Introduction: anti-adhesion therapy in sickle cell disease

John M. Harlan

In this issue of Blood, Kaul et al\(^1\) report that stimulation by platelet-activating factor (PAF) of artificially perfused rat mesoecum ex vivo promotes the adhesion of human sickle red blood cells (SS RBC) to postcapillary endothelium. Notably, this adhesive interaction was blocked by two different monoclonal antibodies (MoAbs) to endothelial αVβ3 integrin receptor, with resulting improvement in microvascular hemodynamics. This study is an elegant and important contribution to our understanding of SS RBC interactions with the vessel wall and, hence, the mechanisms of vasoocclusion. Vasoocclusion of small and sometimes large vessels is the hallmark of sickle cell disease, accounting for much of its morbidity and mortality. The pathophysiology of the vasoocclusive episodes is complex, involving not only the polymerization of the mutant hemoglobin, but also interactions between SS RBC, endothelium, platelets, leukocytes, and plasma constituents. Intracapillary sickling and vasoocclusion occur when transit time through capillaries is longer than the lag time for deoxygenation-induced polymerization of sickle hemoglobin. Thus, processes that delay passage of SS RBC through the microvasculature may participate in the initiation and propagation of vasoocclusion. In particular, an increase in SS RBC adhesion to postcapillary endothelium could initiate vasoocclusion by impairing flow, thereby delaying transit time of less deformable SS RBC and propagating intracapillary sickling.\(^2\) Factors such as inflammatory mediators that activate endothelial cells\(^3\) and enhance endothelial adhesivity for SS RBC might, therefore, promote vasoocclusion. Conversely, anti-adhesive or anti-inflammatory therapies might attenuate vasoocclusion.

Seminal studies by Hebbel et al\(^4\) and Hoover et al\(^5\) two decades ago first demonstrated that SS RBC showed increased adherence to endothelial cells in vitro. Moreover, Hebbel et al\(^6\) showed that vasoocclusive severity correlated with adhesivity of SS RBC in vitro. Subsequently, these and many other investigators have defined adhesion pathways involved in SS RBC adhesion to cultured endothelium under static and flow conditions (Table; also reviewed in references 2 and 7). Adhesion receptors on SS RBC include α4β1 integrin\(^8,9\) and CD36\(^7,10\) whose expression is increased on sickle versus normal reticulocytes\(^8-10\) and is down-regulated by treatment with hydroxyurea.\(^11\) Aggregated, membranous band 3\(^12\) and sulfated glycolipids exposed on the surface of damaged RBC also have been implicated. On the endothelial side, cytokine-induced VCAM-1,\(^9,13,14\) a ligand for α4β1, and αβ3 integrin,\(^10\) which binds von Willebrand factor (vWF) and thrombospondin (TSP), have been demonstrated to mediate SS RBC adhesion. GpIb and CD36 are also potential endothelial adhesion receptors. The adhesive protein TSP\(^15,16\) released by platelets, and vWF,\(^17\) released by endothelium, promote SS RBC adhesion to cultured endothelium, serving as bridging molecules between endothelial and SS RBC receptors.

SS RBC interactions with the vessel wall also may involve interactions with subendothelial matrix components such as laminin (LN),\(^18,19\) TSP,\(^18\) vWF, or fibronectin\(^20\) (Table). Matrix components may be exposed by vascular injury or by endothelial retraction induced by stimuli such as thrombin.\(^21\) A sulfated glycolipid isolated from SS RBC was shown to bind to LN and TSP,\(^18\) and sulfated glycolipids also have been reported to bind to vWF.\(^22\) B-CAM/LU (basal cell adhesion molecule/lutheran protein) was recently shown to be a major LN receptor on SS RBC.\(^19\)

The majority of studies of SS RBC interactions with endothelium or subendothelial matrix have used in vitro approaches. Studies with cultured cells or purified matrix components, under static or flow conditions, have been invaluable in identifying potential adhesive interactions. Ultimately, however, it is necessary to validate concepts and pathways in animal models. Fabry et al\(^23\) used an intact rat model with gamma camera imaging and visualization of the microvasculature by silicone injection. They demonstrated that desmopressin, possibly by releasing vWF, increased the retention of arterially injected deformable SS discocytes without producing overt obstruction, and suggested that narrowing of postcapillary venules by the adhesion of deformable SS RBC facilitated trapping of less deformable SS RBC, triggering vasoocclusion.

