Red cell membrane: past, present, and future

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As a result of natural selection driven by severe forms of malaria, 1 in 6 humans in the world, more than 1 billion people, are affected by red cell abnormalities, making them the most common of the inherited disorders. The non-nucleated red cell is unique among human cell type in that the plasma membrane, its only structural component, accounts for all of its diverse antigenic, transport, and mechanical characteristics. Our current concept of the red cell membrane envisions it as a composite structure in which a membrane envelope composed of cholesterol and phospholipids is secured to an elastic network of skeletal proteins via transmembrane proteins. Structural and functional characterization of the many constituents of the red cell membrane, in conjunction with biophysical and physiologic studies, has led to detailed description of the way in which the remarkable mechanical properties and other important characteristics of the red cells arise, and of the manner in which they fail in disease states. Current studies in this very active and exciting field are continuing to produce new and unexpected revelations on the function of the red cell membrane and thus of the cell in health and disease, and shed new light on membrane function in other diverse cell types. (Blood. 2008;112:3939-3948)

History

Introduction

Jan Swammerdam, a Dutch biologist and microscope, first observed and described red cells in 1668 but it was some years before his observations, recorded in his notebooks, were publicly disseminated. Antonie van Leeuwenhoek, another brilliant Dutch microscopist, was the first to publish, in Philosophical Transactions of the Royal Society in 1675, a remarkable description of the unique features of human red blood cells.¹ He stated, “when he was greatly disordered, the globules of his blood appeared hard and rigid, but grew softer and more pliable as his health returned: whence he infers that in a healthy body they should be soft and flexible, that they may be capable of passing through the capillary veins and arteries, by easily changing their round figures into ovals, and also reassuming their former roundness when they come into vessels where they find larger room.” This striking observation made more than 300 years ago has proven both prescient and accurate. An excellent review by Bessis and Delphech provides a comprehensive description of priorities and credits for the discovery and description of red cells.²

George Gulliver, following the work of William Hewson, published the primary features of red cell membranes in Blood of Vertebrata in 1862, “Not withstanding the current observations that the red corpuscle is absolutely homogeneous, it is really composed of 2 very different parts. One of these is membranous, colourless and insoluble in water; the other is semifluid or viscid, containing the color, and very soluble in water.”³ Gorter and Grendel in 1925 provided the first insights into the structure of the membrane, and indeed biologic membranes generally, by the brilliant deduction that there are “bimolecular layers of lipids on the chromocytes of blood.”⁴ This model has continually evolved over the past 80 years, thanks to a succession of seminal contributions that included outlining of the fluid mosaic model of the structure of cell membranes by Singer and Nicolson,⁵ isolation of spectrin by Marchesi and Steers,⁶ and the definition of the topology of red cell membrane proteins by Steck and colleagues.⁷ This progress has benefited greatly from key discoveries that included methods for isolation of membranes (ghosts); protein component analysis through the development of gel electrophoresis and mass spectrometry; advances in imaging technologies; biochemical, structural, and functional characterization of the various protein components of the membrane; defining of asymmetric distribution of phospholipids in the membrane; and delineating the nature of interactions among various membrane proteins and between proteins and lipids.

While a very large number of investigators contributed to the many exciting advances made in our understanding of the structural organization of the red cell membrane during these intervening years, a few deserve special mention including Peter Agre, Jane Barker, Daniel Branton, Vann Bennett, Jean Delaunay, Bernard Forget, Walter Gratzer, Joseph Hoffman, Philip Low, Samuel Lux, Vincent Marchesi, Jon Morrow, Jiri Palek, Eric Ponder, and Theodore Steck.

The non-nucleated erythrocyte is unique among human cells in that the plasma membrane, its only structural component, accounts for all of its diverse antigenic, transport, and mechanical characteristics. The discoid shape of the red cell evolves from the multilobulated reticulocyte during 48 hours of maturation first in the bone marrow and then in the circulation (Figure 1). An important and distinguishing feature of the discoid human red cell is its ability to undergo large passive deformations during repeated passage through the narrow capillaries of the microvasculature, with cross-sections one-third its own diameter, throughout its 120-day life span. The most dramatic manifestations of this property are seen during red cells’ transit in vivo from the splenic cords to the splenic sinus and during flow-induced deformation in vitro (Figure 1). Several elegant studies from the 1940s through the 1960s by, among others, John Dacie, Lawrence E. Young, Thomas Hale Ham, James H. Jandl, and William B. Castle, documented that splenic sequestration of abnormal red cells with reduced deformability accounts for the decreased life span and resulting hemolytic anemia in several red cell disorders.

