Vasculopathy and renal injury in lupus erythematosus: Does shedding of the endothelial protein C receptor play a role?

Systemic lupus erythematosus (SLE) is a potentially fatal, autoimmune disease that affects about 1.4 million Americans, including 1 in 250 African American women between the ages of 18 and 65. In the past several decades, the prognosis for patients with SLE has greatly improved, with a current 10-year survival rate of close to 90% [1]. This increased survival is due, in part, to the use of steroids and other immunosuppressants, to the success of renal dialysis and transplantation, and to the availability of sensitive diagnostic tests for the earlier and more accurate diagnosis of SLE. Nonetheless, our understanding of the molecular mechanisms involved in the etiology and progression of SLE are still incomplete. SLE is considered to be a classic example of an immune-complex–mediated disease, with manifestations in multiple systems and organs, including joints, skin, heart, liver, lungs, blood vessels, the gastrointestinal system, and kidneys. The immune complexes are believed to induce microvascular injury, with concomitant thrombotic manifestations and inflammation. Numerous autoantibodies have been identified in SLE, including antibodies to circulating, double-stranded DNA, presumably derived from cells undergoing apoptosis or necrosis.

In SLE, a widespread activation of vascular endothelium has been demonstrated. This is associated with abnormal expression of markers of activation, including adhesion molecules such as VCAM-1, E-selectin, and inducible nitric oxide synthase (iNOS) [2, 3]. This activation “primes” the vasculature for subsequent challenges by immune complexes. In this edition of Kidney International, Sesin et al examined circulating levels of the endothelial protein C receptor (EPCR) in a cohort of control patients and patients with SLE in an attempt to correlate plasma levels of soluble EPCR (sEPCR) with SLE disease manifestation and severity [4]. EPCR is the endothelial cell surface receptor for protein C (PC), a vitamin K–dependent plasma glycoprotein that is synthesized in the liver in an inactive form [5]. Thrombin cleaves PC into its active form, APC, which acts as a potent anticoagulant. The transmembrane protein thrombomodulin (TM) can act as a cofactor for thrombin, consequently amplifying thrombin-mediated activation of PC [5]. In contrast, thrombin, a critical enzyme in the coagulation cascade, has greatly reduced procoagulant activity when complexed with TM, and shows greatly reduced activity for cleavage of fibrinogen, for activation of factor V, and for activation of platelets. Activation of PC by the thrombin-TM complex is greatly facilitated by the binding of PC to EPCR. EPCR is constitutively expressed by endothelial cells, and is structurally similar to the MHC complex class I/CDI family of proteins. Activation of PC to APC by the TM-thrombin-EPCR complex thus acts as a potent anticoagulant mechanism. In addition to its anticoagulant role, APC also has potent anti-inflammatory properties; APC down-regulates thrombin generation by affecting factors Va and VIIIa, interfering with thrombin-induced proinflammatory activities, such as platelet activation, chemotaxis of neutrophils and monocytes, and induction of leukocyte adhesion molecules. APC also reduces inflammation by directly inhibiting macrophage activation. APC reduces the expression of TNFα and tissue factor, the activation of NF-kB, and the expression of leukocyte-endothelial adhesion molecules by macrophages. EPCR, expressed on the plasma membrane of endothelial cells in complex with TM and thrombin, activates PC and concentrates it near the surface of the vessel wall. EPCR expression by endothelial cells is suppressed by lipopolysaccharide (LPS), inflammatory cytokines, and thrombin [6]. Cleavage of EPCR from the cell surface by matrix metalloproteinases has also been demonstrated [7]. sEPCR has been shown to bind to PC and APC, and to inhibit the anticoagulant properties of APC. Thus, cleavage of EPCR and its release into the circulation appears to promote a procoagulant and potentially thrombotic pathway, as well as inflammatory responses. The roles of APC, TM, and EPCR in coagulation and inflammation have been elegantly reviewed by Van de Wouwer et al [8].

Sesin et al propose that the initial “priming” of vascular endothelium in SLE sensitizes the vasculature to subsequent challenges by immune complexes. These
challenges will then result in endothelial cell injury, with increased shedding of EPCR, and subsequent thrombotic diathesis and inflammation. Since vascular endothelium is inaccessible from patients, Sesin et al also examined a population of “circulating endothelial cells” (CECs), purified using an endothelial-specific monoclonal antibody (PIH12, Chemicon, Temecula, CA, USA). The mean level of sEPCR was significantly elevated in the cohort of SLE patients compared to healthy controls. One group of patients (28%) showed variable, but consistently substantially elevated sEPCR, which was further elevated when nephritis was present. A second group (36%) showed moderate elevations, while the remainder showed no elevation. These results confirm and expand data in a previous report that sEPCR is elevated in SLE patients [9].

With regard to CECs, EPCR on the surface of CECs from SLE patients was often reduced compared to healthy controls, suggesting in vivo shedding, but an inverse correlation of CEC expression of cell surface EPCR to sEPCR was not always apparent. To determine whether the inflammatory cytokines, IFNγ or IL-1, can induce shedding of EPCR, they were applied to human umbilical vein endothelial cells (HUVECs) in vitro. Shedding was increased by both cytokines, and was blocked by a metalloproteinase inhibitor, suggesting a role for inflammatory cytokines in the induction of increased sEPCR in the plasma of SLE patients.

A novel aspect of the current study involves the analysis of a single nucleotide polymorphism (SNP) in exon 4 of the EPCR gene that is associated with increased shedding of EPCR from endothelial cells [10]. This mutation (A6936G), which converts serine 219 to glycine, is in a region of the molecule that is predicted to be close to the plasma membrane. This may increase exposure of the mutated site to metalloproteinases, thus increasing the potential for shedding when protease expression is induced, for example, in response to inflammatory cytokines, such as IFNγ or IL-1, as discussed above. The high-shedding GG genotype was found to be significantly more prevalent in SLE patients than in controls, but there were clearly individuals with elevated sEPCR who did not carry this G allele. As indicated by the authors, further studies are clearly necessary to evaluate the relationship of this SNP to the vasculopathy and nephropathy of SLE in patient subgroups. In addition, an analysis in endothelial cells of the surface expression and shedding of the mutated, versus the normal protein, in an in vitro expression system might indicate if this mutation really increases susceptibility to metalloprotease-mediated shedding.

Overall, this study provides interesting insight into the role of EPCR in SLE, and suggests that elevated levels of circulating EPCR may be an additional, independent indicator of vasculopathy and renal injury. Measurement of sEPCR in SLE patients may, thus, be an additional diagnostic marker for assessing nephropathy and vascular damage in these patients. The suggestion that an SNP in the EPCR gene increases susceptibility to shedding induced by inflammation in a subgroup of SLE patients, clearly merits further study. It is interesting to note that the use of soluble fragments of EPCR to prevent or treat inflammation has been proposed [9], raising the intriguing possibility that the increased plasma sEPCR may be related to an attempt to limit the pathobiology of the disease, rather than a marker of its severity. Further research on the validity of sEPCR as a marker of vasculitis and nephritis in SLE, as well as on the potential use of soluble EPCR as a therapeutic intervention, are clearly indicated. We look forward to evaluating these studies in the future.

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