Chapter 4
Red Blood Cells and the Vaso-Occlusive Process

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Abstract While the definitive genetic defect in sickle cell disease (SCD) is sickle hemoglobin (HbS), the relationship between the HbS mutation and the pathogenesis of vaso-occlusion in SCD remains incompletely understood and likely involves multiple complex and heterogeneous steps. Since chronic transfusion can prevent stroke and reduce the frequency of acute vaso-occlusive events, it is clear that the sickle red blood cell (RBC) plays a critical role in this process. Numerous sickle RBC factors contribute to the vaso-occlusive process, including: HbS polymerization; RBC cation loss and resultant cellular dehydration; oxidative injury of RBC membrane proteins and lipids; band 3 clustering; loss of phospholipid asymmetry and phosphatidylserine exposure; reduced RBC deformability; irreversibly sickled RBCs; increased adhesion of sickle RBCs to the endothelium and other circulating blood cells; intravascular hemolysis with the release of cell-free hemoglobin, arginase, and adenosine deaminase; and RBC microvesiculation. These sickle RBC properties initiate and propagate endothelial injury, vascular stasis, and activation of the coagulation and inflammatory pathways, precipitating acute vaso-occlusion.

Keywords Sickle red blood cell • Adhesion • Oxidative injury • Vaso-occlusion • Hemolysis

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4.1 The Sickle Red Blood Cell (RBC)

Sickle cell disease (SCD) is caused by a single amino acid substitution in the beta chain of hemoglobin (hemoglobin β Glu6Val) that predisposes deoxyhemoglobin S to polymerize and form long crystals that distort and damage the red cell membrane (Hillery and Panepinto 2004; Hebbel 1991; Bunn 1997). In addition, sickle hemoglobin (HbS) is moderately unstable, with oxidized hemoglobin binding avidly to the lipid bilayer and contributing to multiple membrane defects. The link between HbS polymerization, its many effects on the sickle red blood cell (RBC), and the pathobiology of vaso-occlusion remains incompletely understood and likely involves many complex and heterogeneous steps. The evidence that chronic RBC transfusion effectively prevents most primary or recurrent stroke events (Adams et al. 1998; Russell et al. 1984) and reduces the incidence of pain and acute chest syndrome (Miller et al. 2001) indicates a critical role for the sickle RBC in the pathophysiology of vaso-occlusion. Sickle RBC characteristics that appear to contribute to acute vaso-occlusion include the extent of HbS polymerization, oxidant injury of membrane proteins and lipids, cation loss resulting in cellular dehydration, reduced deformability with a propensity for vesiculation, cellular lysis and enhanced adhesive properties. These sickle RBC characteristics also contribute to chronic endothelial injury, vascular stasis and increased activation of the inflammatory and coagulation pathways. This chapter will focus on the role of the sickle red blood cell (RBC) in the vaso-occlusive process.

4.2 Hemoglobin S Polymerization

The substitution of valine for glutamic acid at the sixth position of the beta chain of sickle hemoglobin creates a hydrophobic pocket in the hemoglobin tetramer that polymerizes upon deoxygenation. This polymerization process is reversed with reoxygenation. The polymerization of deoxy-HbS involves a two-step, double-nucleation process, followed by a rapid increase in polymer/fiber formation that results in RBC "sickling" (Eaton and Hofrichter 1987). There is a delay time between HbS deoxygenation and the onset of exponential polymerization, which is markedly influenced by the intracellular hemoglobin concentration (MCHC), temperature, pH, and the presence of non-S hemoglobins, such as HbF or HbA. For example, the delay time of polymer formation is dependent on the 15th to 30th power of hemoglobin concentration (Eaton and Hofrichter 1987). Thus, the dehydration found in subpopulations of sickle RBCs (described in Sect. 4.3) can greatly promote HbS polymerization.