Although a key role for cellular deformability in regulating red cell function and survival has long been recognized, quantitative characterization of deformability began in 1964 with the publication of the
seminal study by Rand and Burton, based on the micropipette aspiration technique to measure “stiffness” of the membrane. Subsequently, a variety of experimental strategies including rheoscopy, flow channels, ectacytometry, and optical trapping have been used to generate quantitative descriptions of the material characteristics that regulate the ability of red cells to undergo deformation, and of the role of various membrane components in this process. Paul LaCelle, Evan Evans, Robert Hochmuth, Dennis Discher, and several other investigators developed both quantitative measures of deformability and the essential theoretical underpinnings.

Studies during the past 3 decades on red cells from healthy people and from patients with various inherited red cell disorders have illuminated the molecular processes underlying normal and aberrant red cell membrane function. We will survey the current state of understanding of the structural organization of the normal red cell membrane and describe how anomalous structural organization accounts for altered features of membrane and cell in the known red cell membrane disorders.

Present view of structural organization of normal red cell membrane

The structural organization of the human red cell membrane enables it to undergo large reversible deformations while maintaining its structural integrity during its 4-month sojourn in the circulation. The red cell membrane exhibits unique material behavior. It is highly elastic (100-fold softer than a latex membrane of comparable thickness), rapidly responds to applied fluid stresses (time constants in the range of 100 milliseconds), and is stronger than steel in terms of structural resistance. While a normal red cell can deform with linear extensions of up to approximately 250%, a 3% to 4% increase in surface area results in cell lysis. Hence an important feature of induced red cell deformations, both in vitro and in vivo, is that they involve no significant change in membrane surface area. These unusual membrane material properties are the result of an evolution-driven “engineering” process resulting in a composite structure in which a plasma membrane envelope composed of cholesterol and phospholipids is anchored to a 2-dimensional elastic network of skeletal proteins through tethering sites on cytoplasmic domains of transmembrane proteins embedded in the lipid bilayer (Figure 2). Direct interaction of several skeletal proteins with the anionic phospholipids affords additional attachments of the skeletal network to the lipid bilayer.

Membrane lipids. The lipid bilayer is composed of equal proportions by weight of cholesterol and phospholipids. While cholesterol is thought to be distributed equally between the 2 leaflets, the 4 major phospholipids are asymmetrically disposed. Phosphatidylcholine and sphingomyelin are predominantly located in the outer monolayer, while most phosphatidylethanolamine and all phosphatidylserine (PS), together with the minor phosphoinositol constituents, are confined to the inner monolayer. Several
Membrane proteins. More than 50 transmembrane proteins of various abundance ranging from a few hundred to a million copies per red cell have been well characterized. A large fraction—some 25—of transmembrane proteins define the various blood group antigens. The membrane proteins exhibit diverse functional heterogeneity, serving as transport proteins, as adhesion proteins involved in interactions of red cells with other blood cells and endothelial cells, as signaling receptors, and other still undefined activities.

