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Bidirectional Relation Between Inflammation and Coagulation

Marcel Levi, MD; Tom van der Poll, MD; Harry R. Büller, MD

Inflammation and coagulation play pivotal roles in the pathogenesis of vascular disease. Increasing evidence points to extensive cross-talk between these two systems, whereby inflammation leads not only to activation of coagulation, but coagulation also considerably affects inflammatory activity. Activation of coagulation and fibrin deposition as a consequence of inflammation is well known and can be viewed as an essential part of the host defense of the body against, for example, infectious agents or nonidentical cells, in an effort to contain the invading entity and the consequent inflammatory response to a limited area. An exaggerated or insufficiently controlled response may, however, lead to a situation in which coagulation and thrombosis contribute to disease, as illustrated by the fact that thrombus formation on a ruptured atherosclerotic plaque, containing abundant inflammatory cells, is the pathological basis of acute arterial thrombotic events such as myocardial infarction or unstable angina.[1] Expression of procoagulant material by inflammatory cells in the unstable plaque (in particular tissue factor) may initiate activation of coagulation, and the thrombin generated will both activate platelets and result in the formation of a platelet-fibrin thrombus (Figure 1). Another example is the occurrence of systemic coagulation activation in combination with microvascular failure that results from the systemic inflammatory response to severe infection or sepsis and that contributes to multiple organ dysfunction.[2] However, rather than this being a 1-way process with inflammation leading to coagulation, both systems closely interact, whereby coagulation can also substantially modulate inflammatory activity. Coagulation factors (such as thrombin) or anticoagulant proteins (such as activated protein C) may activate specific cell receptors on mononuclear cells or endothelial cells, which may affect, for example, cytokine production or inflammatory cell apoptosis.

Initiation and Propagation of Inflammation-Induced Coagulation Activation

The pivotal initiator of inflammation-induced thrombin generation is tissue factor. Blocking tissue factor activity completely abrogates inflammation-induced coagulation activation in models of experimental endotoxemia or bacteremia, whereas antibodies that inhibit the contact system have no effect on thrombin formation.[3,4] Tissue factor is a transmembrane 45-kDa protein that is constitutively expressed on a number of cells throughout the body.[5] The majority of these cells are in tissues not in direct contact with blood, such as the adventitial layer of large blood vessels. However, tissue factor comes into contact with blood on disruption of the vascular integrity or if cells present in the circulation start expressing tissue factor. The source of tissue factor may be different in various inflammatory situations. In atherosclerotic plaques, macrophages produce abundant tissue factor at their surface; on plaque rupture, there is extensive tissue factor exposure to blood.[6] However, smooth muscle cells and cardiomyocytes also are capable of tissue factor expression, although the exact role of these cells in thrombosis after plaque rupture is not clear.[7] Mononuclear cells in atherosclerotic plaques appear to be primed to express more tissue factor than native, circulating mononuclear cells, which is probably due to sustained exposure to proinflammatory factors in the plaque, such as interleukin (IL)-6, but also platelet-derived growth factor and monocyte chemoattractant protein (MCP)-1.[8] In contrast, in severe sepsis, circulating mononuclear cells, stimulated by proinflammatory cytokines, express tissue factor, which leads to systemic activation of coagulation. Indeed, low-dose endotoxemia in healthy subjects results in a 125-fold increase in tissue factor mRNA levels in blood monocytes.[9] Although many cytokines are capable of inducing tissue factor expression on mononuclear cells in vitro, the in vivo expression of tissue factor appears to be mostly dependent on IL-6. Studies show that inhibition of IL-6 by monoclonal antibodies completely blocks tissue factor–dependent thrombin generation in experimental endotoxemia, whereas specific inhibition of other proinflammatory cytokines had less effect or no effect.[10]

On exposure to blood, tissue factor binds to factor VIIa. The complex of tissue factor–factor VIIa catalyzes the conversion of factor X to Xa, which forms the prothrombinase complex together with factor Va, prothrombin (factor II), and calcium, thereby generating thrombin (factor IIa). One of the key functions of thrombin is converting fibrinogen into fibrin. Amplification loops consist of (1) the activation of factor IX by the tissue factor–factor VIIa complex, generating large amounts of additional factor Xa, (2) activation of the essential