The estimated delay time of greater than 15 s predicts that an unimpeded sickle RBC should return to the lung for reoxygenation before HbS is fully polymerized (Mozzarelli et al. 1987; Du et al. 2015). In agreement, the majority of sickle RBCs in the returning venous circulation are not polymerized. However, any event that delays the return of the sickle RBC to the pulmonary circulation will permit progression to full polymerization. RBC adhesion to the vascular endothelium, either

directly to endothelial cells or via bridging adhesive ligands or bound leukocytes will also promote HbS polymerization due to delay in return to the pulmonary circulation for reoxygenation. Reduced sickle RBC deformability will also slow trafficking through the microcirculation and prolong the time in the hypoxic environment. Finally, any pre-existing polymer that does not completely solubilize in the lung circulation may have a markedly shortened or absent delay time such that polymerization can more rapidly proceed in the microcirculation following delivery of oxygen to tissue beds (Huang et al. 2003).

While the definitive genetic defect in SCD is HbS, the direct link between HbS polymerization and the pathobiology of vaso-occlusion is more complex. Since HbS will only polymerize after delivery of oxygen, uninterrupted blood return to the lungs for reoxygenation is essential to prevent RBC sickling. Risk factors that promote sickling include RBC dehydration, lung or vascular disease that prevents optimal oxygenation, any right shift in oxygen binding curve (acidosis and fever), low HbF levels and delayed microvascular transit time due to leukocyte and sickle RBC adhesion to injured or inflamed endothelium. Because of this, clinical care for sickle cell disease is often targeted to limit HbS polymerization, such as with generous hydration, optimizing oxygenation and raising HbF levels with hydroxyurea therapy.

4.3 Cation Loss and Dehydration

Since the polymerization rate of deoxyHbS is critically dependent on the intracellular concentration of hemoglobin, sickle RBC dehydration will promote sickling and may contribute to the development of vaso-occlusion in SCD cell disease; this may be best exemplified by the papillary necrosis that occurs in the hyperosmolar kidney medulla. Additionally, RBC dehydration status can directly affect the adhesive phenotype, possibly by exposing or altering adhesive components of the membrane (Stone et al. 1996; Hebbel et al. 1989; Wandersee et al. 2005).

A significant proportion of sickle RBCs are inherently dehydrated, primarily due to intracellular K^+ and water losses via the erythrocyte Ca^{2+}-dependent K^+ (Gardos) channel (Brugnara et al. 1986) and the K/Cl cotransport system (Franco et al. 1996). In sickle RBCs, the pathologic activation of the Gardos channel that results in water loss is aggravated by transient increases in Ca^{2+} permeability induced in sickle RBCs with every deoxygenation-reoxygenation cycle (Lew et al. 1997). In SCD, RBC K-Cl cotransport is activated by low pH (Brugnara et al. 1986), low magnesium content, oxidative damage, positively charged hemoglobin (HbS, HbC) and cell swelling. Clotrimazole specifically inhibits the Gardos channel (Brugnara et al. 1993). Magnesium decreases the K^+ and water losses via the K/Cl cotransport system. Both dietary magnesium supplementation (De Franceschi et al. 1996) and oral clotrimazole therapy (De Franceschi et al. 1994) improved the hydration status and hemoglobin levels of a transgenic sickle cell mouse model.

Despite the likely important link between polymerization of HbS with cellular dehydration, and the potential contribution of RBC dehydration to RBC adhesive properties (Wandersee et al. 2005), clinical trials to date using agents to improve

sickle RBC hydration have shown minimal effects on clinically significant vaso-occlusive events. A short term study of five patients with SCD treated with oral clotrimazole also reduced RBC dehydration and resulted in a striking reduction of the number of dense red cells (Brugnara et al. 1996). While the Phase II study using the novel inhibitor of the Gardos channel, ICA-17043, showed improvement of anemia and reduction in reticulocytosis in patients with SCD (Ataga et al. 2006), the subsequent Phase III study was prematurely terminated due to lack of clinical efficacy in reducing acute painful events in patients with sickle cell syndromes (Ataga et al. 2011). In addition, while preliminary studies using Mg pidolate to block the K/CL cotransport system confirmed the beneficial effects on red cell dehydration (De Franceschi et al. 2000; Hankins et al. 2008), the Phase III trial was terminated due to a slow rate of enrollment.