Membrane proteins with transport function include band 3 (anion transporter), aquaporin 1 (water transporter), Glut1 (glucose and L-dehydroascorbic acid transporter), Kidd antigen protein (urea transporter), RhAG (gas transporter, probably of carbon dioxide), Na⁺-K⁺-ATPase, Ca²⁺-ATPase, Na⁺-Cl⁻ cotransporter, Na⁺-Cl⁻ cotransporter, Na⁺-K⁺ cotransporter, K⁺-Cl⁻ cotransporter, and Gardos Channel. Membrane proteins with adhesive function include ICAM-4, which interacts with integrins and Lu, the laminin-binding protein. Detailed discussions of the defined as well as presumptive functions of various membrane proteins can be found in several excellent recent reviews.31-34 Of direct relevance to structural integrity of the membrane are 2 macromolecular complexes of membrane proteins, one ankyrin-based, and the other protein 4.1R-based. Band 3 and RhAG link the bilayer to the membrane skeleton through the interaction of their cytoplasmic domains with ankyrin, and glycoporphin C, XK, Rh, and Duffy through their interaction with protein 4.1R.35-39 Recent studies have indicated that 2 other members of the spectrin-actin-protein 4.1R junctional complex, adducin and dematin, can also serve as linking proteins by interacting with band 3 and Glut1, respectively.39,40 These membrane protein linkages with skeletal proteins may play a role in regulating cohesion between lipid bilayer and membrane skeleton and thus enable the red cell to maintain its favorable membrane surface area by preventing membrane vesiculation. In addition to its linking function, band 3 also assembles various glycolytic enzymes, the presumptive CO₂ transporter, and carbonic anhydrase into a macromolecular complex termed a “metabolon,” which may play a key role in regulating red cell metabolism and ion and gas transport function.41

Skeletal proteins. The principal protein constituents of the 2-dimensional-spectral-membrane-based skeletal network are α- and β-spectrin, actin, protein 4.1R, adducin, dematin, tropomyosin, and tropomodulin.16,42-44 A unique structural feature of the long filamentous spectrin is its large number of triple-helical repeats of 106 amino acids, 20 in α-spectrin and 16 in β-spectrin. First discovered in erythrocytes,45 these triple-helical bundles define a spectrin super family of proteins that includes dystrophin, actinin, and utrophin.46 α- and β-spectrin form an antiparallel heterodimer through strong lateral interaction between repeats 19 and 20 near the C-terminus of α-spectrin with repeats 1 and 2 near the N-terminus of β-spectrin. The 36 triple-helical repeats of spectrin are structurally heterogeneous in terms of their thermal stability.47 Spectrin tetramer, the major structural component of the 2-dimensional skeletal network, is formed by the lateral interaction of a solitary helix at the N-terminus of the α-chain from 1 dimer with 2 helices at the C-terminus of the β-chain from the other to create a stable triple-helical repeat (Figure 3).48,30 While the spectrin dimer-dimer interaction was long thought to be static, recent studies have revealed that dissociation of spectrin tetramers can be induced by membrane deformation.51 The other end of the 100 nm-long spectrin dimer forms a junctional complex with F-actin and protein 4.1R.28,52,53 While actin interacts weakly with N-terminus of β-spectrin; the interaction is greatly enhanced by protein 4.1R.54 The length of the actin filaments in the red cell membrane appears...
to be tightly regulated, probably by tropomyosin, and is made up of 14 to 16 actin monomers. Adducin and tropomodulin cap actin filaments at opposite ends, while the function of the actin bundling protein dematin in the junctional complex has yet to be fully defined. The spectrin dimer-dimer interaction and the spectrin-actin-protein 4.1R junctional complex are key regulators of membrane mechanical stability and play a critical role in preventing deformation-induced membrane fragmentation as the cell encounters high fluid shear stresses in circulation.

While much progress has indeed been made in our understanding of the structural organization of the various lipid and protein components of the normal red cell membrane, the current models are far from comprehensive and continue to evolve. A recent study using state-of-the-art proteomic approaches has generated a comprehensive catalog of red cell proteins and has identified more than 300 proteins including 105 integral membrane proteins. The current membrane models account for fewer than 15% of these molecules!

**Implications of the structural organization of various membrane components for normal red cell function**

In performing its primary function of oxygen delivery to the tissues, the red cell must absorb continuous mechanical punishment throughout its lifetime without structural deterioration. Extensive biophysical studies have identified 3 constitutive features as the primary regulators of the ability of the cell's capacity to undergo the necessary deformations. These are: (1) the geometry of the cell, particularly cell surface area to volume ratio; (2) the cytoplasmic viscosity determined by intracellular hemoglobin concentration; and (3) membrane deformability.