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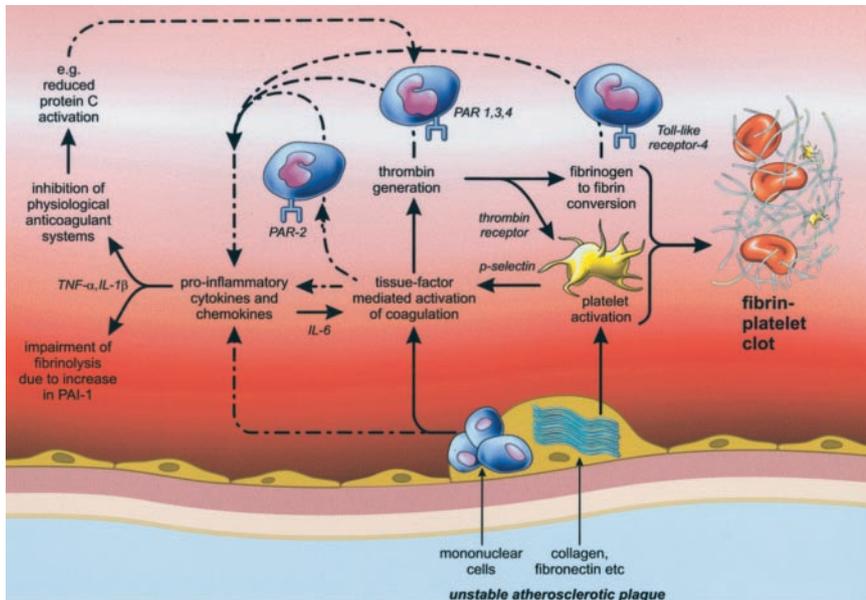
Figure 1. Schematic representation of activation of coagulation and inflammation on rupture of atherosclerotic plaque. Exposure of tissue factor–bearing inflammatory cells to blood results in thrombin generation and subsequent fibrinogen to fibrin conversion. Simultaneously, activation of platelets occurs, both by thrombin and by exposure of collagen (and other subendothelial platelet-activating factors) to blood. Binding of tissue factor, thrombin, and other activated coagulation proteases to specific PARs on inflammatory cells may affect inflammation by inducing release of proinflammatory cytokines, which will subsequently further modulate coagulation and fibrinolysis. Coagulation pathways are indicated by straight arrows; inflammatory mechanisms by dashed arrows.

cofactors V and VIII by thrombin, and (3) thrombin-induced factor XI activation, leading to additional factors IXa and Xa. The assembly of several complexes, such as the "tenase complex," consisting of factor X, factor IXa, factor VIIIa, and calcium, or the prothrombinase complex mentioned above will be markedly facilitated if a suitable phospholipid surface is available and occurs in vivo, presumably on the membrane of activated platelets. Platelets play a pivotal role in the pathogenesis of inflammation-induced thrombosis, in particular in case of acute arterial thrombosis on plaque rupture, such as in acute coronary syndromes. In this setting, erosion of the intima may expose subendothelial collagen to the blood, which will lead to platelet adhesion and activation.[11] Platelets can also be activated directly, for example, by proinflammatory mediators, such as platelet-activating factor.[12] Once thrombin is formed, this will activate additional platelets. Activation of platelets may also accelerate fibrin formation by another mechanism. The expression of P-selectin on the platelet membrane not only mediates the adherence of platelets to leukocytes and endothelial cells but also enhances the expression of tissue factor on monocytes.[13] The molecular mechanism of this effect relies on nuclear factor kappa-B (NFκB) activation, induced by binding of activated platelets to neutrophils and mononuclear cells. P-selectin can be easily shed from the surface of the platelet membrane, and soluble P-selectin levels have been shown to be increased during acute coronary syndromes and systemic inflammation.[13]

Coagulation Affects Inflammation Through Protease-Activated Cell Receptors and Activation of Platelets