4.4 Oxidant Injury of the Sickle RBC Membrane

Hemoglobin S has a higher auto-oxidation rate compared to hemoglobin A; oxidized hemoglobin has an affinity for the lipid bilayer and can expel its heme group with subsequent liberation of free iron (Hebbel et al. 1988; Sheng et al. 1998). Membrane associated iron is catalytically active and likely contributes to the increased susceptibility of sickle RBC membranes to lipid peroxidation (Chiu et al. 1979). This also promotes further hemoglobin denaturation, including the formation of irreversibly oxidized hemichromes located near the membrane inner surface. As a consequence, the sickle RBC membrane is uniquely targeted for oxidant stress, effectively bypassing or depleting the RBC of natural antioxidants, such as vitamin E (α- and γ-tocopherol) glutathione or ascorbic acid (Darghouth et al. 2011). The increased oxidative damage to membrane proteins and lipids contributes to sickle RBC membrane abnormalities, including aberrant clustering of surface proteins, disruption of phospholipid asymmetry, dysregulated cation homeostasis, reduced deformability, formation of irreversibly sickled cells (ISC), increased fragility and release of microvesicles.

4.5 Clusters of Band 3

Clustered Band 3 can also participate in sickle RBC adhesion and promote vaso-occlusion. Band 3 is an abundant RBC anion exchanger that spans the plasma membrane multiple times and is linked to the RBC cytoskeleton. Band 3 is abnormally clustered on the sickle RBC surface due to binding of its cytosolic sections to denatured HbS hemichromes found at the inner sickle membrane (Waugh et al. 1986). Denatured hemoglobin also colocalizes glycophorin and ankyrin on sickle RBC membranes, although to a lesser extent than band 3. Clustering of band 3 binds naturally occurring anti-band 3 autoantibodies (Kannan et al. 1988). Opsonized band 3 promotes sickle RBC phagocytosis by the reticuloendothelial system that

will shorten the sickle RBC lifespan. Band 3 mediates the adhesion of malaria-infected RBCs to the vascular endothelium via exposure of previously cryptic adhesive sites (Crandall et al. 1993). Peptides from sites of clustered Band 3 that are aberrantly exposed on sickle RBCs will also inhibit sickle RBC adhesion to cultured endothelial cells in vitro (Thevenin et al. 1997).

4.6 Increased Phosphatidylserine (PS) Exposure

The normal lipid bilayer maintains phosphatidylserine (PS) and phosphatidylethanolamine sequestered on the inner leaflet. In SCD, PS is abnormally exposed on the outer surface of the sickle RBC membrane (Choe et al. 1986). This impairment of the normal phospholipid asymmetry on the sickle RBC membrane may be due to thiol oxidation of the translocase that moves PS to the inner layer and increased calcium activation of the scramblase that permits PS to move outward (Zachowski et al. 1985).

When PS translocates to the cell surface under normal physiologic circumstances, such as during platelet activation, externalized PS serves as an anchor for factors in the hemostatic system, promoting the activation of the coagulation cascade (Zwaal and Schroit 1997). In agreement, there is a correlation between the level of sickle RBC PS exposure and the activity of the coagulation cascade in human and murine SCD (Setty et al. 2000, 2001). This suggests that this loss of sickle RBC membrane asymmetry, which results in increased PS exposure, contributes to the well described prothrombotic state found in individuals with SCD (Singer and Ataga 2008). Sickle membrane PS exposure also promotes RBC adhesion to endothelial cells (Setty et al. 2002; Schlegel et al. 1985; Manodori et al. 2000). In addition, PS exposure on sickle RBCs shortens RBC survival in sickle mice effectively increasing hemolytic rate (de Jong et al. 2001). Thus, increased PS exposure on sickle RBCs may participate in the vaso-occlusive process by increased adhesion to the microvasculature, activation of the coagulation cascade, and decreased RBC lifespan.

