bjh review

Disorders of red cell membrane

Xiuli An and Narla Mohandas

Red Cell Physiology Laboratory, New York Blood Center, New York, NY, USA

Summary

Studies during the last three decades have enabled the development of detailed molecular insights into the structural basis of altered function in various inherited red cell membrane disorders. This review highlights our current understanding of molecular and mechanistic insights into various inherited red cell membrane disorders involving either altered membrane structural organization (hereditary spherocytosis, hereditary elliptocytosis and hereditary ovalocytosis) or altered membrane transport function (hereditary stomatocytosis). The molecular basis for the vast majority of cases of hereditary spherocytosis, elliptocytosis and ovalocytosis have been fully defined while little progress has been made in defining the molecular basis for hereditary stomatocytosis. Mutations in a number of distinct genes account for hereditary spherocytosis and elliptocytosis, while a single genetic defect accounts for all cases of hereditary ovalocytosis. Based on these molecular insights, a comprehensive understanding of the structural basis for altered membrane function has been developed. Loss of vertical linkage between membrane skeleton and lipid bilayer leads to membrane loss in hereditary spherocytosis, while weakening of lateral linkages between skeletal proteins leads to membrane fragmentation and surface area loss in hereditary elliptocytosis. Importantly, the severity of anaemia in both these disorders is directly related to extent of membrane surface area loss. Splenectomy results in amelioration of anaemia.

Keywords: red cells, hereditary spherocytosis, hereditary elliptocytosis, hereditary ovalocytosis, hereditary stomatocytosis.

The human red blood cell is characterized by its discoid shape and its ability to undergo extensive passive deformation during repeated passage through the narrow capillaries of the microvasculature during its 120-d life span in the circulation. The most dramatic manifestation of red cell deformation is seen during its transit from the splenic cords to the splenic sinus (Fig 1). Loss of cellular deformability not only compromises the ability of the red cell to optimally perform its function of oxygen delivery to the tissues but can also lead to its premature removal from circulation by the spleen. These important features of human red cells were first recognized by Anton van Leeuwenhoek in 1675 when he stated that ''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''. Subsequent studies have validated this truly remarkable and prescient observation made more than three centuries ago.

Extensive studies during the last three decades on red cells from normal individuals as well from individuals with various inherited red cell membrane disorders using biochemical, biophysical and molecular biological approaches have enabled the development of detailed molecular insights into structural basis for normal red cell membrane function and for altered function in various inherited red cell membrane disorders (Mohandas *et al*, 1983; Mohandas & Chasis, 1993; Mohandas & Evans, 1994; Discher, 2000; Delaunay, 2007). This review highlights our current understanding of the molecular and structural basis for various red cell membrane disorders and discusses how these new insights have contributed to the improved understanding of their pathophysiology and also of differences in the severity of clinical manifestations amongst affected individuals.

Structural organization of normal red cell membrane

The structural organization of the red cell membrane is responsible for endowing the cell with its ability to undergo extensive reversible deformations while maintaining its structural integrity during its long lifespan in circulation. The red cell membrane exhibits complex material behaviour. It is highly elastic (100-fold softer than latex membrane of comparable thickness), responds rapidly to applied stresses (time constants in the range of 100 ms) and is capable of undergoing large membrane extensions without fragmentation at constant membrane surface area. These unusual membrane material properties are the consequence of slow evolution-driven ''engineering'', which evolved a composite structure in which a plasma membrane envelope composed of amphiphilic lipid molecules is anchored to a two dimensional elastic network of skeletal proteins through tethering sites (transmembrane

Correspondence: Dr Narla Mohandas, Red Cell Physiology Laboratory, New York Blood Center, 310 East 67th Street, New York, NY 10065, USA. E-Mail: MNarla@NYBloodcenter.org

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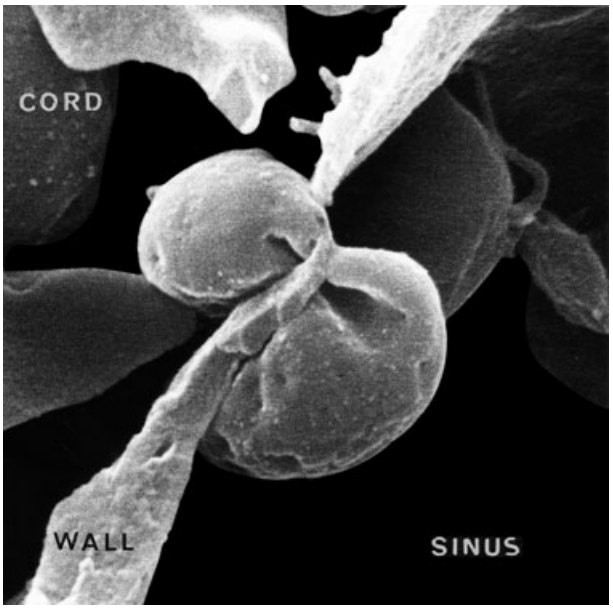