The century-old technique of intravital microscopy, coupled with modern video image analysis, allows precise quantification of blood cell interactions with the vessel wall. This approach has proven to be a powerful tool in defining leukocyte-endothelial interactions, leading to the formulation of the multistep model of leukocyte emigration with selectin-mediated tethering/rolling and integrin-dependent sticking/transmigration.\(^24\) Kaul and colleagues have been leaders in applying intravital microscopy to elucidate SS RBC interactions with the vessel wall, using the rat mesoecocem ex vivo\(^25\) and the sickle transgenic mouse in vivo.\(^26\) Their studies demonstrated that deformable SS RBC are more likely to adhere than dense SS RBC\(^25\) and that adhesion was limited to postcapillary venules.\(^25,26\) They also showed that desmopressin-stimulated human SS RBC adhesion to postcapillary venules in the ex vivo rat mesoecol was inhibited by anti-vWF—but not anti-TSP—antibody, providing the first trial of adhesion blockade with specific reagents in an animal model of sickle disease.\(^27\)

Previous studies in vitro\(^10,28\) suggested that αVβ3 integrin receptor, which is expressed on the luminal surface of endothelial cells,\(^29\) may be involved in SS RBC adhesion to endothelium. Kumar et al\(^28\) found that a conformationally constrained RGD-containing peptide that inhibits αVβ3 significantly reduced plasma-induced SS RBC adhesion to cultured endothelial cells under flow. Sugihara et al\(^10\) reported that the anti-αVβ3 MoAb LM609 and anti-αIIbβ3 MoAb 7E3, which cross-reacts with αβ3 but not the non-cross-reactive anti-αIIbβ3 MoAb 10E5, reduced plasma-dependent SS RBC static adhesion to cultured human endothelial cells. In the current study, Kaul and colleagues used the same interactions with subendothelial matrix components such as laminin (LN), vWF, or fibronectin (Table). Matrix components may be exposed by vascular injury or by endothelial retraction induced by stimuli such as thrombin. A sulfated glycolipid isolated from SS RBC was shown to bind to LN and TSP, and sulfated glycolipids also have been reported to bind to vWF. B-CAM/LU (basal cell adhesion molecule/lutheran protein) was recently shown to be a major LN receptor on SS RBC. The majority of studies of SS RBC interactions with endothelium or subendothelial matrix have used in vitro approaches. Studies with cultured cells or purified matrix components, under static or flow conditions, have been invaluable in identifying potential adhesive interactions. Ultimately, however, it is necessary to validate concepts and pathways in animal models. Fabry et al used an intact rat model with gamma camera imaging and visualization of the microvasculature by silicone injection. They demonstrated that desmopressin, possibly by releasing vWF, increased the retention of arterially injected deformable SS discocytes without producing overt obstruction, and suggested that narrowing of postcapillary venules by the adhesion of deformable SS RBC facilitated trapping of less deformable SS RBC, triggering vasoocclusion. The century-old technique of intravital microscopy, coupled with modern video image analysis, allows precise quantification of blood cell interactions with the vessel wall. This approach has proven to be a powerful tool in defining leukocyte-endothelial interactions, leading to the formulation of the multistep model of leukocyte emigration with selectin-mediated tethering/rolling and integrin-dependent sticking/transmigration. Kaul and colleagues have been leaders in applying intravital microscopy to elucidate SS RBC interactions with the vessel wall, using the rat mesoecocem ex vivo and the sickle transgenic mouse in vivo. Their studies demonstrated that deformable SS RBC are more likely to adhere than dense SS RBC and that adhesion was limited to postcapillary venules. They also showed that desmopressin-stimulated human SS RBC adhesion to postcapillary venules in the ex vivo rat mesoecol was inhibited by anti-vWF—but not anti-TSP—antibody, providing the first trial of adhesion blockade with specific reagents in an animal model of sickle disease. Previous studies in vitro suggested that αVβ3 integrin receptor, which is expressed on the luminal surface of endothelial cells, may be involved in SS RBC adhesion to endothelium. Kumar et al found that a conformationally constrained RGD-containing peptide that inhibits αVβ3 significantly reduced plasma-induced SS RBC adhesion to cultured endothelial cells under flow. Sugihara et al reported that the anti-αVβ3 MoAb LM609 and anti-αIIbβ3 MoAb 7E3, which cross-reacts with αβ3 but not the non-cross-reactive anti-αIIbβ3 MoAb 10E5, reduced plasma-dependent SS RBC static adhesion to cultured human endothelial cells. In the current study, Kaul and colleagues used the same interactions with subendothelial matrix components such as laminin (LN), vWF, or fibronectin (Table). Matrix components may be exposed by vascular injury or by endothelial retraction induced by stimuli such as thrombin. A sulfated glycolipid isolated from SS RBC was shown to bind to LN and TSP, and sulfated glycolipids also have been reported to bind to vWF. B-CAM/LU (basal cell adhesion molecule/lutheran protein) was recently shown to be a major LN receptor on SS RBC.
Potential sickle red blood cell–vessel wall adhesion pathways