4.7 Membrane Deformability and Irreversibly Sickled Cells (ISC)

There is reduced deformability of sickle RBCs even when oxygenated and when HbS is fully solubilized (Chien et al. 1970). Both cellular dehydration and irreversible membrane changes contribute to this effect. This includes abnormal associations and crosslinking of cytoskeletal proteins and membrane components that result from both repeated HbS polymerization and oxidative injury of the membrane lipids and proteins.

Irreversibly sickled RBCs (ISCs) are the predominant form of "sickled" RBCs seen on typical blood smears. ISCs are due to a permanent shape change as a product of damage to membrane and cytoskeletal proteins enabling the retention of the elongated RBC shape regardless of hemoglobin polymerization status (Lux et al. 1976).

Consequently, even when the HbS is oxygenated and fully soluble, the ISC retains its abnormal elongated shape. ISCs tend to be very dense (MCHC greater than 44 g/dL), externalize PS, have low HbF levels and very short survival (Bertles and Milner 1968). Clinically, ISCs are important in diagnosis of a sickling disorder from a blood smear, vary greatly in number between individual patients and contribute to the hemolytic rate from the shortened life span. While ISCs likely participate in RBC blockage associated with vaso-occlusion (Kaul et al. 1986), it is less clear whether the ISC count correlates with vaso-occlusive severity (Barabino et al. 1987b).

4.8 Adhesive Properties of Sickle RBCs

The increased adhesion of sickle RBCs to vascular endothelium in vitro has been described using both static adhesion assays (Hebbel et al. 1980b; Mohandas and Evans 1984) and endothelialized flow chambers (Barabino et al. 1987a). These observations have been confirmed using live animal models by either infusing human sickle RBCs into rats (Fabry et al. 1989; Kaul et al. 1989; French et al. 1997) or by studying transgenic sickle cell mouse models (Kaul et al. 1995; Wood et al. 2004). In addition, leukocyte and platelet interactions with sickle RBC and vascular endothelium are important components of the vaso-occlusive process (Turhan et al. 2002; Dominical et al. 2015; Conran and Costa 2009). The enhanced interactions between sickle RBCs, leukocytes, platelets and the vessel wall play important roles in the pathogenesis of vascular occlusion in sickle cell disease.

The early findings that sickle RBCs adhere to the endothelium to a variable degree and that the level of adhesion may correlate with disease severity (Hebbel et al. 1980a) prompted further investigation into potential receptors and signaling pathways involved in the adhesive processes. Reticulocytes from both normal and sickle individuals express the adhesion molecules integrin α4β1 (Swerlick et al. 1993; Joneckis et al. 1993) and CD36 (GP IV) (Joneckis et al. 1993; Sugihara et al. 1992; Browne and Hebbel 1996). Immature reticulocytes have greater levels of adhesion to endothelial cells compared to mature RBCs, pointing to a potential unique role for reticulocyte adhesion under select experimental and physiologic conditions (Mohandas and Evans 1984; Brittain et al. 1993; Fabry et al. 1992; Joneckis et al. 1993; Sugihara et al. 1992). Potential RBC adhesion molecules that remain present on mature RBCs include basal cell adhesion molecule-1/Lutheran (BCAM/LU), intercellular adhesion molecule-4 (ICAM-4) (Zennadi et al. 2004), integrin associated protein (CD47), phosphatidylserine (PS) (Setty et al. 2002) and sulfated glycolipids (Hillery et al. 1996; Joneckis et al. 1996).