Coagulation activation yields proteases that not only interact with coagulation protein zymogens but also with specific cell receptors to induce signaling pathways that mediate inflammatory responses. Many in vitro observations point to a role of coagulation proteases in upregulating the expression of proinflammatory mediators.[14,15] The most important mecha-

nism by which coagulation proteases influence inflammation is by binding to protease activated receptors (PARs), of which 4 types (PAR 1 to 4) have been identified, all belonging to the family of transmembrane domain, G-protein–coupled receptors.[16] A peculiar feature of PARs is that they serve as their own ligand. Proteolytic cleavage by an activated coagulation factor leads to exposure of a neoamino terminus, which activates the same receptor (and possibly adjacent receptors), initiating transmembrane signaling. PARs 1, 3, and 4 are thrombin receptors, whereas PAR-2 cannot bind thrombin but can be activated by the tissue factor-factor VIIa complex, factor Xa, and trypsin. PAR-1 can also serve as a receptor for the tissue factor–factor VIIa complex and factor Xa. PARs are localized on endothelial cells, mononuclear cells, platelets, fibroblasts, and smooth muscle cells.[16] Binding of thrombin to its cellular receptor may induce the production of several cytokines and growth factors. Binding of tissue factor–factor VIIa to PAR-2 also results in upregulation of inflammatory responses in macrophages (production of reactive oxygen species and cell adhesion molecules) and was shown to affect neutrophil infiltration and proinflammatory cytokine (tumor necrosis factor [TNF]-α, IL-1β) expression.[17] Indeed, tissue factor has attracted considerable attention as a potential mediator of intracellular signaling of established inflammatory pathways, functioning as an intermediate for factor VIIa–induced activation of mitogen-activated protein kinases and calcium signaling.[18] In vivo evidence for a role of coagulation-protease stimulation of inflammation comes from recent experiments showing that the administration of recombinant factor VIIa to healthy human subjects causes a 3- to 4-fold rise in plasma levels of IL-6 and IL-8.[19] There is increasing evidence for a role of PARs in coagulation and inflammation in the setting of (coronary) artery thrombosis and its sequela. PAR-4–deficient mice showed absence of platelet activity in vivo and were protected against experimental arterial thrombosis.[20] Besides, PAR-1 and PAR-4 may mediate cardiomyocyte hypertrophy and cardiac
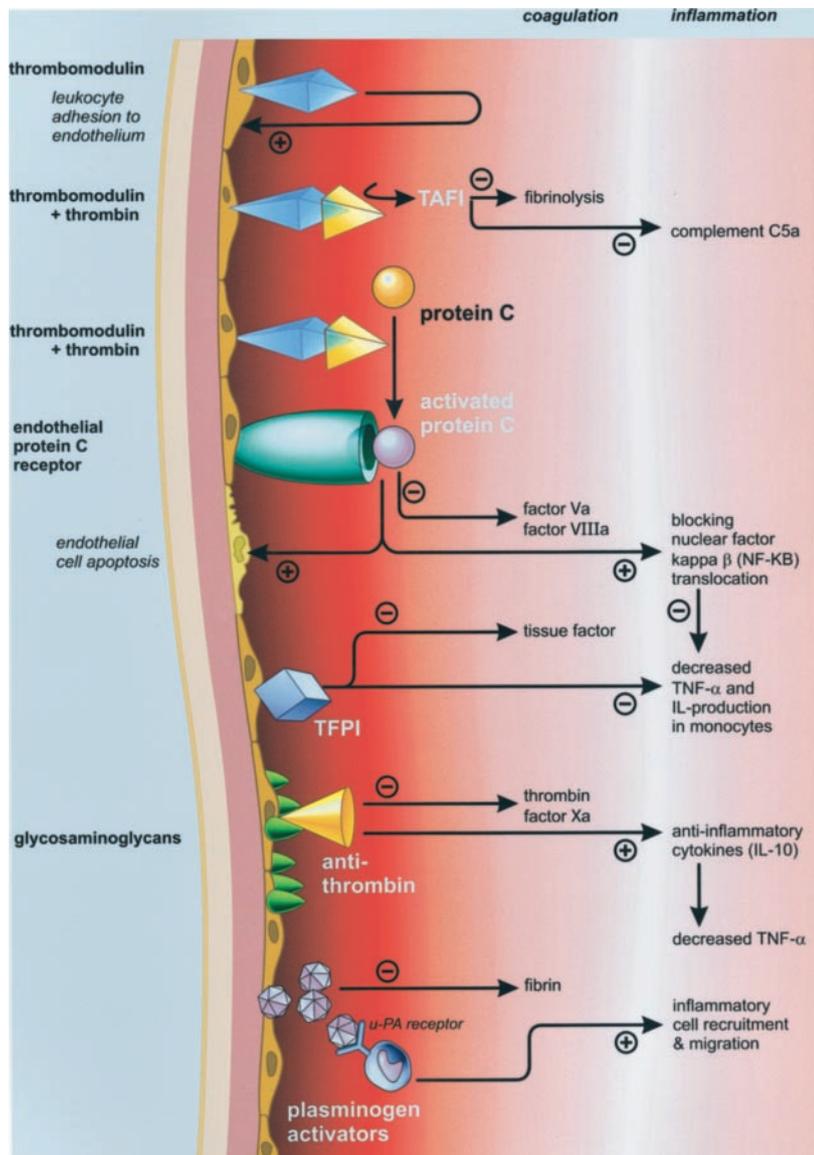
Figure 2. Schematic representation of effects of physiological anticoagulant systems and fibrinolysis on coagulation and inflammation. Thrombomodulin plays a central role as endothelial receptor and activator of protein C and TAFI, respectively, and has direct effects on the endothelium. Activated protein C and TAFI have effects on coagulation/fibrinolysis as well as on inflammatory processes. TFPI is the endothelial cell–associated inhibitor of tissue factor but also has direct effects on cytokine production in mononuclear cells. Antithrombin binds to endothelial glycosaminoglycans, which causes a more efficient inhibition of activated coagulation proteases and affects inflammation as well. Release of plasminogen activators from endothelial cells will affect local fibrinolysis; the complex of u-PA and u-PAR plays an important role in inflammatory cell recruitment and migration. Plus and minus signs indicate stimulatory and inhibitory effects, respectively.

