Commentary

New Mechanisms for Vascular Control of Inflammation Mediated by Natural Anticoagulant Proteins

Charles T. Esmon

Cardiovascular Biology Research Program, Oklahoma Medical Research Foundation, Department of Pathology and Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, and Howard Hughes Medical Institute, Oklahoma City, OK 73104

It has been appreciated for a very long time that infections, particularly bacterial infections of the blood leading to severe sepsis, trigger a hypercoaguable state that sometimes leads to overt disseminated intravascular coagulation. We now recognize that endotoxins and other bacterial, fungal, and viral products can activate the toll receptors, leading to the elaboration of inflammatory cytokines (1) that in turn elicit tissue factor expression to trigger the blood clotting process (2). More recently, we have begun to appreciate the critical role played by natural anticoagulants in controlling the processes leading to septic shock (3). Of these natural anticoagulants, the protein C anticoagulant pathway seems to play a particularly important role in dampening the inflammatory response that occurs with endotoxin and bacteremia. It has now become clear that many of the components in the pathway possess multiple activities that contribute to the regulation of a variety of anticoagulant and antiinflammatory functions. The pathway is illustrated in Fig. S1 (available at http://www.jem.org/cgi/content/full/jem.20021088/DC1). A key feature of the pathway involves protein C activation on the vascular endothelial cell surface leading to the formation of activated protein C (APC). The activation complex is composed of thrombin and protein C bound respectively to the endothelial cell proteins, thrombomodulin (TM) and the endothelial cell protein C receptor (EPCR; references 4–6). APC was originally recognized as an anticoagulant that worked by the proteolytic inactivation of factors Va and VIIIa, thereby shutting down thrombin formation (7, 8). In addition to its anticoagulant activity, APC also possesses antiinflammatory properties. APC is known to inhibit the release of inflammatory cytokines such as tumor necrosis factor α in experimental animals challenged with endotoxin (9, 10). APC also limits leukocyte adhesion (11) and protects against an endotoxin-induced decrease in blood pressure (12). At least some of these activities are thought to be due to the ability of APC to inhibit nuclear factor κB nuclear translocation (13) and reduce nuclear factor κB mRNA levels (14). The multifunctional nature of APC probably accounts for its ability to decrease the death rate in experimental animals (12) and patients (15) with severe sepsis.

Recently, attention has begun to shift toward understanding new functions of the protein C activation complex. Like APC, EPCR appears to have antiinflammatory activities. EPCR can be shed from the endothelium by an inducible metalloproteinase (16). Soluble EPCR binds to proteinase 3, an elastase-like enzyme, and this complex can bind to the adhesion integrin CD11b/CD18 (17). The EPCR–APC complex also seems to be important in preventing leukocyte infiltration into tissues (18). Furthermore, several of the antiinflammatory activities of APC seem to be mediated through APC binding to EPCR (19). A new potential role for soluble EPCR was suggested by the recent solution of the crystal structure (20). EPCR was shown to be remarkably similar in structure to the MHC class 1/CD1 family of proteins, most of which are involved in inflammatory processes. The CD1 family are known to bind lipid antigens and present them to T cells (21, 22). This process seems to be important in host defense against infection. Like the CD1 family, EPCR binds a lipid, in this case a phospholipid, in the groove used by CD1 family members for lipid antigen presentation, which suggests by extrapolation that EPCR may also have a function in lipid antigen presentation (20).

In this issue, Conway et al. (23) provide evidence for one additional and important antiinflammatory activity of the pathway, TM-dependent inhibition of leukocyte adhesion to activated endothelium. TM is a complex membrane protein with a lectin-like domain from the amino terminus, six epidermal growth factor (EGF)-like repeats, a region with O-linked sugar addition sites that also contains the sites for the addition of chondroitin sulfates, a transmembrane domain, and a short cytoplasmic tail. The thrombin binding site required for rapid protein C activation resides in EGF repeats 4–6 (24). Binding of thrombin to this site not only accelerates protein C activation about 100-fold, but also blocks the ability of thrombin to clot fibrinogen and participate in platelet and endothelial cell activation (25). An additional role of TM is to accelerate thrombin

Address correspondence to Charles T. Esmon, Oklahoma Medical Research Foundation, Cardiovascular Biology Research Program, 825 NE 13th Street, Oklahoma City, OK 73104. Phone: 405-271-6474; Fax: 405-271-3137. E-mail: Charles-Esmon@omrf.ouhsc.edu

activation of a plasma procarboxypeptidase B (26). When activated, this class of enzymes removes carboxy terminal arginine and lysine residues. It was originally thought that this enzyme's major target might be fibrin, where removal of lysine residues results in resistance to clot lysis (26). Hence, the enzyme is commonly referred to as thrombin activatable fibrinolysis inhibitor (TAFI). More recently, however, it has been shown that TAFI is probably the primary enzyme responsible for inactivation of the complement-derived anaphylatoxin, C5a (27). Given the high effective TM concentration in the microcirculation (25), the rapid TAFI activation and subsequent rapid inactivation of C5a would be expected to protect against complement-mediated injury to the microcirculation.