Integrin α4β1 is a receptor for both fibronectin and vascular cell adhesion molecule-1 (VCAM-1) (Humphries et al. 1995). Sickle RBCs bind to VCAM-1 on cytokine-stimulated endothelial cells (Swerlick et al. 1993) or transfected COS cells (Gee and Platt 1995), as well as immobilized fibronectin (Kasschau et al. 1996) via α4β1. The activation state of α4β1 is regulated by several factors, including divalent cation concentration and agonist-induced cell signaling (Han et al. 2003). The α4

cytoplasmic domain is directly phosphorylated in vitro by cAMP-dependent protein kinase A (PKA) (Goldfinger et al. 2003), suggesting a role for PKA in activation of α4β1. In agreement, ligation of CD47 on sickle reticulocytes activates α4β1 via a PKA-dependent phosphorylation of the α4 cytoplasmic tail (Brittain et al. 2004). Sickle RBC α4β1 binding to endothelial VCAM-1 likely contributes to the adherence of sickle reticulocytes to cytokine-stimulated retinal microvascular endothelial cells in vitro (Setty and Stuart 1996).

CD36 is a non-integrin adhesive receptor that binds thrombospondin (TSP) and collagen and is present on the surface of endothelial cells, platelets, and a reticulocyte-rich subpopulation of normal and sickle RBCs (Joneckis et al. 1993; Sugihara et al. 1992). Sickle RBCs bind to endothelial cells in the presence of soluble TSP and this adhesion is blocked by anti-CD36 monoclonal antibodies in both static adhesion assays (Sugihara et al. 1992) and under flow conditions (Brittain et al. 1993).

The Lutheran blood group proteins, basal cell adhesion molecule-1 and Lutheran (BCAM/Lu) are derived by alternative splicing from the same gene and differ only in the length of their cytoplasmic tails. Sickle RBCs over express BCAM/Lu, which specifically binds to the alpha 5 subunit of the extracellular matrix protein laminin (Udani et al. 1998; Parsons et al. 2001). RBC intercellular adhesion molecule-4 (ICAM-4), otherwise known as blood group Landsteiner-Weiner (LW), binds β3 integrins, including αvβ3 expressed on vascular endothelial cells (Parsons et al. 1999). In a rat ex vivo microvascular flow model, ICAM-4-specific peptides inhibited human sickle RBC adhesion to the activated ex vivo microvascular endothelium (Kaul et al. 2006). Interestingly, both BCAM/Lu and ICAM-1 can be activated by epinephrine in a subset of sickle RBCs via a cAMP-dependent pathway that likely involves PKA (Zennadi et al. 2004; Hines et al. 2003).

Integrin-associated protein (CD47) is a 50 kDa integral membrane protein found on RBCs and many other cells that associates with integrins and binds to the C-terminal cell binding domain of thrombospondin-1 (TSP) (Gao et al. 1996). CD47 is expressed in RBCs and protects normal RBCs from immune clearance (Oldenborg et al. 2000). CD47 on sickle RBCs binds immobilized TSP under both static and flow conditions (Brittain et al. 2001). Furthermore, soluble TSP binds CD47 and induces an increase in sickle RBC adhesion via shear stress-dependent and G protein-mediated signal transduction pathways (Brittain et al. 2001).

Lipids naturally present in the red cell membrane that have been abnormally exposed or modified on the sickle RBC also contribute to their adhesive properties. For example, increased exposure of phosphatidylserine (PS) on the sickle RBC likely contributes to its proadhesive phenotype (Setty et al. 2002; Schlegel et al. 1985; Manodori et al. 2000). Sulfated glycolipids avidly bind TSP, von Willebrand factor, and laminin and may also play a role in sickle red cell adhesion (Hillery et al. 1996; Joneckis et al. 1996; Barabino et al. 1999; Zhou et al. 2011).

A disturbed endothelium contributes to sickle RBC, leukocyte and platelet adhesion. Endothelial adhesive molecules that bind sickle RBCs include VCAM-1, integrin αVβ3, E-selectin and P-selectin (Swerlick et al. 1993; Gee and Platt 1995;

Brittain et al. 1993; Natarajan et al. 1996; Matsui et al. 2001). For example, monoclonal antibodies directed against αVβ3 inhibited human sickle RBC adhesion to platelet-activating factor (PAF)-treated rat mesocecum vasculature ex vivo (Kaul et al. 2000b). In agreement, αVβ3 antagonists also reduced sickle RBC adhesion to human endothelial cell monolayers under venular shear flow conditions (Finnegan et al. 2007). P-selectin is rapidly expressed on the surface of activated endothelial cells and promotes sickle RBC rolling and adhesion (Embury et al. 2004). Optimal surface expression of these endothelial adhesion molecules requires induction by cytokines, shear stress or other perturbations of the endothelium. In fact, exposure of endothelium to inflammatory agonists is associated with increased RBC adhesion (Wick and Eckman 1996; Manodori 2001).