remodeling on ischemia,[21] whereas endothelial PAR-2 plays a role in relaxation of epicardial coronary arteries.[22]

Activated platelets play an important role in inflammation, in particular in chronic inflammation, which is associated with atherosclerosis.[23] First, platelet adhesion to the subendothelial matrix supports leukocyte rolling, adhesion, and transmigration through interaction of platelet P-selectin with leukocyte P-selectin glycoprotein ligand-1.[24] Indeed, a deficiency in P-selectin delays atherosclerotic plaque formation.[25] Firm leukocyte adhesion to the vessel wall is stimulated by platelet activating factor–mediated activation of macrophage 1 antigen (Mac-1) and interaction of this integrin with fibrinogen bound to the platelet glycoprotein IIb/IIIa receptor.[26] Also, activated platelets release various proinflammatory cytokines (such as CD40 ligand and IL-1β) and chemokines (such as RANTES and platelet factor-4), which may result in (further) activation of monocyte integrins and thereby lead to monocyte recruitment to atherosclerotic plaques.[27]

Modulation of coagulation and inflammation may be of benefit in various situations in which these two processes

appear to play a pivotal role in the pathogenesis. Conventional antithrombotic agents, such as heparin or aspirin, are typically directed at modulation of coagulation but are likely to affect inflammatory activity as well. At the interface between coagulation proteases and inflammation, tissue factor may also be an interesting target. Administration of tissue factor pathway inhibitor (TFPI) was indeed shown to reduce thrombus formation and intimal hyperplasia in the setting of a ruptured atherosclerotic plaque.[28] An increase in levels of TFPI was also successful in experimental and initial clinical studies of severe systemic inflammation,[29–31] although no beneficial effect on survival has been observed so far in a large study in patients with severe sepsis.[32]

Inflammation-Induced Downregulation of Physiological Anticoagulant Pathways

Activation of coagulation is regulated by 3 major anticoagulant pathways: antithrombin, the protein C system, and TFPI (Figure 2). During inflammation-induced activation of coagulation, the function of all 3 pathways can be impaired. There

is a differential pattern of expression of the various antico-agulant pathways in different vascular beds; for example, in the coronary circulation, TFPI is mostly expressed in microvessels, whereas the protein C system is more universally present.

