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Tissue Factor and Thrombin in Sickle Cell Anemia

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Abstract

Sickle cell anemia is an inherited hematologic disorder associated with hemolytic and vaso-occlusive complications. An activation of coagulation is also a prominent feature of sickle cell anemia. Growing evidence indicates that coagulation may contribute to the inflammation and vascular injury in sickle cell anemia. This review focuses on tissue factor expression and its contribution to the activation of coagulation, thrombosis and vascular inflammation in sickle cell anemia.

Introduction

Sickle cell anemia (SCA) is caused by a single nucleotide mutation that substitutes glutamic acid with valine at the 6th position of the β-globin gene [1–3]. Acidosis or hypoxia leads to abnormal polymerization of hemoglobin tetramers resulting in the formation of sickled red blood cells that are less flexible, prone to hemolysis and adhere to the endothelium [1–3]. Interaction of sickled red blood cells with leukocytes and the vascular endothelium results in vaso-oclussive episodes within postcapillary venules, leading to tissue ischemia, hemolysis and inflammation. Subsequent reperfusion of the ischemic tissue leads to oxidative stress, vascular injury, increased expression of adhesion molecules and further enhancement of inflammation [1–3]. In addition to these pathological processes, activation of coagulation is also a prominent feature of SCA, as demonstrated by an increased expression of tissue factor (TF), high plasma levels of procoagulant microparticles and markers of thrombin generation, platelet activation, depletion of natural anticoagulants and abnormal activation of fibrinolysis [4]. This review focuses on TF expression and its contribution to the activation of coagulation, thrombosis and vascular inflammation in SCA.

Increased TF expression in SCA

TF is a primary activator of the coagulation cascade [5]. Formation of the TF:factor VIIa (FVIIa) complex leads to the activation of both FX and FIX, with subsequent thrombin generation, fibrin deposition and activation of platelets [5]. Sickle cell patients demonstrate elevated whole blood TF procoagulant activity [6]. Furthermore, circulating endothelial cells isolated from sickle cell patients showed increased levels of TF antigen, mRNA and activity [7]. In addition, blood of sickle cell patients contains monocyte- and endothelial cell-derived

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TF-positive microparticles [8]. TF expression in whole blood and circulating endothelial cells was similarly increased in patients in pain crisis and those with steady-state disease [6, 7]. In contrast, higher numbers of TF-positive microparticles were observed during pain crisis episodes compared to steady-state disease [8].

Consistent with the observations in sickle cell patients, TF expression was also increased in the endothelium of the lung microvasculature and in circulating monocytes in mouse models of SCA [9]. Mouse studies have also shed light on the possible mechanism responsible for the increased expression of TF in SCA. Two recent publications demonstrated that endothelial cell TF expression was regulated by EC nitric oxide synthase and activation of the NFκB pathway in mononuclear cells [10, 11]. In addition, in mouse model of SCA, hypoxia/reoxygenation increases TF staining in both endothelial cells and monocytes [9]. In vitro stimulation of endothelial cells with free heme, a hemoglobin degradation product, induces TF expression [12]; however, it is not known if heme contributes to the increased expression of TF in sickle cell patients and mouse models of SCA.

Histone deacetylase inhibitor or lovastatin treatment reduces the increased TF staining observed in pulmonary endothelial cells of sickle cell mice [9, 13]. However lovastatin treatment in sickle cell mice did not attenuate inducible TF expression in monocytes [9]. Furthermore, lovastatin had no effect on the constitutive TF expression, as demonstrated by similar TF staining observed in perivascular cells of sickle cell and control mice [9]. In sickle cell patients, short term use of simvastatin had only modest effect on plasma levels of TF antigen [14].

Increased thrombin generation and thrombosis in SCA

There are several pieces of evidences supporting the concept that thrombin generation is increased in SCA. Plasma levels of prothrombin fragment 1.2 and thrombin anti-thrombin complexes are increased in sickle cell patients [4, 15, 16]. Recently, higher rates of thrombin formation, higher thrombin peak height and higher endogenous thrombin potential has been reported in platelet-poor plasma of sickle cell patients compared to age-matched controls, reflective of a “hypercoagulable state” [17]. In addition, plasma levels of D-dimers, fibrinopeptide E, fibrin-fibrinogen peptide E and plasmin-antiplasmin complexes are also elevated indicating that thrombin-dependent fibrinogen cleavage, clot formation and subsequent fibrin degradation occurs in sickle cell patients [4, 15, 16]. In contrast, plasma levels of tissue factor pathway inhibitor, a natural inhibitor of TF, were not changed in the sickle cell patients [6]. A hypercoagulable state in SCA is further supported by the presence of multiple thrombotic complications observed in sickle cell patients, including venous thromboembolism, in situ pulmonary embolism and stroke [18–20]. Furthermore, pulmonary microthrombi have been observed in the sickle cell patients during episodes of the acute chest syndrome [21].

