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Forum Editorial

Redox Control of Blood Coagulation

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Any disturbance in the integrity of the endothelium that allows the exposure of blood to the extravascular tissue will trigger blood coagulation and platelet-mediated primary hemostasis leading to the formation of a platelet plug that initially occludes the vascular lesion. Careful control of blood coagulation is assured by anticoagulant mechanisms that, under normal conditions, prevail over the procoagulant forces, as well as by an efficient fibrinolytic system that helps to limit the amount of cross-linked fibrin. Disturbances to this delicate balance between procoagulant, anticoagulant, and fibrinolytic systems may result in bleeding or thrombotic diseases (6).

In addition to the hemostatic response increased formation of reactive oxygen species (ROS) is readily observed at sites of vascular injury (1). ROS have been made responsible for promoting many diseases of the cardiovascular system, but also multiple other disease states, including diabetes and cancer. Interestingly, the majority of these diseases are associated with a prothrombotic state suggesting a close link between ROS formation and activation of the blood coagulation system. Therefore, this Forum issue is focused to shed light on the role of ROS and the redox state in the control of hemostasis, thrombosis, and fibrinolysis.

THE BLOOD COAGULATION CASCADE—A TIGHT BALANCE BETWEEN BLEEDING AND THROMBOSIS

Endothelial cells are central in regulating blood flow because, under physiological conditions, they provide a nonthrombogenic surface that helps to prevent undesired hemostasis. After blood-vessel injury or under inflammatory conditions, the endothelium rapidly becomes an adhesive surface with intercellular gaps allowing the passage of soluble plasma and blood cells out of the vascular lumen into the underlying tissue (23). Within seconds, circulating platelets adhere to collagen fibrils in the vascular subendothelium, a process mediated mainly by integrin $\alpha 2\beta 1$ and glycoprotein Ib/IX. After adherence, they aggregate to form an initial plug, thereby providing cell-surface phospholipids for the assembly of blood-clotting enzyme complexes.

The extrinsic pathway of blood coagulation is initiated when blood is exposed to non–vascular-cell–bound tissue factor (TF) in the subendothelial space. TF binds to activated factor VII, and the resulting enzyme complex activates factors IX and X of the intrinsic and common coagulation pathways, respectively. Factor IX activated by the TF pathway, in turn, activates additional factor X, in a reaction that is greatly accelerated by a cofactor, factor VIII. Once activated, factor X converts prothrombin to thrombin (factor IIa) in a reaction that is accelerated by factor V. In the final step of the coagulation pathway, thrombin cleaves fibrinogen to generate fibrin monomers, which then polymerize and link to one another to form a chemically stable clot. Thrombin also feeds back to activate cofactors VIII and V, thereby amplifying the coagulation process. Moreover, by binding to thrombin receptors on platelets, it contributes to the activation of platelets (27).

Thus, the blood-coagulation cascade has the ability to transduce a small initiating stimulus into a large fibrin clot. This potentially explosive cascade is offset by natural anticoagulant mechanisms. The maintenance of adequate blood flow and the regulation of cell-surface activity limit the local accumulation of activated blood-clotting enzymes and complexes. Antithrombin III is a plasma protein that inhibits the activity of the serine proteases of the intrinsic and common coagulation pathways, a process that is potentiated by endogenous heparan sulfate. In the presence of thrombomodulin bound to endothelial cells, thrombin activates protein C, which in turn cleaves activated factors VIII and V accelerated by the cofactor protein S. The TF factor-pathway inhibitor (TFPI) is a lipoprotein-associated plasma protein that forms a quaternary complex with TF and activates factors VII and X, thereby inhibiting the extrinsic coagulation pathway.

Finally, proteolytic degradation of fibrin (fibrinolysis) is critical for preventing excess thrombus growth and restoring blood flow following thrombotic vascular occlusion.

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Fibrinolysis is mediated by plasminogen and its activators, tissue-type plasminogen activator (tPA) and urokinase plasminogen activator (uPA), and is controlled by plasminogen activator inhibitor-1 (PAI-1), a member of the serine protease inhibitor (serpin) superfamily that acts as the principal inhibitor of tPA and uPA in the fibrinolytic system (2).

Increased procoagulant and/or decreased fibrinolytic activity may result in a thrombotic phenotype, which has been shown to contribute to cardiovascular disorders, including atherosclerosis, (pulmonary) hypertension and diabetes, as well as to cancer. Thus, mechanisms controlling the activities of these systems may provide an essential clue for better understanding and treating these disorders.