Antithrombin is a serine protease inhibitor and the main inhibitor of thrombin and factor Xa. During severe inflammatory responses, antithrombin levels are markedly decreased as the result of consumption (as a result of ongoing thrombin generation), impaired synthesis (as a result of a negative acute phase response), and degradation by elastase from activated neutrophils.[33,34] In atherosclerotic disease, a more moderate but locally important reduction in antithrombin function may be caused by a reduction in glycosaminoglycan availability at the endothelial surface, because glycosaminoglycans act as physiological heparin-like cofactors of antithrombin.[35] Antithrombin also may be important as a mediator of inflammation, for example, by direct binding to neutrophils and other leukocytes and thereby attenuation of cytokine and chemokine receptor expression.[36]

Endothelial dysfunction is even more important in the impairment of the protein C system during inflammation. Under physiological conditions, protein C is activated by thrombin bound to the endothelial cell membrane–associated thrombomodulin. Thrombomodulin is a membrane protein with several domains.[37] The binding of thrombin to thrombomodulin not only results in an approximately 100-fold increase in the activation of protein C but also blocks the thrombin-mediated conversion of fibrinogen into fibrin and inhibits the binding of thrombin to other cellular receptors on platelets and inflammatory cells. Binding of protein C to the endothelial protein C receptor (EPCR) results in a further 5-fold augmentation of the activation of protein C by the thrombomodulin-thrombin complex.[38] Activated protein C regulates coagulation activation by proteolytic cleavage of the essential cofactors Va and VIIIa (Figure 2). In addition, thrombomodulin accelerates the activation of the plasma carboxypeptidase thrombin–activatable fibrinolysis inhibitor (TAFI), an important inhibitor of fibrinolysis.[39] Histological studies indicate that the protein C system may play a role in coronary atherothrombosis.[40] Endothelial cells overlying an atherosclerotic plaque in coronary arteries of explanted hearts expressed less thrombomodulin as compared with control cells with no or more moderate atherosclerosis. This downregulation of thrombomodulin may potentially lead to more extensive thrombin generation at the site of the atherosclerotic lesion. Indeed, patients with a heterozygous mutation in the thrombomodulin gene appeared to have a higher risk of myocardial infarction.[41] Also, on systemic inflammation, in addition to low levels of protein C caused by impaired synthesis[33] and degradation by neutrophil elastase (which has been described at least in vitro),[42] the protein C system is defective as the result of downregulation of thrombomodulin at the endothelial surface, mediated by the proinflammatory cytokines TNF-α and IL-1β.[43] Underlying mechanisms are a decreased gene transcription and cleavage of the extracellular domain. Animal experiments of severe inflammation-induced coagulation activation convincingly show that compromising the protein C system results in increased morbidity and mortality rates, whereas restoring an adequate function of activated protein C improves survival and organ failure.[44]

A third inhibitory mechanism of thrombin generation involves TFPI, the main inhibitor of the tissue factor–factor VIIa complex. A recent study showed that overexpression of TFPI by local gene transfer reduced the extent of intimal hyperplasia and thrombus formation in balloon-injured atherosclerotic arteries in rabbits.[31] Other experiments in a setting of more systemic inflammation showed that administration of recombinant TFPI (thereby achieving higher than physiological plasma concentrations of TFPI) blocked inflammation-induced thrombin generation in humans.[45] The observation that pharmacological doses of TFPI are capable of preventing death during systemic infection and inflammation suggests that high concentrations of TFPI are capable of importantly modulating tissue factor–mediated coagulation.[29] However, the endogenous concentration of TFPI is presumably insufficiently capable of regulating coagulation activation and downstream consequences during inflammation.