**Cell geometry.** The normal biconcave human red cell with a volume of 90 fl and surface area of 140 $\mu$m$^2$ possesses an excess surface area of 40% compared with a sphere of the same volume. Without excess surface area to volume ratio the cell cannot deform, for any deviation from the spherical state at constant volume implies an increase in surface area, which is forbidden by the lipid bilayer properties. Maintenance of membrane surface area is mediated by strong cohesion between the bilayer and the membrane skeleton that prevents membrane vesiculation, and by a mechanically stable spectrin-based membrane skeleton that prevents membrane breakup. Maintenance of cell volume is mediated by various membrane-associated ion transporters. Surface area loss as a result of membrane vesiculation due to decreased membrane cohesion, or cell fragmentation as a consequence of reduced membrane mechanical stability, as well as increase in cell volume due to defective ion transporters, will all compromise the ability of the cell to deform and lead to its premature removal from circulation.

The determinant of normal membrane cohesion is the system of "vertical" linkages between bilayer and membrane skeleton, formed by the interactions of the cytoplasmic domains of various membrane proteins with the spectrin-based skeletal network (Figure 2). Band 3 and RhAG provide such links by interacting with ankyrin, which in turn binds to $\beta$-spectrin. Protein 4.2 binds to both band 3 and ankyrin and can regulate the avidity of the interaction between band 3 and ankyrin. Glycophorin C, band 3, XK, Rh, and Duffy all bind to protein 4.1R, the third member of the ternary junctional complex with $\beta$-spectrin and actin. Recent studies reveal that band 3 and Glut1 can also link the bilayer to the skeleton through their interactions with the skeletal proteins, adducin and dematin, respectively. Of the various linkages documented, those mediated by band 3 appear to be the dominant determinant of membrane cohesion followed by the linkage mediated by RhAG.

A dominant regulator of membrane mechanical stability that enables the red cell to maintain its structural integrity through the vicissitudes of the circulation is the stability of the network. Network stability is critically dependent on both the avidity of interaction between spectrin dimers and the interactions that define the junctional complex at the distal ends of spectrin tetramers. While the spectrin tetramer is formed through the association of the solitary helix at the N-terminals of the $\alpha$-chain from one dimer with 2 helices at the C-terminals of the $\beta$-chain from the other to create a stable triple helical repeat, the adjacent triple helical repeats are needed for effective interaction between the dimers. The dimer-dimer interaction is not static, but dynamic in the sense that it opens reversibly under tensile forces imposed by deformation, and indeed flickers continuously between the open and closed states, as reflected by the relatively low association constant. This association constant is further strengthened by the binding of ankyrin to the $\beta$-chain. The principal constituents of the junctional complex at the end of spectrin tetramers are spectrin, F-actin, and protein 4.1R and avidity of this complex is another major determinant of membrane stability. At least 4 other proteins, tropomyosin, adducin, tropomodulin, and dematin, are present at lower copy numbers of 1 to 2 per junction. These proteins appear to stabilize the short actin filaments in the complex, but their influence on membrane stability appears modest compared with the primary constituents, spectrin, actin, and protein 4.1R.

While a major role for protein-protein interactions in regulating mechanical stability has been well documented, the contribution of...
protein-lipid interactions has received much less attention. Recent studies have determined that certain of the triple-helical repeats of both α- and β-spectrin bind PS (Figure 3) and this binding in situ increases membrane mechanical stability. It was inferred thatPIP2 binds to N-terminus of β-spectrin and decreases the propensity of spectrin to form a ternary complex with actin and protein 4.1R and may thereby decrease membrane mechanical stability.

Cytoplasmic viscosity and cell volume regulation. The ability of normal red cells to rapidly change their shape in response to fluid shear stresses is governed by cytoplasmic viscosity, which is determined by intracellular hemoglobin concentration. Non-nucleated mammalian red cells regulate their mean cell hemoglobin concentration within a very narrow range (30-35 g/dL) while their mean cell volumes range can vary widely (20-200 fL). While the mean cell hemoglobin concentration of normal human red cells is 33 g/dL, the distribution of hemoglobin concentrations in individual red cells in whole blood ranges from 27 to 37 g/dL. The viscosity of hemoglobin solution increases steeply starting at 37 g/dL. While hemoglobin viscosity is only 5 centipoise (cp) [5 times greater than water] at 27 g/dL increasing to 15 cp at 37 g/dL, it rises abruptly to 45 cp at 40 g/dL, up to 170 cp at 45 g/dL, and 650 cp at 50 g/dL. By tightly regulating hemoglobin concentration within a narrow range, red cells minimize the cytoplasmic viscous dissipation during cell deformation. Increases in hemoglobin concentrations above 37 g/dL markedly decrease the rate at which the cell recovers its initial shape after both extensional and bending deformations. Thus the ability of the cell to accommodate rapidly to a narrow capillary in the microcirculation will be compromised by increased cytoplasmic viscosity, and with it, its efficacy in tissue oxygen delivery. Of note, however, cell dehydration and the resultant increase in cytoplasmic viscosity only minimally affect red cell survival.

