REVIEW ARTICLE

MECHANISMS OF DISEASE

The Hemostatic System as a Modulator of Atherosclerosis

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Cardiovascular disease is one of the leading causes of death and complications worldwide. The classic concept of atherosclerosis assigns a pivotal role to inflammation in the onset and progression of this disease.[1,2] Various inflammatory cell types (e.g., macrophages, neutrophils, and lymphocytes) play crucial roles in the destabilization and subsequent rupture or erosion of an atherosclerotic plaque, ultimately resulting in atherothrombosis.[3] Inflammation is closely linked to coagulation in several pathologic conditions.[4] Intriguingly, extensive bidirectional cross-talk between the two systems has been established in many complex diseases,[5,6] including atherosclerosis.

Although there is no clinical evidence of a role for the hemostatic system in the progression of atherosclerosis, ample experimental data indicate that platelets and the coagulation system are important determinants of both atherogenesis and atherothrombosis. In numerous clinical trials, the administration of antiplatelet or anticoagulant therapy has not been associated with attenuation or regression of plaque growth. Nevertheless, the hemostatic system is well known for its capacity to exert a multitude of actions on the vasculature, which may influence the molecular and cellular composition of the arterial wall and presumably of the atherosclerotic plaque. This review covers recent advances in this field and discusses mechanisms of hemostasis as potential modulators of plaque phenotype.

Cross-Talk Mechanisms Linking the Hemostatic System with Atherosclerosis

Hemostasis

Hemostasis is accomplished through a network of processes that include the platelet system, coagulation, and anticoagulant and fibrinolytic pathways, which all support the dynamic equilibrium that provides proper blood flow.[7,8] Such processes evolved to maintain the blood in a fluid state under physiologic conditions and to arrest bleeding after vascular injury[9-15] (Fig. 1A and 1B). Disruption of this well-regulated balance leads to pathologic conditions, such as thrombosis and bleeding.

Molecular and Cellular Responses in the Vasculature

The targeting of genes that encode distinct hemostatic factors and their effect on arterial thrombosis in vivo has been extensively studied (see Table 1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). Abundant experimental data suggest a role for various constituents of the platelet membrane and coagulation system in the regulation of atherosclerosis progression. Beyond their traditional hemostatic functions, platelets are considered important in proinflammatory conditions, such as atherosclerosis.[16] In addition, numerous coagula-

tion proteins have been implicated in processes such as the disruption of the endothelial barrier, oxidative stress, leukocyte recruitment, inflammation, migration and proliferation of vascular smooth-muscle cells (VSMCs), immune responses, apoptosis of platelets and other cell types, and angiogenesis.[17,18] Some of these actions, mostly mediated by the complex of tissue factor and factor VIIa (TF–FVIIa), factor Xa, and thrombin, involve the activation of G-protein–coupled protease-activated receptors (PARs) 1, 2, 3, and 4. PARs are widely distributed on vascular cells under normal conditions and are overexpressed during atherogenesis.[19]

PLATELETS, THE CELLULAR INTERFACE BETWEEN HEMOSTASIS AND ATHEROSCLEROSIS

Pioneering studies have documented a prominent role of platelets in experimental studies of atherogenesis.[20,21] Platelets exert a plethora of proatherogenic activities and create an interface between hemostasis, innate immunity, and inflammation in atherosclerosis.[16] A systemic inflammatory environment, independent of vessel-wall injury, induces a phenotypic switch to a proatherogenic endothelium. This results in enhanced expression of cell-adhesion molecules, such as P-selectin and E-selectin. The primary adhesion of platelets on a compromised vascular endothelial surface is accomplished through the binding of platelet glycoprotein Ibα receptors to von Willebrand factor, whereas firm adhesion is mediated through β_3 integrins. Once adherent, platelets also secrete atherogenic mediators, such as cytokines, chemokines, growth factors, adhesion molecules, and coagulation factors. The upregulation of P-selectin expression on the surfaces of both platelets and endothelial cells potentiates the interactions with P-selectin glycoprotein ligand 1, which is expressed on leukocyte membranes. The binding between platelets and circulating leukocytes (monocytes and neutrophils), dendritic cells, and progenitor cells produces coaggregates that support further leukocyte activation, adhesion, and transmigration, processes considered to be critical for plaque formation and progression[22-29] (Fig. 2).

COAGULATION SYSTEM DURING ATHEROSCLEROTIC PLAQUE PROGRESSION

We have found a local synthesis of several functionally active coagulation proteins, which suggests an active cell-based coagulation network, within human atherosclerotic lesions. The role of these coagulation proteins in atherogenesis is indicated by increased thrombin-generating activity in early atherosclerotic lesions, as compared with that in stable, advanced lesions.[30] These findings are supported by experimental data[31] and a clinical study showing that increased plaque echogenicity (more fibrous structure), rather than plaque echolucency (lipid-rich, higher content of inflammatory cells and thinner fibrous caps), is associated with thrombin generation in plasma from patients with carotid-artery stenosis.[32] The abundance of coagulation factors within early atherosclerotic vessels and local generation of thrombin or fibrin may be attributable to primary protective mechanisms against vascular injury. However, the persistent inflammatory environment within the arterial wall, supported in part by coagulation-mediated actions, may maintain local thrombin generation, which will eventually turn into a vicious cycle, contributing to the formation of intraplaque thrombi[33,34] and thus ultimately leading to plaque instability.

TISSUE FACTOR (EXTRINSIC) PATHWAY

Tissue factor is a transmembrane class II cytokine receptor, which is considered the primary physiologic trigger of the coagulation cascade.[8] Tissue factor is also physiologically essential for vascular development. In mice, tissue factor deficiency is associated with a high rate of embryonic death and impaired vascular integrity. Tissue factor is differentially distributed among the various cell types of the vessel wall. Under physiologic conditions in normal blood vessels, the inner endothelial lining does not express tissue factor, whereas the surrounding layers, consisting of VSMCs, adventitial fibroblasts, and pericytes, show abundant synthesis of tissue factor. This specific vascular localization of tissue factor is generally attributed to its role in the prevention of bleeding after injury, also referred to as a hemostatic envelope.[35]

Within the atherosclerotic lesion, tissue factor is predominantly localized on macrophages, VSMCs, and foam-cell–derived debris within the necrotic core.[30,36-38] Tissue factor activity is significantly higher in lesions obtained from patients with unstable angina or myocardial infarction than in those from patients with a stable