ROS AND THE CELLULAR REDOX STATE—A TIGHT BALANCE BETWEEN SIGNALING AND TOXICITY

Reduction–oxidation or redox reactions are central not only to intermediary metabolism, but also to the integration and control of a diverse array of interactions, including gene regulation by transcription factors, protein–protein interactions, and DNA synthesis (9). ROS have long been considered as unwanted by-products of aerobic mitochondrial metabolism or other electron transfer reactions when dioxygen is not completely reduced to H_2O. Superoxide anions, hydrogen peroxide (H_2O_2), and hydroxyl radicals are widespread ROS that can give rise to more toxic species. To limit cellular damage, counteracting mechanisms have evolved, including antioxidative enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase.

In recent years, it has become evident that a variety of cells is able to generate ROS. These ROS serve as second messengers and are involved in various cellular signaling mechanisms (26). Indeed, modulation of ROS production has been shown to regulate a variety of genes, most of them linked to proliferation, migration, growth, and development, as well as to inflammation and chemotaxis. A number of enzymes, including NADPH oxidases, cyclooxygenases, xanthine oxidase, and lipoxygenases, have been identified in vascular cells to contribute to inducible ROS production in response to a variety of agonists and/or physicochemical stress. Among them, NADPH oxidases are now considered to play a major role in vascular ROS production (22). They have been shown to be involved in inflammation, proliferation, hypertrophy, remodeling and angiogenesis, but also in prothrombotic responses of the vascular wall, suggesting that they may play a pathogenetic role in many diseases associated with these processes (3, 15).

ROS AND THE REDOX STATE IN THE CONTROL OF HEMOSTASIS, THROMBOSIS, AND FIBRINOLYSIS

Platelets represent a key element in the pathogenesis of atherosclerosis. Once activated, they secrete mitogenic factors, such as platelet-derived growth factor, transforming growth factor, and epidermal growth factor, which lead to ROS production of the vessel wall and subsequently can promote smooth muscle cell proliferation and progression of atherosclerotic lesions (13, 20). In addition, platelets themselves provide important targets for ROS produced or released in the vascular lumen. In this issue, Ferroni *et al.* (11) summarized the current literature that addresses the mechanisms by which oxidant stress affects platelet function and ultimately contributes to vascular diseases in the context of hypercholesterolemia, a major risk factor of atherosclerosis. *In vitro* studies showed that low levels of oxidants may promote aggregation, whereas exposure to high concentrations of exogenous H_2O_2 may result in platelet inhibition. However, as pointed out by the authors, the *in vivo* situation is clearly more complex, because the net effect of ROS on intravascular thrombosis is also dependent on the integrity of the endothelium, as well as on oxidant-mediated alterations of other major players of thrombosis, such as coagulation factors. The complexity of the *in vivo* situation is demonstrated by a study in this issue by Peng *et al.* (25) investigating platelet-dependent thrombus formation at sites of vascular injury in hemoxygenase-1 (HO-1)-deficient mice. Although HO-1 has been considered to exert antioxidative capacities, platelet-dependent thrombus formation was not affected by HO-1 under basal conditions. However, under oxidative stress conditions associated with enhanced HO-1 levels, thrombus formation was significantly reduced in wild-type mice compared with knockout animals, suggesting that up-regulation of HO-1 by ROS may act as an adaptive mechanism in reducing platelet-dependent thrombus formation under these conditions.

The important role of the redox state in platelet activation and adhesion is further delineated in great detail by the article of David Essex in this issue (10), providing insights into the role of the redox cofactor protein disulfide isomerase (PDI), which is involved in disulfide-bond formation and isomerization, on platelet responses. PDI and sulfhydryl groups are present on the platelet surface and provide redox-sensitive sites that regulate platelet aggregation and secretion, as well as activation of platelet integrin receptors.

Many members of the integrin family play a central role in hemostasis, because they control not only adhesion and aggregation of platelets, but also the contractility and barrier function of endothelial cells, thereby acting as key elements in the interplay between the coagulation system and the vascular wall (17). Whereas, on the one hand, integrin activity can be controlled by ROS, these receptors have also been shown to generate ROS—possibly via activation of vascular NADPH oxidases and lipoxygenases—and to activate redox-sensitive signaling cascades, thereby modulating the thrombotic balance as summarized by Gregg *et al.* in this issue (17). In addition to their role in endothelial ROS production, integrins may also participate in ROS production by platelets. Indeed, activation of platelets is accompanied by enhanced formation of ROS. As shown by Chlopicki *et al.* in this issue (4), platelets contain, similar to neutrophils, a gp91phox-containing NADPH oxidase. Activation of ROS generation by this enzyme was involved in the control of thromboxane A_2 production and thrombin-induced platelet aggregation. Interestingly, platelet ROS formation was shown to potentiate neutrophil ROS production, suggesting

that the proinflammatory effects frequently associated with coagulation may be at least partially due to the promotion of neutrophil ROS production (4).