The ability of the red cell to regulate its hemoglobin concentration within narrow limits is critically dependent on its ability to control its volume. As red cell volume is primarily determined by total cation content, a host of transport proteins, which transport sodium and potassium across the membrane play a role in regulating cytoplasmic viscosity.

Membrane deformability. A unique feature of the normal red cell membrane is its high elasticity, which enables the cell to rapidly respond to applied fluid stresses in the circulation. While both theoretical and experimental evidence implicates a critical role for the spectrin-based skeletal network in general, and spectrin in particular, in determining membrane elasticity, the precise structural basis of the effect remains uncertain. An important structural feature of the long filamentous spectrin dimer is the succession of 36 spectrin repeats, 20 in α-spectrin and 16 in β-spectrin, which behave in part as independently folding units. A recent study revealed that the thermal stabilities of the 36 individual repeats, expressed in terms of the mid-point unfolding temperature, vary widely, ranging from 21 to 72°C. It was inferred that unfolding of the least stable spectrin repeats might affect membrane elasticity. A recent elegant study, based on labeling by fluorophores of sterically shielded cysteines of spectrin in intact membranes in conjunction with quantitative mass spectrometry, demonstrated that such cysteines indeed became available to the reagent when the cell was mechanically stressed, and that these groups were located in repeats of low stability. These findings support the concept that the unfolding and refolding of distinct spectrin repeats make a major contribution to the elasticity of the normal red cell membrane.

During senescence, normal red cells lose surface area and volume with little loss of hemoglobin and as a consequence cell density progressively increases during the red cell’s 120-day life span. Recent studies have documented that after extensive dehydration, normal red cells as well as sickle red cells, lose their ability to maintain their cation homeostasis and cell volume increases. It has been suggested that this cell population may represent the “end-stage” normal red cells destined to be eliminated from cell circulation. Alternate postulated mechanisms for removal of senescence normal red cells include phagocytosis of senescent cells by macrophages either through recognition of clustered band 3 or PS exposure on the outer monolayer of this cell population. While it is difficult to assign the relative contribution of each of the various documented cellular changes to removal of senescent red cells from circulation, it is likely that all of them play some role in the process.

Thus, our understanding of the mechanistic basis for normal red cell deformability appears reasonably well developed and comprehensive. We may anticipate that ongoing structural work aimed at determining the structures of major red cell membrane proteins at atomic resolution will provide more refined insights into normal red cell membrane structure and function.

Mechanistic basis for altered membrane and cell function in inherited red cell disorders

Inherited red cell disorders with altered membrane and cell function can be broadly divided into 2 classes. (1) Altered function due to mutations in various membrane or skeletal proteins. Such conditions include hereditary spherocytosis (HS), hereditary elliptocytosis (HE), hereditary ovalocytosis, and hereditary stomatocytosis. (2) Altered function due to secondary effects on the membrane resulting from mutations in globin genes; these conditions include sickle cell disease, Hb SC disease, Hb CC disease, unstable hemoglobins and thalassemias. A consistent feature of red cells in all of these disorders is decreased cell deformability. We will discuss briefly these various inherited disorders based on the dominant cellular features responsible for impaired cell deformability, and on our current understanding of the molecular and mechanistic basis for the documented cellular alterations.

Altered cell geometry. Decreased cell surface area to volume ratio and consequent increased cell sphericity is a distinguishing feature of red cells in HS, HE, and overhydrated hereditary stomatocytosis (OHS). In the case of HS and HE, increased sphericity is the result of loss of cell surface area while in the case of OHS it results from increased cell volume.