Because thrombin activation of endothelial cells contributes to a variety of inflammatory events including the expression of P selectin on the endothelial cell surface and the formation of the neutrophil agonist, platelet-activating factor (28), the ability of TM to block these activities has an indirect antiinflammatory effect. Furthermore, binding thrombin to TM dramatically increases the rate at which thrombin is neutralized by plasma proteinase inhibitors (29). Once neutralized, the thrombin inhibitor complex dissociates from TM. Other functions for TM have also been suggested, including thrombin internalization and degradation (30) and the ability of the EGF repeat regions to stimulate fibroblast growth (31). Until now, however, no function had been identified for the lectin-like domain of TM. In the report by Conway et al. (23) in this issue, they not only show that deletion of the lectin domain from TM in mice increases leukocyte infiltration in a variety of experimental conditions, but also demonstrate that the isolated soluble domain possesses this function. The lectin domain, whether soluble or as part of intact cellular TM, provides protection against systemic and inhaled endotoxin, and ischemia/reperfusion injury. At least in part, these activities appear to be mediated by the suppression of the mitogen-activated protein kinase pathway. These exciting results not only suggest a novel role for TM and an interesting new therapeutic candidate for the treatment of acute inflammatory diseases, but they also help to explain some of the interesting results seen when TM expression is altered in vivo.

As discussed above, the protein C anticoagulant pathway limits the expression of inflammatory mediators and dampens leukocyte extravasation into the tissues. However, the pathway is not static. Inflammatory cytokines can down-regulate TM and EPCR expression by inhibiting gene transcription and in the case of EPCR, by promoting shedding from the endothelium. When neutrophils are bound to the endothelium and become activated, they release potent oxidants that reduce the ability of TM to bind thrombin and activate protein C (32). They also release proteases that can proteolytically remove TM from the endothelium (33, 34) with the released form being much less active than the endothelial cell form. TM expression is also subject to down-regulation by fluid shear force (35). As a result of these and potentially other mechanisms for regulating expression, the protein C activation complex can be impaired in a variety of clinically relevant situations. In a subset of patients with severe sepsis, TM expression on the vasculature is down-regulated, leading to decreased protein C activation (36). The loss of direct activities targeted toward inhibiting leukocyte extravasation would further compromise the already damaged and fragile vasculature in these patients.

Another example of TM down-regulation is in Wegener's granulomatosis (37). Here, leukocyte infiltrates in the vasculature are prevalent. The plot thickens, however, as the autoantibodies are thought to target proteinase 3 in particular. Proteinase 3 is the molecule through which EPCR binds to the activated leukocyte surface (17). It would seem likely that in some vascularities, both compromised TM expression and impaired interaction with EPCR would contribute to the progression of the disease.

Atherosclerotic plaque development is another situation in which the protein C system could play an important role. The expression of both TM and EPCR is dramatically reduced on endothelium overlying atherosclerotic plaque in humans (38), suggesting reduced protein C activation in these areas. Based on the studies presented herein by Conway et al (23) and the other studies reviewed above, the decreased expression would be anticipated to facilitate leukocyte attachment and possibly infiltration into the atheroma. In turn, this could contribute to the growth of the atheroma, the fragility of the fibrous cap, and the thrombogenicity of the ruptured plaque.

Direct examples of important roles for TM therapies and disease processes involving the heart have begun to emerge. In model systems, TM overexpression has been shown to prevent thrombosis, leukocyte infiltration, and restenosis in rabbit systems involving deep arterial injury (39). Although some of the inhibition of leukocyte infiltration seen in this system is likely due to APC formation, the presence of increased concentrations of direct leukocyte inhibitory activity related to the increased expression of TM probably also played a significant role and could have been the dominant contribution responsible for preventing the restenosis. In vein bypass grafts, recent studies have shown that TM down-regulation occurs within days after graft placement and likely contributes to the thrombotic complications that ensue (40). Loss of the leukocyte inhibitory functions of TM seems likely to contribute to both the thrombosis and restenosis that is prevalent in these grafts.

The report in this issue by Conway et al (23) adds an important new insight into the control of the inflammatory process by the protein C anticoagulant pathway specifically, and the role of the vasculature in general. In particular, it provides greater insights into the pathophysiological sequelae that follow the down-regulation of TM in a variety of common, clinically relevant situations. Given the rapid rate of new discoveries of the roles of the protein C pathway in the regulation of inflammation, it seems likely that many more novel mechanisms involving this pathway will be identified in the near future and this is

likely to be followed by the application of these findings to new therapies.

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