Figure 1 (facing page). Platelets and Coagulation Factors in the Regulation of Thrombus Formation.
Panel A shows platelet adhesion and aggregation, in which atherothrombosis begins with an endothelial injury or rupture of an atherosclerotic plaque. This process triggers transient neurohumoral vasoconstrictor mechanisms, which are reinforced by the release of endothelium-derived factors, such as endothelin. The platelet membrane receptors glycoprotein Ib/IX/V and glycoprotein VI elicit platelet tethering to the exposed thrombogenic subendothelial proteins, von Willebrand factor, and collagen. In addition, glycoprotein VI generates intracellular signals to mediate platelet adhesion and aggregation through the activation of integrin receptors, such as glycoprotein Ia/IIa and glycoprotein IIb/IIIa, with the latter also serving as a receptor for fibrinogen. These molecular events ultimately contribute to the formation of the primary hemostatic plug.[10] Panel B shows the tissue factor (extrinsic) pathway, in which tissue factor, the major trigger of coagulation, is exposed at the site of plaque erosion or rupture. Tissue factor forms a catalytic complex with factor VIIa that leads to the subsequent activation of factors IX and X. In a so-called prothrombinase complex, activated factor X together with activated factor V promotes a downstream enzymatic cleavage of prothrombin, which yields small amounts of thrombin.[11] Thrombin is a pleiotropic, central coagulation enzyme[12] that not only converts fibrinogen into fibrin but also has a substantial role in the activation of platelets and activates factor XIII to induce fibrin polymerization, a fundamental process for the formation of a stable clot, or thrombus. Furthermore, by supporting positive-feedback activation of the upstream factors V, VIII, and XI, thrombin plays a crucial part in the amplification and propagation phases of coagulation. The activated platelet surface is also a critical catalyst for the coagulation cascade. Platelets actively participate in the clotting process by introducing extra amounts of tissue factor, factor V, fibrinogen, and factor XIII into the system, derived from various local sources (fibrinogen and factors V and XIII stored in α granules),[13] and facilitating the direct activation of factor XI by thrombin and the subsequent activation of factor IX on the platelet surface. Factor IXa forms the so-called tenase complex together with factor VIIIa, thereby igniting a burst of additional thrombin generation, which is essential in forming sufficient fibrin and sealing the defect. Panel C shows the contact activation (intrinsic) pathway, which is not considered to be essential for protection against bleeding in vivo, even though its components may be involved in the pathogenesis of arterial thrombosis.[14] The exposure of plasma prekallikrein, high-molecular-weight kininogen, and factors XI and XII to anionic surfaces[15] results in the conversion of prekallikrein to kallikrein, which activates factor XII into factor XIIa but also cleaves high-molecular-weight kininogen, leading to the release of the inflammatory mediator and vasodilator bradykinin. Factor XIIa activates factor XI and favors the conversion of more prekallikrein to kallikrein, thereby reciprocally amplifying the cascade. This sequence of proteolytic reactions leads to the activation of factor IX, which ultimately cleaves factor X into its active form and culminates in the convergence of both coagulation pathways. Gray circles indicate the inactive form of a coagulation protein, and green circles indicate the active form.

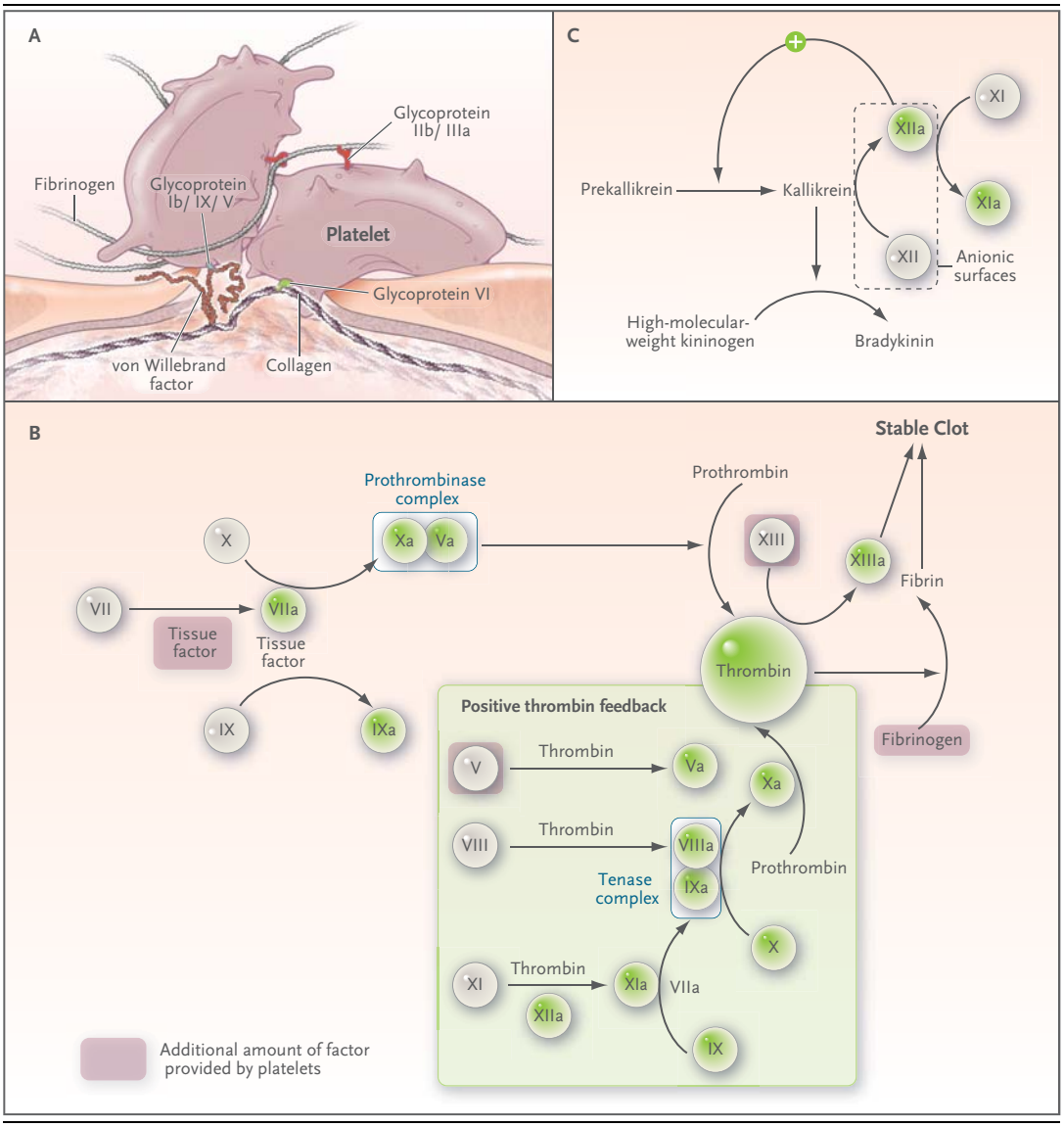
form of cardiovascular disease,[39-41] suggesting a role of this coagulation protein in plaque thrombogenicity. Factor VII is also extrahepatically expressed within both normal and atherosclerotic vessels and colocalizes with tissue factor on macrophages and VSMCs.[30] Apart from its coagulation properties, the TF–FVIIa complex is multifunctional, with a capacity to promote cell signaling, gene transcription, and subsequent protein synthesis. PAR-2 activation is essential in the mediation of TF–FVIIa–induced signaling. The latter may engage several proatherogenic processes, such as monocyte and fibroblast chemotaxis, inflammation, VSMC migration and proliferation (vascular remodeling), angiogenesis (contributing to plaque destabilization), induction of oxidative stress in macrophages, and apoptosis[42] (Fig. 3). Surprisingly, reduced vascular expression of tissue factor does not affect atherosclerosis progression in transgenic mice.[43]