HS is a common inherited hemolytic anemia affecting all ethnic groups, but is particularly common in people of northern European ancestry (1 in 3000). HS is usually associated with dominant inheritance (75%), although nondominant inheritance (25%) is not uncommon. “Typical” HS is characterized by evidence of hemolytic anemia, jaundice, splenomegaly, reticulocytosis, gallstones, and the presence of spherocytes on peripheral blood smears. However, the clinical manifestations of HS are highly variable ranging from mild to very severe anemia. A common feature of all forms of HS is loss of membrane surface area and resultant change in cell shape from discocytes to stomatocytes to spherocytes (Figure 4). As red cells with decreased membrane surface area are unable to effectively traverse the spleen, they are sequestered and removed from circulation by the spleen. Importantly, the severity of anemia is related to extent of decrease in membrane surface area. Splenectomy reduces the severity of anemia by increasing the survival of spherocytic red cells. The mechanistic basis for membrane loss in HS is decreased membrane cohesion due to a
reduced number of “vertical” linkages between bilayer and membrane skeleton. Reduced anchoring results from deficiencies of transmembrane proteins that link the bilayer to the membrane skeleton (band 3 or RhAG), or of anchoring proteins (ankyrin or protein 4.2) or of spectrin due to too little membrane skeleton available for linkage (Figure 5). Thus, HS is the result of defects in genes encoding any of the protein components involved in vertical linkages between skeletal network and the membrane.

HE is a relatively common, clinically and genetically heterogeneous disorder, characterized by presence of elliptically shaped red cells on peripheral blood smear. HE has a worldwide distribution, but is more common in malaria endemic regions with prevalence approaching 2% in West Africa. Inheritance of HE is autosomal dominant. The overwhelming majority of HE is asymptomatic but approximately 10% of patients have moderate to severe anemia including a few reported cases of hydrops fetalis. Typically, people heterozygous for an elliptocytic variant are asymptomatic while people with homozygosity or compound heterozygosity for HE variants experience mild to severe anemia, including the severe variant hereditary pyropoikilocytosis. A common feature of all forms of HE is a mechanically unstable membrane that results in progressive transformation of cell shape from discocyte to ellipsoidocyte with time in the circulation (Figure 4), and in severe cases, membrane fragmentation and cells with reduced membrane surface area (Figure 4). Importantly, the severity of the disease is related to extent of decrease in membrane mechanical stability and resultant loss of membrane surface area. While very few patients with HE need splenectomy, as with HS, splenectomy reduces the severity of anemia in patients with severe anemia by increasing the circulatory life span of fragmented red cells. The mechanistic basis for decreased membrane mechanical stability in HE is weakened “horizontal” linkages in membrane skeleton due either to defective spectrin dimer-dimer interaction or a defective spectrin-actin-protein 4.1R junctional complex. Thus, HE is the result of defects in genes encoding for α-spectrin, β-spectrin or protein 4.1R, all of which are involved in “horizontal” linkages in the skeletal network (Figure 3).

OHS is a rare disorder characterized by presence of large numbers of stomatocytes on blood smears in association with moderately severe to severe anemia. Inheritance pattern of OHS is autosomal dominant. The distinctive feature of red cells is their increased sphericity due to increased cell volume without concomitant increase in membrane surface area. Stomatocytes with increased sphericity are sequestered by the spleen. However, while splenectomy is highly beneficial in the management of HS and HE patients, it is contraindicated in OHS, because it leads to increased risk of venous thromboembolic complications. The mechanistic basis for increased volume is the cell’s inability to regulate its cation homeostasis. Stomatocytes exhibit a marked increase in total cation content due to elevation of intracellular sodium, resulting in increased cell volume. The molecular basis for OHS has not yet been defined.

**Increased cytoplasmic viscosity.** Red cell dehydration and hence increased cytoplasmic viscosity is a feature of the inherited
red cell membrane disorder, dehydrated hereditary stomatocytosis (xerocytosis; DHS). Inheritance of DHS is autosomal dominant. The distinctive feature of DHS is the increased MCHC of red cells as a consequence of decreased total cation content and loss of cell water. However, in contrast to significantly compromised survival of hydrated cells of OHS, cell dehydration has only a marginal effect on survival of DHS red cells. DHS is therefore associated with well-compensated anemia with only a mild to moderately increased membrane rigidity. The mechanism for decreased volume is the red cell’s inability to regulate cation homeostasis resulting in decreased total cation content due to decreased intracellular potassium. The molecular basis for DHS is, like OHS, still unknown.