Fig 1. A reticulocyte traversing from the splenic cord to splenic sinus. Note the marked deformation the cell undergoes during its passage through the narrow endothelial slit separating the cord from the sinus. Original magnification ×15 000.

proteins) embedded in the lipid bilayer (Fig 2) (Mohandas & Evans, 1994; Mohandas & Reid, 2006). There is also evidence suggesting direct interaction of skeletal proteins with the anionic phospholipids (Rybicki *et al*, 1988; An *et al*, 2004).

The lipid bilayer is composed of equivalent amounts of cholesterol and phospholipids. A significant feature of bilayer lipid organization is that various phospholipids are asymmetrically distributed. Phosphatidylcholine and sphingomyelin are predominantly localized in the outer monolayer of the lipid bilayer while most of phosphatidylethanolamine and all of phosphatidylserine (PS) and phosphoinositides are localized in the inner monolayer (Zwaal & Schroit, 1997). A number of proteins, such as scramblases and flippases have been postulated to be involved in maintaining asymmetric distribution of phospholipids(Comfurius *et al*, 1996; Tang *et al*, 1996; Zwaal & Schroit, 1997; Sims & Wiedmer, 2001) but their molecular identity has proven to be elusive. The asymmetric distribution of phospholipids is of functional relevance since both PS and phopsphoinositides interact with skeletal proteins spectrin and protein 4.1R, thereby anchoring the skeletal network to the bilayer. Recent studies have documented that spectrin binding to PS enhances membrane mechanical stability (Manno *et al*, 2002) while phosphoinositide binding to 4.1R regulates its interaction with transmembrane proteins band 3 and glycophorin C