There are few clinical data regarding the role of TF–FVIIa on atherosclerosis progression. Levels of plasma tissue factor antigen, modulated by known polymorphisms of the tissue factor gene, are positively associated with both an increased risk of death from cardiovascular causes[44] and an increased carotid intima–media thickness,[45] which is considered a marker of subclinical atherosclerosis. A similar relation between factor VII and increased intima–media thickness has been documented both in healthy young adults and in patients with peripheral arterial disease.[46,47]

COMMON COAGULATION PATHWAY

PLEIOTROPIC FACTOR Xa

Once activated, factor Xa initiates intracellular signaling in various cell types of the cardiovascular system, preferentially mediated by PAR-2 or, when in ternary complex with TF–FVIIa, through both PAR-1 and PAR-2.[17] PAR-1, PAR-2, or both are present in abundance on endothelial cells, leuko-



cytes, VSMCs, fibroblasts, and dendritic cells. Factor Xa–dependent, PAR-mediated signaling contributes to the production of proinflammatory cytokines, including interleukin-6, interleukin-8, and chemokine (C-C motif) ligand 2 (CCL2), and to the expression of cell-adhesion molecules, including E-selectin, intracellular adhesion molecule 1 (ICAM-1), and vascular-cell adhesion molecule 1 (VCAM-1), along with tissue factor up-regulation, VSMC proliferation, and the release of growth factors (vascular endothelial growth factor, platelet-derived growth factor, and transforming growth factor β).[17] All these may contribute to the progression of atherosclerotic plaque, involving inflammation, leukocyte transmigration, restenosis, and angiogenesis (Fig. 3). Of note, vascular remodeling and neointimal formation were reduced on targeted delivery of nonspecific factor Xa inhibitors (heparin and low-molecular-weight heparins) coupled to an antifibrin antibody.[48]

THROMBIN

Thrombin is a unique serine protease that is pivotal to coagulation and that may also display various actions toward other systems (e.g., immune, nervous, gastrointestinal, and musculoskeletal systems). Governed by the interaction and proteo-

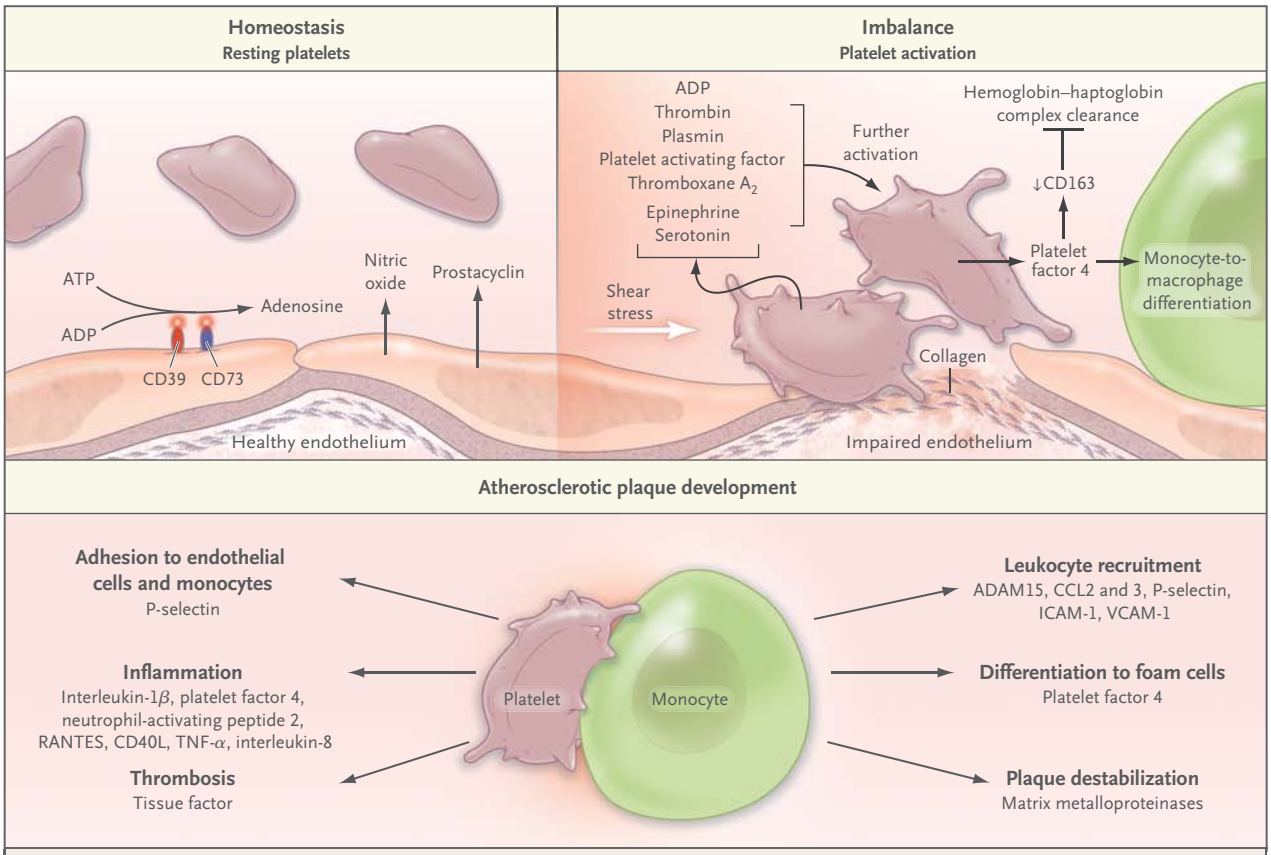


Figure 2. Platelets in Atherogenesis.