We and others have shown that plasma levels of thrombin-antithrombin complexes are also increased in mouse models of SCA [22, 23]. Furthermore, microthrombi were observed in the lungs, liver and kidneys [24]. Exposing sickle cell mice to hypoxic conditions resulted in further increase in the plasma TAT levels and thrombosis within the lung vasculature [23, 25].

Contribution of TF and thrombin to the pathology of SCA

Using a hematopoietic stem cell transplant model of SCA, Hillery and colleagues demonstrated a reduction of vascular congestion in the livers of mice expressing very low levels of TF in non-hematopoietic cells, providing the first evidence that TF may contribute to vascular inflammation and cellular stasis [26]. Recently we have investigated the role of

TF in the activation of coagulation in sickle cell mice. We found that treatment with an inhibitory anti-TF antibody completely abrogated the activation of coagulation indicating that the activation of coagulation was TF-dependent [22]. Gavins and colleagues demonstrated that inhibition of TF or thrombin attenuates enhanced thrombosis in cerebral microvessels of mice expressing the sickle form of hemoglobin [27]. Since TF expression was not observed in cerebral microvessels, the authors proposed that TF expressed by blood cell- and/or microparticle-associated TF contributed to enhanced thrombosis in this model [27]. We are currently investigating what cellular sources of TF contribute to the activation of coagulation in sickle cell mice. Possible cellular sources include monocytes and lung microvascular endothelial cells in which increased TF expression has been previously reported [27]. In addition, perivascular TF exposed to plasma FVII/FVIIa after injury to the endothelium could activate coagulation. Indeed, endothelial cell injury and increased vascular permeability have been demonstrated in mouse models of SCA [28, 29].

TF activates coagulation and enhances inflammation in animal models of endotoxemia, sepsis and ischemia-reperfusion injury, indicating a crosstalk between coagulation and inflammation [30–32]. Interestingly, we found that inhibition of TF reduced plasma levels of interleukin-6, serum amyloid P and soluble vascular cell adhesion molecule-1 in sickle cell mice [22]. In addition, we observed decreased levels of myeloperoxidase in the lungs of sickle cell mice treated with the anti-TF antibody [22]. Together, these data indicate that TF not only activates coagulation but also contributes to inflammation and endothelial cell injury in mouse models of SCA. Ongoing studies in our group will determine if TF:FVIIa complex itself and/or downstream proteases of the coagulation cascade, including FXa and thrombin, promote vascular inflammation in sickle cell mice. A recently presented abstract, demonstrating that low molecular weight heparin reduces plasma levels of sVCAM-1, suggests that TF-dependent thrombin generation may contribute to endothelial cell injury in sickle cell mice [33]. Although, the beneficial, anti-inflammatory effects of low molecular weight heparin could also be due to interruption of P-selectin-mediated cellular interactions [34].

Several clinical trials evaluating the effect of different forms of anticoagulant therapy on vaso-occlusive crises in sickle cell patients have been summarized in recent reviews [4, 16]. Most of these were small studies that used frequency of pain crisis as the only endpoint and were inconclusive [4, 16]. Notably, however, in one larger randomized placebo-controlled clinical trial with low molecular weight heparin, a reduction in the severity and duration of pain crisis in SCA, without major bleeding complication, was observed [35]. Together with our data, this study suggests that anticoagulation therapy is a valid approach to attenuate not only coagulation but also vascular inflammation and subsequent organ damage in sickle cell patients.

Conclusions

TF plays an important role in the activation of coagulation in both sickle cell patients and in mouse models of SCA. Growing evidence from animal models indicates that activation of coagulation is not only a secondary event, but also significantly contributes to inflammation and vascular injury in sickle cell mice. The precise mechanism by which TF-dependent activation of coagulation contributes to the pathophysiology of SCA needs to be further elucidated in animal models. In addition, future clinical studies of new orally available anticoagulants (rivaroxaban and dabigatran etexilate) using a variety of clinical endpoints could further characterize the contribution of increased coagulation to the vascular inflammation in SCA.

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