Adhesive plasma and extracellular matrix proteins may also contribute to sickle RBC adhesion. Thrombospondin (TSP) is a 450 kDa, homotrimeric glycoprotein present in the subendothelial matrix, plasma and platelet alpha storage granules; it can be released in high local concentrations by activated platelets (Santoro and Frazier 1987). In SCD, both soluble and immobilized TSP can bind sickle RBCs. In its soluble form, TSP may serve as a linker molecule between sickle RBCs and endothelial cells (Brittain et al. 1993; Gupta et al. 1999). TSP also interacts with sickle RBC CD47 (Brittain et al. 2001), sulfated glycolipids (Barabino et al. 1999), and a normally cryptic domain of the dominant membrane protein, band 3, which is subject to rearrangement in hematologic disorders (Thevenin et al. 1997; Sherman et al. 1992). Laminin, a major constituent of the extracellular matrix, is composed of a family of large heterotrimeric glycoproteins that support cell adhesion and migration (Tryggvason 1993). Sickle RBCs avidly bind both immobilized and soluble laminin (Udani et al. 1998; Hillery et al. 1996). Vitronectin, fibrinogen, and von Willebrand factor also support sickle RBC adherence (Wick and Eckman 1996).

Sickle RBCs also bind leukocytes and platelets (Sakamoto et al. 2013; Frenette 2004). In fact, the leukocyte-endothelial cell adhesive event may initiate and precede sickle RBC adhesion in the microvascular bed (Turhan et al. 2002; Dominical et al. 2015; Conran and Costa 2009). The sickle RBC likely utilizes multiple adhesive pathways, potentially first binding to the endothelium and inducing localized pathologic changes, followed by a second adhesive event with the sickle RBC binding to leukocytes, platelets, or the newly exposed endothelial or subendothelial adhesive ligands.

4.9 Increased Fragility and Microvesiculation

Sickle RBCs have increased fragility with a propensity for vesiculation and cellular lysis. The shortened lifespan of sickle RBCs includes both extravascular mechanisms of removal, primarily through the reticuloendothelial system, and intravascular hemolysis. Intravascular RBC lysis releases intracellular components and generates RBC microvesicles and likely contributes most directly to the vaso-occlusive process.

4.9.1 Intravascular Hemolysis

Intravascular hemolysis contributes to the vascular pathologies associated with SCD. RBC lysis releases Hb into the plasma compartment; consequently plasma levels of cell-free Hb (CF-Hb) from individuals with SCD are elevated. CF-Hb is present mainly in the ferrous oxygenated form (oxyHb) with a smaller contribution of the ferric form (metHb) (Reiter et al. 2002). Normal individuals have plasma CF-Hb levels of less than 1 μM, whereas individuals with SCD have variable levels up to ~20 μM (Reiter et al. 2002). CF-Hb is an efficient scavenger of nitric oxide (NO), a critical regulator of vascular homeostasis (Datta et al. 2004; Gladwin et al. 2004; Jison and Gladwin 2003; Liao 2002; Pawloski 2003; Jeffers et al. 2006; Kim-Shapiro et al. 2006; Lancaster Jr 1994). OxyHb reacts with NO with a rate constant in excess of 10^7 $M^{-1}s^{-1}$ to form metHb and inert nitrate. In individuals with SCD, oxidation of CF-Hb by NO inhalation therapy improves forearm blood flow in response to nitrovasodilators, suggesting that CF-Hb has an acute effect on the bioavailability of NO (Reiter et al. 2002). However, chronic vascular dysfunction in isolated vessels has been observed in animal models of SCD and other intravascular hemolytic models (Kaul et al. 2000a; Frei et al. 2008; Ou et al. 2003). The role played by CF-Hb in chronic vascular dysfunction is less clear, but it is conceivable that long-term loss of NO bioavailability, due to the presence of CF-Hb, could lead to significant changes in endothelial function, including a switch to alternate mechanisms of vascular control (Godecke and Schrader 2000; Zatz and Baylis 1998). The chronic presence of CF-Hb is also associated with other pathological presentations of SCD, including hemoglobinuria, increased blood pressure and vasoconstriction, decreased inhibition of platelet activation, a prothrombotic tendency, and increased expression of endothelial cell adhesion molecules such as ICAM-1, VCAM-1 and E-selectin (Rother et al. 2005; Villagra et al. 2007; Silva et al. 2009).