Intact endothelium normally expresses CD39 (ecto-ATPase) and CD73 (ecto-5′-nucleotidase), which act in tandem to induce the breakdown of the prothrombotic adenosine 5′-triphosphate (ATP) and adenosine diphosphate (ADP) into the largely antiinflammatory adenosine, thus preventing platelet activation and aggregation. Healthy endothelium also secretes vasodilators, such as prostacyclin and nitric oxide, which have potent antiadhesive and antiaggregating effects. At the time of activation, platelets undergo a substantial change in shape and promptly release a variety of autocrine and paracrine mediators such as ADP, epinephrine, and thromboxane A_2. Studies investigating how platelets orchestrate these widely differing atherogenic actions have provided an increased understanding of the mechanisms involved. Much attention has focused on cytokine-like and chemokine systems such as the CD40–CD40L dyad, CCL5 (RANTES), and platelet factor 4.[23,24] Platelet factor 4 supports monocyte differentiation into macrophages and down-regulates the atheroprotective receptor CD163, which accounts for the clearance of hemoglobin–haptoglobin complexes. Transgenic mice lacking platelet factor 4 have diminished progression of atherosclerosis. Furthermore, CD40 and its ligand, CD40L, which belongs to the superfamily of tumor necrosis factor receptor and ligand, is widely expressed in the vessel wall (e.g., in endothelial cells, vascular smooth-muscle cells, and fibroblasts) and several immune constituents (monocytes or macrophages, neutrophils, mast cells, T and B cells, and dendritic cells).[25] The complex array of proinflammatory, immune-modulating effects and prothrombotic features[26] assert an integral role for CD40–CD40L in atherogenesis. Overall, these findings support the hypothesis that platelets are important proinflammatory players that elicit multifaceted cellular interactions and are directly involved in the early development of atherosclerotic lesions. Platelets are primary mediators in both adaptive and innate immunity.[27] Hence, the targeting of platelet chemokines appears to be therapeutically unsuitable in the context of atherosclerosis because of the severe impairment of multiple systemic immune responses, which may also result in carcinogenesis.[28,29] ADAM15 denotes ADAM metallopeptidase domain–containing protein 15, CCL2/3 chemokine (C-C motif) ligand 2/3, ICAM-1 intercellular cell-adhesion molecule 1, TNF-α tumor necrosis factor α, and VCAM-1 vascular-cell adhesion molecule 1.

lytic activation of its direct cellular targets (PAR-1, 3, and 4),[49,50] thrombin is entwined with the regulation of vascular physiology and pathophysiology[51] (Fig. 3). Thrombin is an example of a multifaceted molecule with broad physiologic properties. By binding to thrombomodulin, thrombin favors the transformation of protein C into activated protein C, a potent anticoagulant and antiinflammatory molecule. Moreover, thrombin can diminish the release of interleukin-12 and promote the up-regulation of interleukin-10 in monocytes, thus inducing immunosuppressive

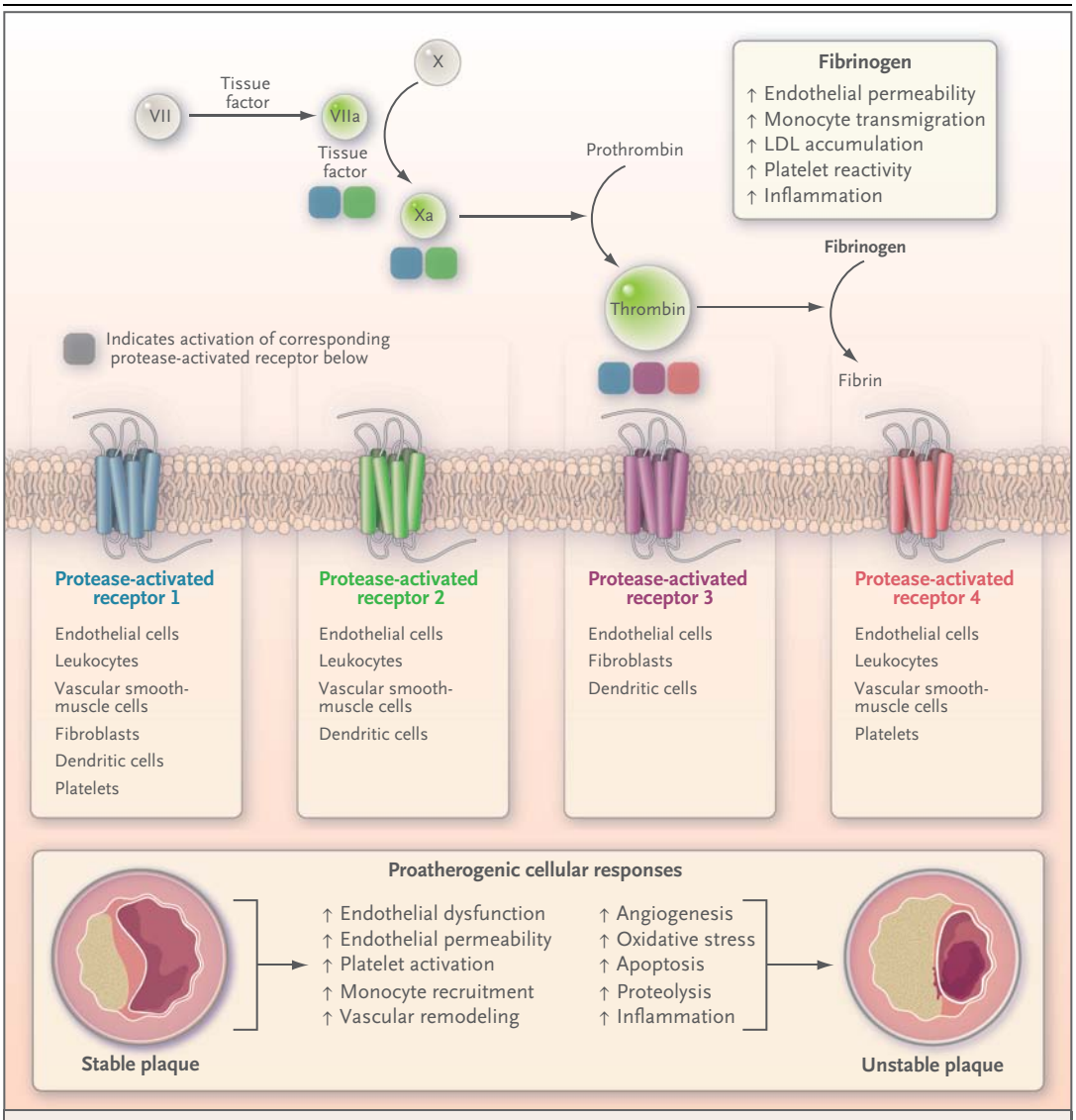


Figure 3. Nonhemostatic Actions Triggered by the Tissue Factor and Common Activation Pathways in the Phenotypic Modulation of the Arterial Wall.