The Protein C Pathway and Inflammation

Apart from its central role in regulation of coagulation activation, there is mounting evidence that the protein C system also has an important function in modulating inflammation.[37] Indeed, activated protein C has been found to inhibit endotoxin-induced production of TNF-α, IL-1β, IL-6, and IL-8 by cultured monocytes/macrophages.[46] Furthermore, activated protein C abrogates endotoxin-induced cytokine release and leukocyte activation in rats in vivo.[47] Blocking the protein C pathway in septic baboons exacerbates the inflammatory response, whereas administration of activated protein C ameliorates the inflammatory activation in various models of severe systemic inflammation.[44,46] Mice with a heterozygous protein C deficiency not only have a more severe coagulation response to endotoxin but also demonstrate significant differences in inflammatory responses.[48] It is likely that the effects of activated protein C on inflammation are mediated by the EPCR, which may mediate downstream inflammatory processes.[37] Binding of activated protein C to EPCR influences gene expression profiles of cells by inhibiting endotoxin-induced calcium fluxes in the cell and by blocking NFκB nuclear translocation, which is a prerequisite for increases in proinflammatory cytokines and adhesion molecules.[49,50] The EPCR-activated protein C complex itself can translocate from the plasma membrane into the cell nucleus, which may be another mechanism of modulation of gene expression, although the relative contribution of this nuclear translocation and cell surface signaling is unclear at present.[51] In addition, recent experiments demonstrate that binding of activated protein C to the protein C receptor inhibits endotoxin-induced tissue factor expression on mononuclear cells. EPCR binding of activated protein C can also result in activation of PAR-1, although the in vivo relevance of this observation is unclear.[52] Last, activated protein C is capable of inhibiting endothelial cell apoptosis, which also appears to be mediated by binding of activated protein C to EPCR and appears to require PAR-1.[53]

Thrombomodulin can also exert significant antiinflammatory activity. As described above, thrombomodulin enhances

thrombin-induced activation of TAFI.[39] TAFI has recently been suggested to be the primary enzyme responsible for inactivation of complement factor C5a.[54] Considering that thrombomodulin is abundantly present in the microcirculation, TAFI-mediated inactivation of C5a would be expected to protect against complement-mediated injury in the microvasculature. The lectin-like domain of thrombomodulin has a function in inhibiting leukocyte adhesion to activated endothelium.[55] Hence, thrombomodulin occupies a central position at the crossroads between coagulation and inflammation, by activating protein C (with its anticoagulant and antiinflammatory properties), by accelerating TAFI activation (and thereby affecting fibrinolysis and inhibiting complement), and by binding to thrombin (which is thereby less available for fibrinogen to fibrin conversion, platelet activation, and binding to PARs, which will affect inflammatory activity).

The therapeutic perspective of the pivotal role of the protein C system in inflammation and coagulation is best illustrated by the fact that administration of pharmacological doses of recombinant activated protein C results in a significant reduction of organ failure and mortality rates in patients with severe sepsis.[56] From the clinical studies, it is not clear whether the beneficial effect can be attributed to inhibition of coagulation or modulation of inflammation, although it is likely that both mechanisms play a role. Administration of activated protein C in acute thrombosis after rupture of an atherosclerotic plaque or in ischemia-reperfusion syndromes is an attractive option, and clinical studies in these areas have recently been initiated.