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<th>Endothelial Cell Adhesive Bridging Protein</th>
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SS RBC, sickle red blood cell; TSP, thrombospondin; VWF, von Willebrand factor; VCAM-1, vascular cell adhesion molecule-1; GP Ib, glycoprotein Ib; FN, fibronectin; LN, laminin; B-CAM/LU, basal cell adhesion molecule/lutheran protein.

reagents in the ex vivo rat model to address the role of αvβ3 integrin. Platelet-activating factor, a mediator that is increased in plasma of sickle cell patients, was used to stimulate SS RBC adhesion to postcapillary venules, possibly by releasing vWF or by provoking endothelial cell retraction. As shown in the dramatic videomicroscopy (vide infra), treatment with the αvβ3-blocking MoAb 7E3 largely abolished PAF-stimulated SS RBC adhesion to the vessel wall. MoAb 7E3 and the αvβ3-specific MoAb LM609, but not the αIbβ3-specific MoAb 10E5, also improved hemodynamics in the PAF-treated vessels.

This study provides compelling evidence for a role of endothelial αvβ3 integrin in SS RBC adhesion to PAF-stimulated postcapillary venules in the rat. There are, of course, a number of obvious cautions in extrapolating these exciting observations in the animal model to sickle cell vasoocclusive crises. First, this is an ex vivo model with artificial perfusion of isolated cells in a plasma-free medium, certainly not reflecting the complex hemodynamics and rheology that occur in the microcirculation during inflammation and hypoxia. Second, although the in vitro studies with large vessel human endothelial cells are consistent with the ex vivo results in the rat regarding the role of αvβ3 integrin, the interaction of human SS RBC with human microvascular endothelium in vivo may involve adhesion pathways different from those observed in the rat microvasculature. Third, the model examines only a single inflammatory stimulus, PAF, in the absence of other blood cells, whereas vasoocclusion occurs in a complex milieu of platelets, leukocytes, plasma, and multiple inflammatory mediators that may markedly alter the adhesion pathways used.

Despite these caveats, the current study by Kaul et al suggests that blockade of endothelial αvβ3 integrin might be beneficial in treatment of vasoocclusion in sickle cell disease. Integrin receptors have emerged as important therapeutic targets. Anti-adhesion therapies directed to platelet αIbβ3 integrin receptor have been proven to be of considerable benefit in acute coronary syndromes.30 These drugs include abiciximab, a derivative of MoAb 7E3 that cross-reacts with αvβ3 integrin.31 Antagonists of leukocyte α4β1 and β2 integrin receptors have demonstrated efficacy in diverse animal models32 and are now in clinical trial in several acute inflammatory disorders. Small molecule and antibody-based specific inhibitors of αvβ3 integrin are being developed as anti-angiogenic agents.33 If αvβ3 antagonists continue to prove safe in other indications, what additional studies would be required before testing them for the prevention or acute treatment of vasoocclusive episodes in sickle cell disease? Certainly, confirmatory results in other animal models of sickle cell disease would be encouraging. However, given the strong rationale, the supporting ex vivo and in vitro evidence, and the limitations of any of the animal models, it would seem reasonable even now to consider a clinical trial.

References

Video announcement

In addition to its scientific excellence, the work by Kaul and colleagues is also notable in that it launches a new technical feature of Blood: electronic links to video imagery. Perhaps the most compelling data presented by these authors is real-time intravital microscopy of red cells flowing through vascular beds of the rat mesocolon, now available to all members and subscribers of the Journal. I would encourage all readers of the paper to visit the Blood website (www.bloodjournal.org) to see the video relating to Figure 3. Take the necessary 2 minutes to download and view the short video comparison of red cell flow in the absence and presence of αVβ3 integrin blockade. Hematology is a visual science, initiated by Leeuwenhoek’s microscopes and extended by the electron microscopy of sickle erythrocytes and of individual fibrinogen or von Willebrand factor molecules. In addition, most of the cells that concern hematologists are either intrinsically or passively motile. The trafficking of lymphocytes and neutrophils to sites of inflammation, the phagocytosis of macrophages, the growth and maturation of a platelet-rich clot, and intravascular blood cell flow patterns serve as outstanding examples of how the capacity to present video data might enhance the dissemination of hematologic knowledge. The editors of Blood sincerely hope that providing its readers the opportunity to view video data images that support hematologic and oncologic studies will enhance their understanding and enjoyment of modern scientific research.

Kenneth Kaushansky, MD
Editor-in-Chief
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