Thrombin, factor Xa, and the tissue factor–factor VIIa complex can activate protease-activated receptors, which are widely expressed on endothelial cells, leukocytes, vascular smooth-muscle cells, fibroblasts, dendritic cells, and platelets, resulting in a plethora of proatherogenic actions. Gray circles indicate the inactive form of a coagulation protein, and green circles indicate the active form. LDL denotes low-density lipoprotein.

and antiinflammatory actions. Thrombin may also play a role in normal vasomotor regulation.[18]

The endothelial decay of thrombomodulin during atherogenesis may allow thrombin to potentiate atherogenic processes, such as endothelial dysfunction and barrier disruption, oxidative stress, apoptosis, inflammation (overexpression of cytokines or chemokines), activation of platelets and leukocytes, leukocyte recruitment, migration and proliferation of VSMCs, and angiogenesis, which suggests an important role in the pathogenesis of cardiovascular disease.[18] Thrombin, factor Xa, factor XIa, factor IXa, and plasmin also show enzymatic activity for cleavage of complement proteins C3 and C5 into their active forms.[52] Proteins C3 and C5 are known to induce inflammation and chemotaxis of inflammatory cells. Human coronary atherosclerotic lesions overexpress anaphylatoxin receptors C3aR and C5aR, as compared with healthy vessels,

primarily localized on macrophages but also on endothelial cells, intimal VSMCs, T cells, and mast cells. Overall, these data establish a new interface between coagulation and inflammation in atherosclerosis.

The administration of thrombin-specific inhibitors reduces restenosis in rabbits with atherosclerosis after angioplasty.[53,54] Another piece of evidence for the in vivo relevance of these effects comes from a study showing that the direct thrombin inhibitor melagatran reduces atherosclerosis progression in apolipoprotein E–knockout mice and promotes plaque stability by inhibiting proinflammatory transcription factors and attenuating the synthesis of matrix metalloproteinases.[55] Furthermore, mice with combined deficiency of factor VIII and apolipoprotein E had significantly less development of atherosclerotic lesions than control mice, despite having more pronounced hyperlipidemia.[56] In contrast, hypercoagulability has been linked with atherosclerosis progression in murine studies, showing that homozygosity for factor V Leiden, a known prothrombotic mutation, promotes atherogenesis.[57] However, a recent study showed an increase in the size of atherosclerotic plaques in procoagulant mice, indicating that a hypercoagulable state contributes to a more stable plaque phenotype.[31] Overall, these findings suggest that hemostasis exerts various effects on the vasculature and, by the action of distinct regulators, may ultimately contribute to determining the plaque phenotype.

The clinical evidence in this regard remains inconsistent. Despite the fact that prothrombotic genetic variants have not been consistently linked to the progression of cardiovascular disease in patients,[44] clinical data show a positive association between markers of thrombin generation and the atherosclerotic plaque burden.[58,59] Low levels of factor VIII have not shown atheroprotective effects in patients with hemophilia,[44] whereas there is clinical evidence that elevated levels of factor VIII promote cardiovascular disease.[60] In plasma, factor VIII circulates in a complex with von Willebrand factor, which modulates factor VIII activity in the circulation. Since mice that are deficient in von Willebrand factor have significantly fewer atherosclerotic plaques than control mice, von Willebrand factor may also play a role in atherosclerosis.[61] Like the data regarding factor VIII and other coagulation proteases, clinical data on the association between von Willebrand factor and cardiovascular disease have been inconsistent.[44,60] More experimental and clinical data are needed to clarify these relationships.

FIBRINOGEN, FIBRIN, AND FACTOR XIII

In clinical studies, there have been strong associations between increased plasma fibrinogen levels and the risk of cardiovascular disease, which suggests hyperfibrinogenemia as an independent predictor of vascular events.[62] Furthermore, the distribution of fibrinogen and fibrin degradation products in atherosclerotic lesions during progression has been clearly documented.[63,64] Elevated levels of plasma fibrinogen, a major determinant of the amount of thrombin that is formed,[65] are closely related to an enhanced rate of coronary-artery calcification and increased intima–media thickness, both measures of premature atherosclerosis.[66] From a cellular and molecular perspective, fibrinogen may affect the plaque phenotype through several distinct mechanisms: favoring the permeability of endothelial cells, extracellular accumulation of low-density lipoprotein (LDL) cholesterol, and the formation of foam cells; inducing the migration of monocytes and VSMCs; increasing platelet reactivity or aggregation; and enhancing inflammation[67] (Fig. 3). Studies in animals have shown distinct results on the role of fibrinogen in atherosclerosis, with some studies indicating that fibrinogen deficiency in transgenic mice is associated with accelerated atherogenesis in a thrombin-dependent manner,[68] and others showing that fibrinogen deficiency is not a prerequisite for the development of advanced atherosclerotic plaque.[69] Increased plasma levels of D-dimer fragments are also associated with enhanced inflammation and an increased incidence of cardiovascular disease and are considered a biomarker of atherothrombosis.[70] However, the effect of fibrin degradation products on the vascular-wall phenotype is less clear. Although the results of one study suggested that D-dimers promote a proatherogenic phenotype in human monocytes,[71] other studies have shown that both fragments D and E may prevent the proliferation of VSMCs in vitro.[72]

Finally, blood coagulation factor XIII may also be related to atherogenesis. Factor XIII not only cross-links fibrin chains to fibrin on activation, which contributes to clot stability, but also appears to facilitate the formation of hyperactive dimers of angiotensin II type 1 receptor, thus

leading to chronic sensitization of circulating monocytes and exacerbating atherosclerosis.[73]

CONTACT ACTIVATION (INTRINSIC) PATHWAY

The contact activation pathway is considered nonessential for hemostasis in vivo (Fig. 1C and Fig. 4). However, it may be involved in the pathogenesis of arterial thrombosis.[14] Although experimental data have clearly shown that mice deficient in factor XII are protected against arterial thrombosis and stroke,[14] in several epidemiologic studies, data on the association between factor XII and the risk of cardiovascular disease in humans are inconsistent.[74-76] Although additional research is needed in this field, the pharmacologic inhibition of factor XII activation represents a potential therapeutic target,[77,78] considering that hereditary deficiency of factor XII is not associated with bleeding disorders or other pathologic conditions.