Inhibition of Fibrin Removal During Inflammation

The acute fibrinolytic response to inflammation is the release of plasminogen activators, in particular, tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA), from storage sites in vascular endothelial cells. However, this increase in plasminogen activation and subsequent plasmin generation is counteracted by a delayed but sustained increase in plasminogen activator inhibitor type-1 (PAI-1).[57] The resulting effect on fibrinolysis is complete inhibition and, as a consequence, inadequate fibrin removal, thereby contributing to microvascular thrombosis. The pivotal regulators of PAI-1 in this respect are TNF-α and IL-1β.[15] Experiments in mice with targeted disruptions of genes encoding components of the plasminogen-plasmin system confirm that fibrinolysis plays a major role in inflammation-induced coagulation. Mice with a deficiency of plasminogen activators have more extensive fibrin deposition in organs when challenged with endotoxin, whereas PAI-1 knockout mice, in contrast to wild-type control mice, have no microvascular thrombosis on endotoxin administration.[58] In addition, inhibitors of PAI-1 have been shown to be able to prevent coronary thrombosis in a model of endothelial damage and coronary stenosis.[59]

Fibrin, Fibrin Degradation Products, and Fibrinolytic Proteins as Mediators of Inflammation

Fibrinogen and fibrin can directly stimulate expression of proinflammatory cytokines (such as TNF-α and IL-1β) on mononuclear cells and induce production of chemokines (including IL-8 and MCP-1) by endothelial cells and fibroblasts.[60] The effects of fibrin(ogen) on mononuclear cells are at least in part mediated by Toll-like receptor-4, which is also the receptor of endotoxin.[61] Fibrinogen-deficient mice show inhibition of macrophage adhesion and less thrombin-mediated cytokine production in vivo.[60]

Fibrinolytic factors, in particular u-PA and its receptor (u-PAR), may modulate the inflammatory response by their effect on inflammatory cell recruitment and migration. U-PAR mediates leukocyte adhesion to the vascular wall or extracellular matrix components (such as vitronectin), and the expression of u-PAR on leukocytes is strongly associated with their migratory and tissue-invasive potential.[62,63] Recruitment of mononuclear cells to the infarcted area in patients with myocardial infarction has been shown to be related to enhanced u-PAR expression on their surface and results in increased inflammatory activity.[64] The underlying mechanism by which u-PAR and u-PA affect cell migration may be related to extracellular matrix degradation by proteases that are activated by u-PAR-associated u-PA (such as elastase, plasmin, and metalloproteinases). However, u-PAR also exerts protease-independent properties, which involve transmembrane signal transduction after interaction with proteins or receptors, such as vitronectin and Mac-1, which lead to cytokine and growth factor production.[65] PAI-1 can bind vitronectin, thereby preventing integrin association to this extracellular matrix component and hence cell adhesion and migration. Moreover, PAI-1 competes with u-PAR for binding to vitronectin, thereby further inhibiting cell adhesion and migration.[66] Studies with u-PAR gene–deficient mice have emphasized the preeminent role of this receptor in leukocyte trafficking.[67] In these models, the function of u-PAR in chemotaxis was independent from its interaction with u-PA. In accordance, u-PA gene–deficient mice have a normal neutrophil recruitment during pneumonia caused by bacteria or fungi,[67,68] although u-PA appears to facilitate the accumulation of other inflammatory cells in infected lungs.[68]

Mediators of fibrinolysis can also affect cytokine synthesis. The active end product of the fibrinolytic system, plasmin, induces activation of mitogen-activated protein kinases and proinflammatory cytokine production by monocytes in vitro.[69] PAI-1 inhibits endotoxin-induced TNF-α production by mononuclear cells in vitro[70] Interference of u-PAR binding to its ligands or modulation of u-PAR–dependent cell signaling may affect leukocyte recruitment and invasion in inflamed tissue areas as a result of infarction (and thereby reduce infarct size) or may modulate the inflammatory response to infection.

Conclusions

There is ample evidence that inflammation and coagulation are intricately related processes that may considerably affect each other. This cross-talk occurs at the levels of platelet activation, fibrin formation, and resolution as well as physiological anticoagulant pathways. Increased insight into the molecular mechanisms that play a role in the close relation between inflammation and coagulation may lead to the identification of new targets for therapies that can modify excessive activation of these systems. On the basis of exper-

imental and clinical studies, it can be hypothesized that simultaneous modulation of both coagulation and inflammation, rather than specific therapies aimed at one of these systems, will be more successful. In particular, therapies aimed at tissue factor or at physiological regulatory pathways, such as the protein C system, may be most promising.

Acknowledgment

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