Failure to regulate cell volume with ensuing cell dehydration has long been recognized as a feature of red cells in sickle cell disease, Hb SC disease and Hb CC disease. However, in contrast to DHS, in which cell dehydration is the only dominant feature, red cells in hemoglobinopathies are not only dehydrated but also exhibit significant membrane alterations, including increased membrane rigidity. The membrane disturbances are the result of oxidation-induced structural reorganization. While the Gardos channel and K-Cl cotransporter have been implicated in an altered state of hydration of red cells in hemoglobinopathies, the mechanistic understanding of disordered volume regulation in these disorders is far from complete.

**Altered membrane deformability.** Decreased membrane deformability is a distinguishing feature of red cells in hereditary ovalocytosis. Ovalocytosis is very common in Southeast Asia where in malaria endemic areas its prevalence ranges from 5% to 25%. The condition is characterized by the presence of oval-shaped red cells with 1 or 2 transverse ridges or a longitudinal slit on blood smears. Inheritance of ovalocytosis is autosomal dominant and to date only heterozygotes have been identified in regions of high incidence implying that homozygosity is embryonic lethal. Membranes of ovalocytes are 4 to 8 times less elastic than normal membranes as assessed by ektacytometry and by micropipette aspiration. In terms of clinical manifestations, it is important to note that despite a marked increase in membrane rigidity most affected people experience minimal hemolysis. In all cases of ovalocytosis studied to date, only 1 mutation has been identified: a genomic deletion of 27 bp encoding amino acids 400 to 408 of band 3. This hereditary ovalocytosis is unique among red cell membrane disorders in that the same mutation in a single gene is responsible for the morphologic phenotype. Although several hypotheses have been proposed regarding how a mutation in band 3 could lead to a marked increase in membrane rigidity, the mechanism of the effect has yet to be established.

Our current understanding of molecular basis for inherited red cell membrane disorders, hereditary spherocytosis, hereditary elliptocytosis and hereditary ovalocytosis is wide-ranging, yet there are still cases where the molecular and genetic pathobiology are unknown. In contrast, the molecular and genetic basis for red cell disorders due to membrane transport defects such as the dehydrated and overhydrated hereditary stomatocytosis syndromes are largely unknown, remaining a continuing challenge.

**Future**

Studies on the red cell membrane have shed much new, often unexpected light on structure and function of plasma membranes generally, and have, we would argue justified the claims, so often made, of its broad paradigmatic value. There can, at the same time, be no doubt as to the advances in hematology that have flowed from this work, for now we have a far-reaching understanding of the causes of membrane defects in genetic diseases. As result of these many advances, it might be felt that we have a complete understanding of all aspects of red cell membrane structure and function and very few new and novel insights will be forthcoming from future endeavors. This narrow point of view ignores the great continued potential of red cell research as revealed by reports published in 2008 that outlined unexpected new and novel insights into red cell membrane structure and function. Furthermore, just a few examples of the great unexhausted potential of red cell research concern: nature and function of macromolecular complexes in the membrane; dynamic regulation of skeletal protein and membrane protein complexes by phosphorylation, and other posttranslational modifications, and by phosphoinositides; dynamics of assembly and function of the membrane microdomains, for which there is now strong evidence; molecular basis for cell volume regulation; detailed clarification of the sequence of events in regulated assembly of plasma membrane, and its associated protein skeleton; regulation of membrane properties by biodynamic ligands, including adrenergic agents, nucleotides and hormones; the nature of membrane perturbations in diabetes and many other diseases; the problems presented by membrane damage in sickle cell and related diseases; interaction of red cells with endothelial surfaces in health and disease; and of course the many problems, basic and practical, presented by malaria. While it is clear these or related topics are currently under intensive investigation in many other cell types, because of its simplicity and elegance, it is very likely that the red cell will continue to provide deep insights into these complex issues.

**Acknowledgments**

We very much regret that many important papers from several very talented people who made significant contributions to red cell research could not be cited or discussed due to strict space limitations. We sincerely apologize for this omission. We gratefully acknowledge the camaraderie and friendship of a very large number of investigators associated with red cell research who over the years have enriched our careers in red cell research. This work is dedicated to the memory of Sally Marchesi, a dear friend and a wonderful colleague.