At a molecular level, factor XII influences distinct processes mostly through the plasma kallikrein–kinin system.[79] Factor XII–mediated bradykinin formation not only regulates vasodilatation and vascular permeability but also induces activation of the complement and fibrinolytic systems by activating components C3 and C5 and facilitating the synthesis of tissue-type plasminogen activator from endothelial cells, whereas kallikrein activates urokinase-type plasminogen activator and plasminogen. Platelet-derived inorganic polyphosphates[80] and misfolded proteins, which are found abundantly in atherosclerotic arteries,[81] can also activate factor XII, leading to kallikrein formation without triggering coagulation.[82] Levels of tissue kallikrein and plasma prekallikrein are associated with the severity of cardiovascular disease[83,84] and have been found to be critical in the process of vascular repair.[85] Given the proangiogenic and proinflammatory nature of factor XII[86] and the plasma kallikrein–kinin system, chronic stimulation of these responses may promote a proatherogenic intraarterial environment over time.

ANTICOAGULANT PATHWAYS IN VASCULAR INFLAMMATION

Tissue factor pathway inhibitor (TFPI), which is widely distributed in healthy arterial vessels, tends to be overexpressed in atherosclerotic lesions[87] (Fig. 5A). Although TFPI is expressed on endothelial cells, VSMCs, and macrophages in the fibrous cap and shoulder areas of the plaques, it also colocalizes with tissue factor and attenuates its activity within atherosclerotic lesions.[30,96,97] This finding suggests a role for TFPI not only in the regulation of tissue factor procoagulant activity but also in the control of tissue factor–induced proatherogenic signaling. The administration of recombinant TFPI has reduced the rates of inflammation and death in an animal model by decreasing the expression of tumor necrosis

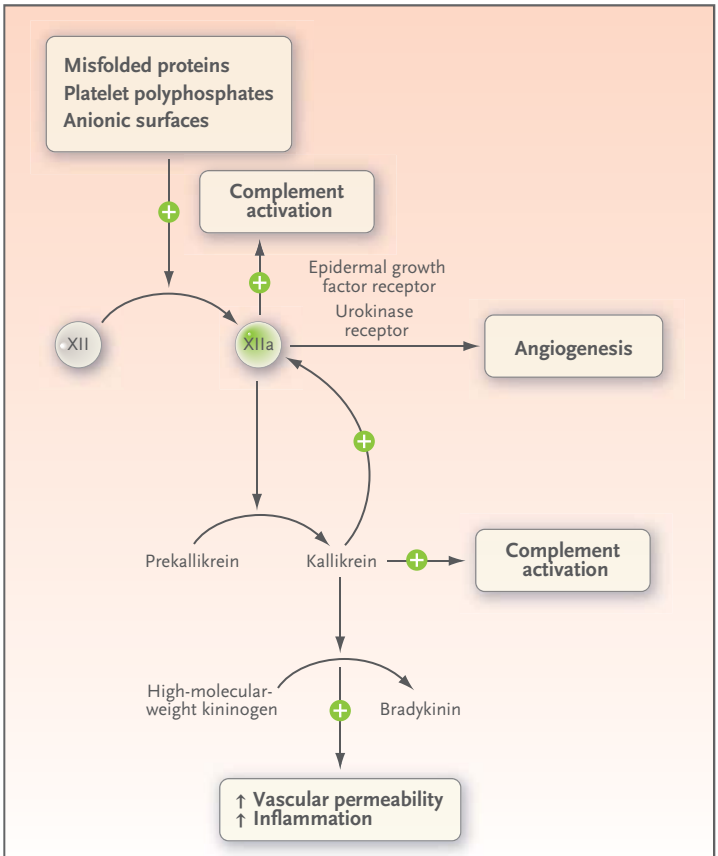


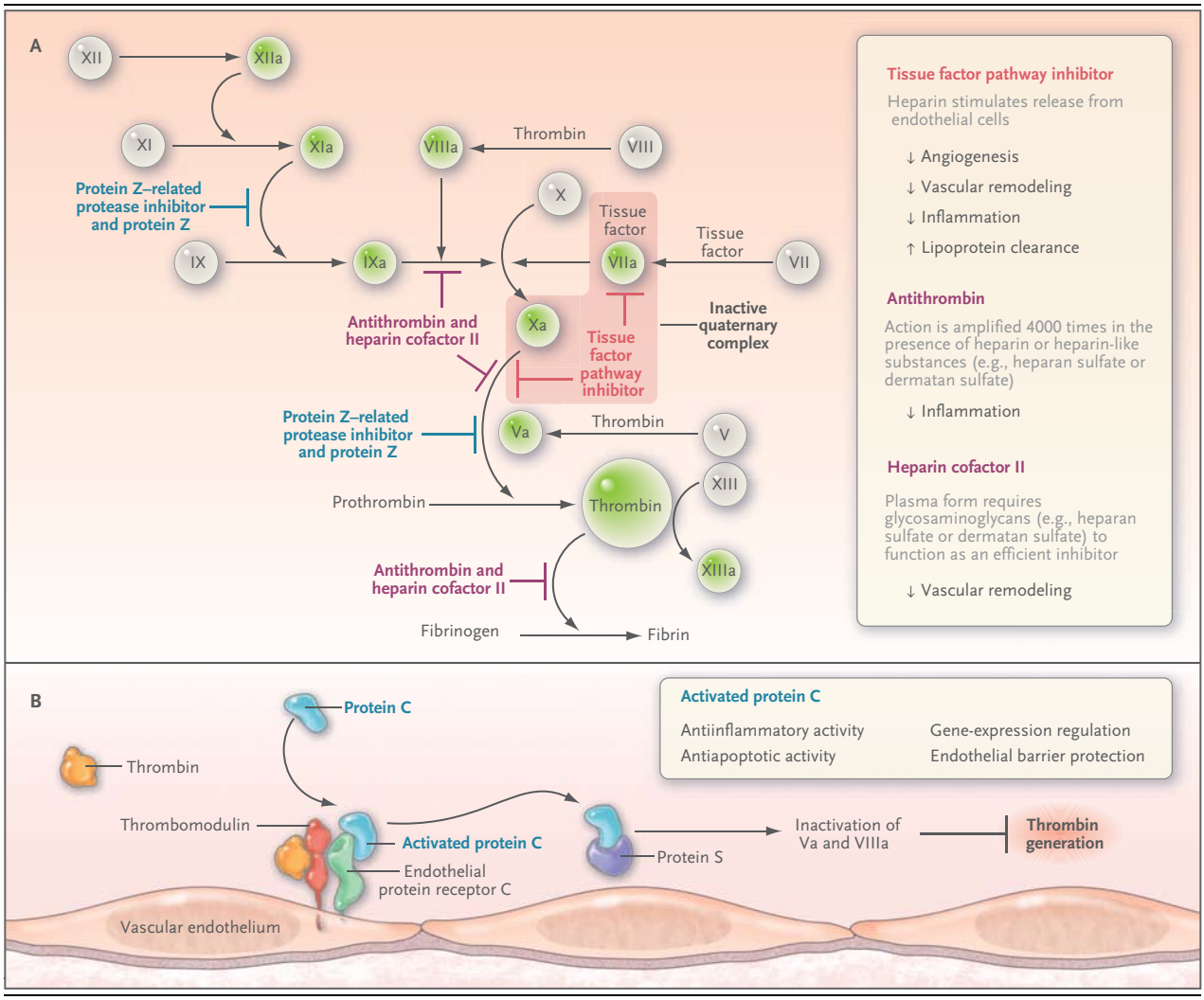
Figure 4. Contact Activation Pathway and Its Proinflammatory and Proangiogenic Properties.