Other cytoplasmic components of lysed RBCs also accumulate in the plasma during chronic intravascular hemolysis, and may be important contributors to overall vascular dysfunction. RBC arginase has been specifically highlighted as arginase will deplete the substrate for nitric oxide formation with a negative impact on vasoreactivity. In this regard it is worth highlighting that there is significant evidence that RBC arginase, in humans, may contribute to loss of NO function through its ability to deplete arginine, the substrate for nitric oxide synthase (Rother et al. 2005; Gladwin 2006; Morris et al. 2008).

In addition, hemolysis releases adenosine deaminase (ADA) from the RBC into plasma, reducing extracellular adenosine stores via the conversion of adenosine to inosine (Tofovic et al. 2009). Since adenosine is involved in protective responses against vasculopathy, the reduction of adenosine by ADA released from RBCs may exacerbate vascular pathology initiated by cell-free hemoglobin and heme (Tofovic et al. 2009).

4.9.2 Microvesiculation

Patients with SCD have elevated RBC, platelet, monocyte, and endothelial microvesicles that increase further during crisis (Shet et al. 2003). RBC sickling, induced by hypoxia and subsequent reoxygenation, causes the loss of 2–3 % of sickled RBC lipids in the form of microvesicles (Allan et al. 1982). RBC-derived microvesicles house hemoglobin, which scavenges NO with comparable kinetics to soluble hemoglobin (Donadee et al. 2011). Circulating RBC fragments and microparticles may directly injure the endothelium and promote coagulation and inflammation (Setty et al. 2001). Interestingly, when children with SCD were treated with hydroxyurea therapy, which should improve sickling and provide a new source of nitric oxide, there were reduced levels of RBC and platelet-derived microvesicles compared to untreated counterparts (Nebor et al. 2013).

Incubation of sickle RBC microvesicles with cultured endothelial cells induced reactive oxygen species (ROS) formation to a much greater extent than control RBC microvesicles (Camus et al. 2012). The ROS formation was also inhibited by pretreating the microvesicles with annexin V to "cover" microvesicle anionic phospholipids. When RBC microvesicles were injected into a mouse model of sickle cell disease, acute "vaso-occlusion" of the kidneys was observed, suggesting a potential role for microvesicles in the evolution of vaso-occlusion (Camus et al. 2012, 2015).

In summary, the sickle RBC is a critical participant in the vaso-occlusive process, which is the major clinical manifestation of sickle cell disease. HbS directly injures the sickle RBC through polymerization of deoxyHbS that distorts and perturbs the red blood cell membrane and through oxidized HbS that binds to the lipid bilayer, causing further membrane damage. This results in a wide array of sickle RBC abnormalities, including cellular dehydration, clustering of band, increased PS exposure, reduced RBC deformability, increased hemolysis with release of intracellular contents and microvesicles, and increased adhesion to the vascular endothelium and non-erythroid blood cells. These aberrant sickle RBC properties initiate and propagate endothelial injury, vascular stasis, and activation of the coagulation and inflammatory pathways, ultimately precipitating acute vascular occlusion.

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