by thrombin-activated platelets have major consequences on the development of atherosclerotic lesions.

Angiogenesis, the elaboration of new blood vessels from pre-existing blood vessels, is necessary for both wound healing and metastatic tumor growth; and it has become more apparent that hemostasis and angiogenesis are closely interrelated processes [18]. Specifically, platelets adherent to sites of vascular injury also secrete agents that both enhance and inhibit angiogenesis [18]. Platelet-derived agents that favor angiogenesis include epidermal growth factor [126, 127], hepatocyte growth factor [128, 129], platelet-derived growth factor [130], platelet factor 4, insulin-like growth factor I [131], vascular endothelial growth factor-A [128], and vascular endothelial growth factor-B [132]. In addition to stimulating angiogenesis [129], fragments of hepatocyte growth factor also inhibit angiogenesis [133]. Specifically, alternatively spliced constructs of hepatocyte growth factor α-chain mRNA consisting of the first, the first two, or all four kringle domains of this growth factor lead to synthesis of proteins that suppress hepatocyte growth factor-induced angiogenesis and tumor growth [133]. Thus, expression of these products, or their generation from hepatocyte growth factor (by proteolytic cleavage), could decrease the angiogenic effect of this growth factor. Platelet factor 4 released also inhibits angiogenesis *in vivo* [134, 135], and thereby inhibits tumor growth. Also secreted from platelet α-granules are thrombospondin, transforming growth factor-β, and plasminogen activator inhibitor type-1, all known to inhibit angiogenesis [18, 136, 137].

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