The contact system plays a role in various physiologic processes, such as blood-pressure regulation, coagulation, fibrinolysis, angiogenesis, and inflammation. It consists of factor XII, prekallikrein, and high-molecular-weight kininogen. The activation of the proinflammatory kallikrein–kinin and complement systems is triggered by the proteolytic cleavage of factor XII (autoactivation) in reaction to contact with negatively charged artificial or biologic surfaces. The gray circle indicates the inactive form of a coagulation protein, and green circles indicate the active form. Green circles with plus signs indicate either positive-feedback reactions or induction of a process.

Figure 5 (facing page). Anticoagulant Pathways and Their Nonhemostatic Features.
The regulation of coagulation operates at three levels: inhibition of thrombin, factor Xa, and factor IXa by antithrombin; inhibition of factor Xa, the tissue factor–factor VIIa (TF–FVIIa) complex, and hence thrombin formation by tissue factor pathway inhibitor; and proteolytic inactivation of factor V and factor VIII by activated protein C. As shown in Panel A, antithrombin is a serine protease that inhibits key coagulation enzymes such as thrombin, factor Xa, and factor IXa. Its action is amplified by as much as 4000 times in the presence of heparin or heparin-like substances, such as heparan sulfate proteoglycan. Antithrombin has apparent antiinflammatory effects,[88] as seen in an increase in the release of prostacyclin and a decrease in nuclear factor κB signaling, which is known to have multiple proinflammatory responses. Similar effects have been found after the administration of synthetic direct thrombin inhibitors, which has contributed to plaque stability in vivo.[55] Antithrombin attenuates leukocyte recruitment during inflammation, which hints at another potential atheroprotective role. Heparin also stimulates the release from endothelial cells of tissue factor pathway inhibitor, which then binds to factor Xa and the TF–FVIIa complex to form an inactive quaternary complex, thus showing a multitude of antiatherogenic functions. Like antithrombin, heparin cofactor II has the ability to inactivate thrombin, factor Xa, and factor IXa, whereas the plasma form of heparin cofactor II is an inefficient inhibitor in the absence of glycosaminoglycans (e.g., heparan sulfate and dermatan sulfate). Heparin cofactor II is implicated both in vascular remodeling and in atherogenesis. Mice that are deficient in heparin cofactor II have enhanced intimal hyperplasia after vascular injury.[89] Such mice have increased neointima formation and enhanced atherogenesis, as compared with control mice. However, the findings in clinical studies have been inconsistent, with some indicating that heparin cofactor II is a strong predictive marker against atherosclerosis[90,91] and one indicating that its presence is not predictive.[92] Protein Z is a cofactor of another protein, named protein Z–related protease inhibitor, which inhibits factor Xa and factor XIa in the coagulation cascade. Although the roles of protein Z and protein Z–related protease inhibitor in inflammation and the onset of atherosclerosis are poorly understood, a few clinical trials have shown a significant inverse relationship between levels of these proteins and the clinical severity of atherosclerosis.[93-95] As shown in Panel B, thrombin also behaves as an anticoagulant molecule physiologically. Binding to the endothelial protein C receptor, protein C is transformed into activated protein C by an activation complex established between thrombin and thrombomodulin. This process is followed by dissociation of activated protein C from the endothelial protein C receptor and the formation of a complex between activated protein C and protein S. The latter allows the inactivation of factor Va and factor VIIIa and thus limits further thrombin generation. Gray circles indicate the inactive form of a coagulation protein, and green circles indicate the active form.

factor α (TNF-α), chemokines, and myeloperoxidase.[88] Moreover, TFPI is a potent inhibitor of matrix metalloproteinases, which are considered key players in plaque destabilization and atherothrombotic complications. Decreased TFPI expression has been associated with up-regulation of the synthesis of matrix metalloproteinases in plaques with a vulnerable phenotype. In addition, TFPI has inhibited endothelial migration and angiogenesis in mice. Several studies in animals have shown that TFPI attenuates neointimal hyperplasia and stenosis but also suppresses the release of proatherogenic platelet-derived growth factor BB, CCL2, and matrix metalloproteinase 2.[98-101] In agreement with these findings, TFPI-deficient mice have significantly more atherosclerotic plaques than control mice,[102] whereas vascular-directed TFPI overexpression appears to regulate lipoprotein clearance and temporarily lowers plasma cholesterol levels, also reducing atherosclerotic plaque development.[103] Clinical data suggest that plasma TFPI is a marker of endothelial dysfunction; high levels of both free and total TFPI levels are associated with an increased atherosclerotic burden and coronary-artery calcification,[104,105] whereas low levels of total TFPI are associated with an increased risk of atherothrombosis.[106,107]

In addition to its anticoagulant properties, the protein C pathway is known for its protective effects on vascular gene-expression profiles involving antiapoptotic and antiinflammatory responses, as well as its stabilizing effect on the endothelial barrier (Fig. 5B).[108] Studies of atherosclerosis have shown a substantial downregulation of the local expression of endothelial protein C receptor and thrombomodulin within atherosclerotic vessels, suggesting impaired activation of protein C and hence a reduced antiatherogenic response. Several mechanisms, such as enhanced shedding of thrombomodulin from dysfunctional endothelium, an abundance of LDL-cholesterol deposits, and local inflammation within the arterial wall, may account for the attenuation of the anticoagulant activities of protein C within the atherosclerotic plaque. Overexpression of thrombomodulin has been shown to limit neointimal formation in rabbits,[109] whereas a genetic impairment of the protein C–activating cofactor function of thrombomodulin, resulting



in diminished formation of activated protein C, is associated with an increased atherosclerotic burden in mice.[31]