As shown by Cuzzocrea *et al.* in this issue (5), treatment with a synthetic SOD mimetic decreased ROS production and activation of the nuclear factor-κB (NFκB) pathway in mice with an acute inflammatory response. Although platelet activity was not determined in this study, one might speculate that such a treatment may also be beneficial in treating a hypercoagulant or a prothrombotic state. This is supported by the findings described by Djordjevic *et al.* in this issue (8). Expression and activity of TF as the key component triggering intravascular thrombus formation *in vivo* were up-regulated by thrombin via activation of the GTPase Rac, which stimulates the NADPH oxidase, and subsequent activation of NFκB, in pulmonary artery smooth muscle cells. As these cells are responsible for most of the remodeling processes observed in pulmonary hypertension, such a mechanism might link these processes with a hypercoagulable state frequently observed in this disease (19). This assumption is further supported by the article from Herkert *et al.* (18), summarizing evidence for a role of ROS in regulating TF expression and thrombin action in the vascular wall. ROS generated by the NADPH oxidase act as signaling molecules in thrombin-stimulated vascular cells, activating specific signaling pathways that lead to enhanced TF expression and activity. As TF itself promotes the formation of thrombin and may itself be able to induce ROS production, ROS and NADPH oxidases may play an important role in promoting a prothrombotic state in vascular diseases by supporting a thrombogenic cycle in the vascular wall.

Indeed, enhanced NADPH oxidase expression and ROS production have been observed in cardiovascular diseases associated with thrombosis as, for example, in atherosclerosis (3,15), and increased levels of TF have been observed in atherosclerotic plaques. Surprisingly, enhanced expression of the endogenous TF inhibitor TFPI is also found in these lesions (21). In this issue, Ohkura *et al.* (24) showed that oxidized low-density lipoproteins, which are important proatherogenic factors, impair the anticoagulant activity of TFPI by direct interaction of oxidized phospholipids with the protein. These findings provide a further mechanism to indicate how an oxidized environment can lead to a prothrombotic state in the vasculature.

Moreover, as discussed by Dimova *et al.* in this issue (7), ROS and reduced oxygen levels as observed in ischemic or thrombotic diseases play an important role in regulating the expression of PAI-1 in different cell systems. PAI-1 counteracts the fibrinolytic activity of the plasmin system, thus supporting a prothrombotic state. Whereas antioxidants and depletion of NADPH oxidase subunits inhibited thrombin-induced PAI-1 expression in smooth muscle cells (14), PAI-1 expression in liver cells induced by hypoxia was inhibited by expressing a constitutively active Rac mutant activating ROS production and the NADPH oxidase (16). These seemingly contradictory findings suggest that the signaling role of ROS is cell type- and stimulus-specific. This assumption is further substantiated by the *in vivo* study by Franke *et al.* in this issue (12) using mice with hepatocyte-specific overexpression of SOD or GPX. Compared with wild-type mice, PAI-1 expression was enhanced in SOD- as well as in GPX-overexpressing mice.

In summary, modifications of the redox state and ROS production have significant impact on the coagulation system, acting on multiple sites in a cell type-specific fashion. Oxidative stress, which is related to many disease states, is able to modulate platelet function, as well as the function of a variety of coagulation factors. Moreover, ROS act as key players in mediating the interaction between the coagulation system and the vessel wall. This interaction may promote a hypercoagulable state in the blood, but may also provide an essential mechanism to indicate how coagulation factors and platelets activate the vessel wall or other organs thereby modulating gene expression, inflammatory or chemotactic responses, proliferation or angiogenesis. The elucidation of the complex involvement of ROS on the diverse functions associated with hemostasis, thrombosis, and fibrinolysis is still a challenging task but may provide the basis for novel therapeutic strategies targeting thrombosis and thrombosis-related diseases.

ABBREVIATIONS

GPX, glutathione peroxidase; HO-1, hemoxygenase-1; H_2O_2, hydrogen peroxide; NFκB, nuclear factor-κB; PAI-1, plasminogen activator inhibitor-1; PDI, protein disulfide isomerase; ROS, reactive oxygen species; SOD, superoxide dismutase; TF, tissue factor; TFPI, tissue factor-pathway inhibitor; tPA, tissue-type plasminogen activator; uPA, urokinase plasminogen activator.

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