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**Authorship**

Contribution: N.M. and P.G.G. cowrote the paper.

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I arrived in the United States in 1968 with a degree in chemical engineering to begin graduate studies at the School of Engineering at Washington University, St Louis. It was there it all started—and not the way I had envisioned. My thesis supervisor, Robert Hochmuth, introduced me to the field of red cell research. It became my first professional love and I have stayed faithful to it ever since. After completing my DSc, I spent 3 formative years in Paris working with Marcel Bessis. Using the ektacytometer that we developed together, we found interesting differences in the deformability of red cells in a variety of disorders. Realizing that my engineering background had not equipped me for a broad understanding of the pathophysiologic implications of the phenomenon I was studying, in 1976 I began to look for collaborators with expertise in hematology, biochemistry, biophysics, cell biology, molecular biology, and genetics. This journey, spanning the past 3 decades, has been extraordinarily rewarding both professionally and personally. During these years I have had the privilege of working closely with an array of wonderful colleagues who have become close friends. I would like especially to acknowledge my collaborators of many years, Xiuli An, Joel Ann Chasis, Margaret Clark, John Conboy, Ross Coppel, Dennis Discher, Evan Evans, Gil Tchernia, and Yuichi Takakuwa, and my many good friends, always generous with their support and help, including Peter Agre, David Anstee, Anthony Baines, Jane Barker, Vann Bennett, David Bodine, Pat Gallagher, Walter Gratzer, Kasturi Haldar, Bob Hebbel, Joe Hoffman, Phil Low, Sam Lux, Jon Morrow, Luanne Peters, and Stan Schrier. A happy feature of the red cell field is its collegiality, and it has been a particular pleasure to associate with so many excellent scientists, whose friendship and support has meant so much to me. My fascination with red cells is still unabated after devoting the past 4 decades of my professional life to it.

Patrick G. Gallagher

The little boy with sickle cell disease in Room 316A was hospitalized with the diagnosis of “impending crisis.” “What did his peripheral smear show?” Dr Beatrice Lampkin asked me on my fourth day of internship at Cincinnati Children’s Hospital. “Let’s go look.” Listening to her describe the morphology and its significance and discuss the clinical manifestations and pathobiology of his disease and what it meant to this boy on a daily basis was a life lesson in hematology. People in the field of hematology care, they are thoughtful, they want to understand how things work, and they want to improve people’s lives. I also learned another lesson of hematology: you can learn a lot more sitting around a microscope with a group of hematologists than just what’s on the peripheral smear. After residency, I moved to Yale University for fellowship training, where I began training in the laboratory of Dr Bernard Forget. The research training I received from Bernie was priceless, going from experimental conception, design, execution, and interpretation to presentation. Group meetings in the Hematology Division at Yale at that time were exciting, with presentations on hemoglobin switching, gene expression, receptor signaling, gene structure and function, membrane biology, and inherited and acquired human disease, which instilled an interest in a broad range of topics that continues today. I also greatly benefitted from William Tse’s laboratory tutelage, for which I am most grateful. I think of my experiences in hematology as vignettes. Bernie Forget coaching the art of peer review: “First, always say something nice.” Watching the influence of Edward Benz’s smile and encouraging words on the people around him. Sitting in an airport listening to Peter Agre comparing academics to drug addiction, explaining that no matter the ups and downs, we just love academics and can’t stop. Introducing myself to Samuel Lux when he was visiting New Haven; he replied, “I know who you are. Are you putting in a K?” Meeting my collaborator and friend David Bodine at a Gordon Conference reception to discuss erythroid-specific promoters, and the Red Sox. David Nathan rescuing me from a tough questioner at a Children’s Health Research Center meeting. Reading the name badge of the kind man who came over to talk to me about my poster at ASH while I was a fellow: “Mohanad Narla” I read. Mohan is now a great friend and colleague and I am proud to coauthor this review with him. I have found the field of hematology to be exciting, collegial, and compassionate. I look forward to many, many more years.
Red cell membrane: past, present, and future

Narla Mohandas and Patrick G. Gallagher