The determinants of soluble levels of thrombomodulin in patients with atherosclerosis are poorly understood. The results of various clinical studies that have examined the relationship between thrombomodulin and the extent of atherosclerotic burden have been inconsistent.[110-114] In monkeys, progressive atherosclerosis is associated with impaired formation of activated protein C, whereas dietary regression of atherosclerosis was found to enhance the anticoagulant response.[115] Mice with a heterozygous deficiency in protein C have enhanced focal arterial inflammation and thrombosis, leading to increased neointima formation and localized thrombosis.[116] In agreement with these findings, several clinical studies have confirmed a significant association between circulating low levels of activated protein C and a greater extent or severity of atherosclerosis.[117-119]

Furthermore, protein S, which has been described as linking hemostasis, inflammation, and apoptosis, forms a complex with the complement system regulator C4b-binding protein (C4BP), a major inhibitor of the classical complement pathway, localizing it on the surface of apoptotic cells[120] and thus promoting phagocytic activity by macrophages.[121] Intriguingly, protein S significantly inhibits the expression of macrophage scavenger receptor A and diminishes the uptake of acetylated LDL cholesterol mediated by this receptor, resulting in a decreased intracellular lipid content in macrophages.[122] These actions are mostly attributable to the ability of protein S to

bind to and induce phosphorylation of the Mer receptor tyrosine kinase. In addition, protein S plays a role in the protection of the integrity of the blood–brain barrier.[123] The expression of protein S is reduced within atherosclerotic plaques obtained from patients with unstable angina, as compared with specimens from patients with stable angina.[124] Hereditary deficiency of both proteins C and S has been associated with an increased incidence in arterial thromboembolic events[125] and peripheral-artery disease (Fig. 5B).[89-95,126]

FUTURE PERSPECTIVES

Hemostasis is anatomically and functionally entwined with the vasculature. Besides its essential roles in protecting vascular integrity and maintaining normal blood flow, accumulating data suggest an intimate cross-talk between hemostasis and inflammation, underscoring the role of both systems in many complex diseases, including atherothrombosis. Intriguingly, numerous studies in animals have also documented that hemostasis is closely linked to the pathophysiology of atherogenesis. Is this association, mostly based on experimental data, corroborated by clinical data as well?

The current concept of a vulnerable plaque suggests that repeated plaque microruptures, followed by subclinical thrombosis, are critical for plaque growth and vulnerability.[127-129] In agreement with these findings, histopathological studies showed that two thirds of coronary thrombi obtained from patients who died suddenly from cardiovascular causes were in later stages of maturation, suggesting that thrombi may exist long before a rupture occurs.[33,34] In addition, the contemporary understanding of atherothrombosis has evolved substantially, establishing new roles for the hemostatic system beyond thrombosis. We have summarized the potential array of actions of hemostasis in relation to the phenotype of the atherosclerotic vascular wall, presumably linked to plaque stability. But is all of this clinically relevant?

Antithrombotic therapy with the use of antiplatelet or anticoagulant agents is the key to atherothrombosis prevention in various clinical situations.[130-133] The role of antiplatelet therapy in secondary prevention is no longer questioned, given the strong overall effect of drugs such as aspirin.[134] A meta-analysis of primary-prevention trials has indicated that the use of aspirin is associated with a reduction of approximately 30% in the risk of myocardial infarction, with more limited effects on the risk of stroke.[135] In addition to aspirin's antiplatelet actions, the efficacy of this drug may be due in part to its antiinflammatory actions.[136-138] It is difficult to dissect the contribution of platelets in any of these antiinflammatory effects of aspirin. Also, for more selective antiplatelet drugs, including clopidogrel, prasugrel, and ticagrelor, which target platelet receptors, resulting in impaired platelet activation, antiinflammatory and atherosclerosis-delaying effects have been reported.[139] However, clinical trials of platelet inhibitors for the prevention of atherosclerosis progression have not shown diminished development of plaque with any consistency.[140]

For many years, oral anticoagulants have been used for short- and long-term indications. Studies of heparin and vitamin K antagonists have shown that short-term use of these drugs is not likely to have a major effect on chronic disorders such as atherosclerosis.[141,142] Despite the fact that long-term administration of vitamin K antagonists did not have any visible effects on angiographic progression in patients who had undergone coronary-artery bypass grafting, an additional follow-up assessment 3 years after discontinuation of therapy showed a significant 35% reduction in overall mortality in the warfarin group.[143] Given the powerful effects on risk reduction in thrombotic cardiovascular outcomes, one might speculate that this effect was at least partially mediated by effects of vitamin K antagonists on plaque phenotype rather than plaque size. At the same time, the principal vascular side effect of the long-term administration of these drugs is accelerated calcification. This effect is mainly due to direct inhibition of other vitamin K–dependent proteins in the vessel wall, including matrix Gla protein. It is not known whether any additional influence of inhibition of thrombin formation may occur.[144,145] The role of the hemostatic system in atherosclerosis in humans requires further investigation. Only a handful of molecules relevant to hemostasis are targeted by existing medications. As more specific interventions are developed, new therapeutic avenues and research approaches may open up. With the introduction of new oral anticoagulants (e.g., direct inhibitors of factor Xa and thrombin),[146,147] which are small

molecules that can access the vessel wall, it will be possible to document the effects of these drugs on plaque formation and especially on plaque stability. Since both thrombin inhibition[55] and a prothrombotic state[31] have been suggested as promoters of plaque stability in atherogenic mice, the net effects in humans, if any, are unpredictable.

In conclusion, given the potential of hemostasis to influence molecular and cellular responses in the vasculature, new scientific approaches are required. Notably, the majority of experimental data are entirely based on quantification of plaque burden, rather than on extensive phenotyping of the lesions. This is a major drawback in vascular medicine. Furthermore, most clinical studies predominantly focus on establishing the thrombotic and mortality outcomes, whereas few investigate plaque progression. During the past decade, ultrasonography has been a major tool in vascular imaging. Unfortunately, this approach is characterized by poor tissue penetration, providing no information on plaque characteristics, and is subject to intraobserver and interobserver variability. With the development of high-resolution magnetic resonance imaging, the assessment of plaque characteristics will improve vessel-wall phenotyping as a means of addressing the role of the hemostatic system in atherosclerosis.

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