Hemoglobin S Polymerization and Red Cell Membrane Changes

Frans A. Kuypers, PhD

KEYWORDS

• Polymerization • Oxidative damage • Membrane lipids • Microparticles

KEY POINTS

- Although the pathophysiology of sickle cell disease may be uniquely related to the polymerization of sickle hemoglobin under low oxygen conditions, it has become apparent that many factors are involved in the vasculopathy that characterizes this disorder affecting millions of individuals worldwide.
- The altered red blood cell (RBC) membrane plays an important role in the dysfunctional interactions of the sickle RBC with other blood cells and vascular endothelium, and leads to premature recognition and removal, an imbalance in hemostasis, vaso-occlusive events, and intravascular hemolysis, and may be involved in acute chest syndrome.
- The complex, well-orchestrated RBC membrane phospholipid organization is apparently lost in subpopulations of RBC during erythropoiesis as well as in the circulation. Increased oxidant stress may play an important role in the inability of the RBC to maintain composition and asymmetry in phospholipid molecular species, but the mechanisms that lead to phosphatidylserine (PS) exposure are poorly understood.
- This lack of knowledge is in part due to the incomplete characterization of the proteins involved in the maintenance of phospholipid asymmetry in the RBC, as well as the complexity of studying a complete plasma membrane in which several protein entities act in synchronization with each other and are governed by protein-protein and protein-lipid interactions.
- The purification and/or expression of the proteins thought to be involved in membrane organization in well-defined lipid bilayers, their 3-dimensional structural modeling, and detailed functional characterization may lead to a better understanding of their individual functions and their interaction with other entities in the bilayer.
- This knowledge will also lead to a better understanding of how the function of these proteins is impaired or altered in leading to PS exposure in hemoglobinopathies, and to a definition of the molecular underpinnings of this complex pathophysiology.

INTRODUCTION

It has been 100 years since Herrick published the first medical case report of the anemia describing abnormal shapes of red blood cells (RBCs) and gave sickle cell anemia its name.[1] In 1949, Pauling and Itano[2] defined the underlying molecular reason by identifying hemoglobin S (HbS), and defined sickle cell anemia as the first

Children's Hospital Oakland Research Institute, 5700 Martin Luther King Jr. Way, Oakland, CA 94609, USA
E-mail address: fkuypers@chori.org

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"molecular disease." The last 60 years have resulted in an increasingly coherent detailed molecular-level description of the pathophysiology of sickle cell disease (SCD). While great progress has been made in describing the basic disease process that accounts for hemolytic anemia and the obstructive events underlying vaso-occlusive events (VOE), many questions remain. The simple mutation in the β6 location of globin has a profound effect on all tissues and organs in the SCD patient, and because the vasculopathy affects a large variety of physiologic mechanisms, the varied genetic background of individual patients makes prediction of the clinical severity highly complex. However, it assuredly starts with the mutated hemoglobin (Hb) and the changes in the membrane that result from it. Hb is a complex molecule that undergoes conformational changes in response to oxygen, and is affected by environmental changes, allosteric effectors, and mutations. The polymerization of deoxy HbS, which forms long fibers inside the RBC, leads to the typical distorted sickle RBC (SRBC) morphology that was noted by Herrick. Together with changes in cytosol, which result from the relatively unstable pro-oxidant character of HbS,[3] the morphology and ability of the SRBC to deform are affected. Both the mechanical stress on the RBC membrane and oxidation-induced damage affect both lipid and protein components of the RBC membrane and alter the interaction of the SRBC with its environment, including endothelial cells, white blood cells, and platelets, and leads to the loss of bioactive membrane material (particles). A loss of normal ion permeability of the SRBC membrane leads to an altered hydration status of the cell. In turn this affects polymerization, and an altered cytosolic calcium status affects a variety of processes, which will affect the plasma membrane and lead to apoptosis during erythropoiesis, in addition to hemolysis and early removal of the adult SRBC. While different pathways may lead from the simple point mutation in hemoglobin to the membrane alterations, the changes in the SRBC membrane and the downstream effects on its environment result in the vasculopathy that characterizes the disease.

POLYMERIZATION

As the predominant cell type, the RBC largely determines the rheologic and hemodynamic behavior of blood. The intricate mechanisms that govern the interaction of the RBC membrane skeleton with membrane proteins and the lipid bilayer[4,5] provide the ability of the RBC to deform under shear stress in the circulation and regain its typical shape as a biconcave disk. In SCD, mechanically fragile, poorly deformable RBCs contribute to impaired blood flow and other pathophysiologic aspects of the disease.[6,7] Formation of hemoglobin polymers in the SRBC negatively affect the RBC's ability to maintain its normal morphology, and it has long been considered that the radical shape change of the SRBC under low oxygen leads to the inability of the SRBC to properly deform and pass though the microvasculature, leading to VOE.[8–11] In addition to the change in shape, HbS polymer formation will cause mechanical stress on the RBC membrane, resulting in membrane changes as well as loss of membrane material, evidenced by the increased circulating RBC-derived microparticles (MPs). The kinetics of HbS polymerization are affected by the presence of normal Hb (HbA) or fetal Hb (HbF). Although sickle cell trait individuals (HbAS) can experience VOE, this is a rare event and seems related to extreme dehydration or low oxygen tension as experienced at higher altitudes. The replacement of 50% of HbS by HbA slows polymerization by approximately 100-fold.[12] Increased levels of gammaglobulin ameliorate the severity of SCD because HbF can effectively replace HbS and lower the rate of polymerization, providing a treatment protocol by reversing the