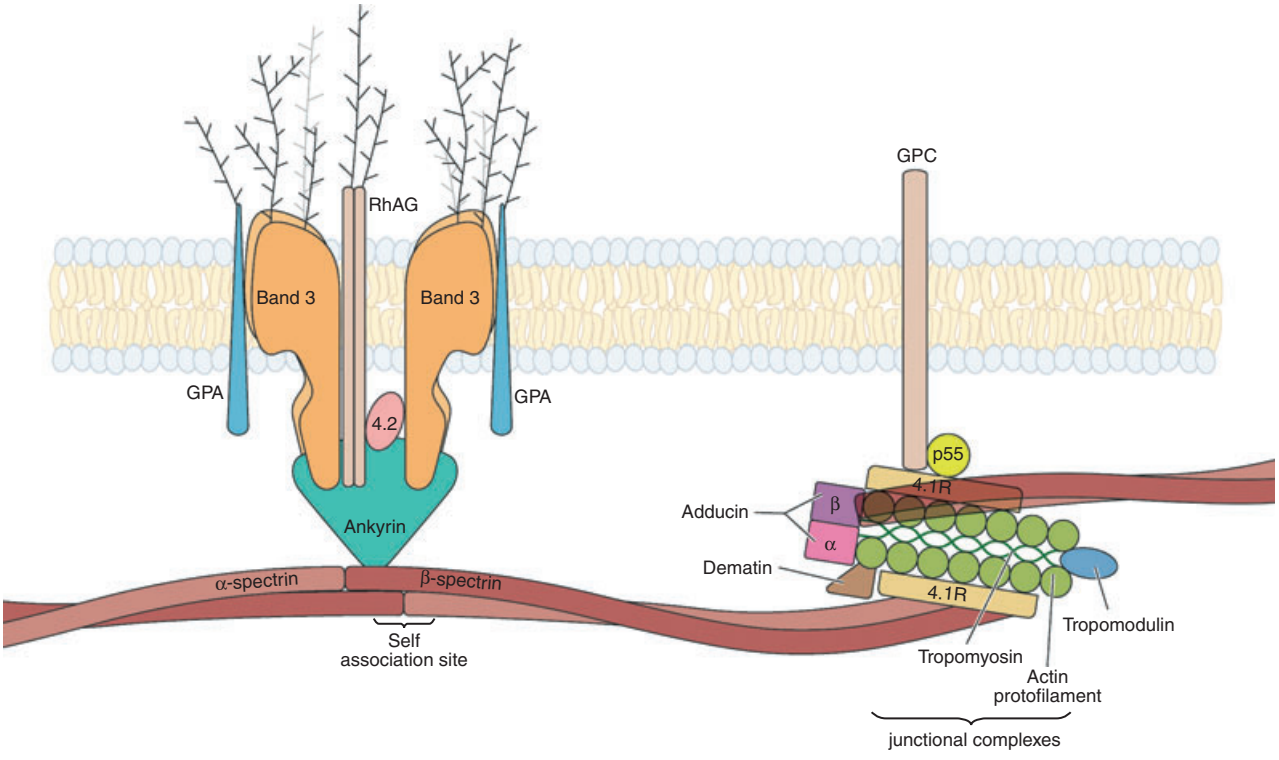

Fig 2. A schematic representation of the red cell membrane. The membrane is a composite structure in which a plasma membrane envelope composed of amphiphilic lipid molecules is anchored to a two dimensional elastic network of skeletal proteins through tethering sites (transmembrane proteins) embedded in the lipid bilayer. Of particular relevance to the present review is the vertical interaction involving cytoplasmic domains of band 3 and RhAG, ankyrin, protein 4.2 and β-spectrin and the lateral linkages in the membrane skeletal network involving spectrin self-association and spectrin-actin junctional complex.

(An *et al*, 2006a). Furthermore, loss of lipid asymmetry resulting in translocation of PS to the outer monolayer leads to recognition and phagocytosis of the aberrant red cells by macrophages. This recognition mechanism is thought to play a role in premature destruction of sickle and thalassemic red cells (Wood *et al*, 1996; Kuypers *et al*, 1998; Yasin *et al*, 2003).

More than 50 transmembrane proteins of varying abundance, ranging from a few hundred to approximately one million copies per red cell, have been identified. A large fraction of these transmembrane proteins, approximately 25, specify the various blood group antigens (Reid & Mohandas, 2004). Transmembrane proteins exhibit diverse functional heterogeneity serving as cation, water and urea transporters, as adhesive proteins involved in interactions of red cells with other blood cells and endothelial cells, in cell signalling events and in some yet-to-be defined functions (Reid & Mohandas, 2004). Of direct relevance to structural integrity of the membrane are membrane proteins, band 3, glycophrin C and RhAG that link the bilayer to the spectrin based membrane skeleton. Band 3 and RhAG link the bilyer to the membrane skeleton through the interaction of their cytoplasmic domains with ankyrin (Bennett, 1983; Nicolas *et al*, 2003) while glycophorin C links through its interaction with protein 4.1R (Reid *et al*, 1990; Marfatia *et al*, 1994, 1995). The linkages play a key role in regulating cohesion between bilayer and membrane skeleton. Loss of linkages results in lipid loss and decreased membrane surface area, thus compromising the ability of red cells to deform in circulation. Loss of membrane surface area is a key contributor to decreased cell survival in various red cell disorders (Mohandas & Chasis, 1993; Walensky *et al*, 2003). In contrast, increased number of linkages leads to increased membrane cohesion and increased membrane rigidity(Mohandas *et al*, 1992; Knowles *et al*, 1994).

The principal protein constituents of membrane skeletal network are α- and β-spectrin, actin, protein 4.1R, adducin, dematin, tropomyosin and tropomodulin (Bennett, 1989; Bennett & Baines, 2001; Mohandas & Reid, 2006). A unique structural feature of the long filamentous spectrin is the triple helical repeats of 106 amino acids, 21 in α-spectrin and 15 in β-spectrin (Speicher & Marchesi, 1984; Yan *et al*, 1993). α- and β-spectrin form an anti-parallel heterodimer through strong lateral interaction between repeats 20–21 near the C-terminus of α-spectrin with repeats 1–2 near the N-terminus of β-spectrin (Ursitti *et al*, 1996). The 36 triple helical repeats of spectrin are structurally heterogeneous and deformation-induced unfolding of specific repeats appears to account for membrane elasticity (An *et al*, 2006b; Johnson *et al*, 2007). Spectrin tetramer, the major structural component of the two-dimension skeletal network is formed by the lateral interaction of the single helical repeat at the N-terminus of α-spectrin of one spectrin dimer with a two helical repeat at the C-terminus of β-spectrin of the second dimer (DeSilva *et al*, 1992; Speicher *et al*, 1993). The spectrin dimer-dimer interaction is dynamically regulated in intact red cell membranes and the loss of avidity of the interaction leads to decreased membrane mechanical stability (Liu & Palek, 1980; An *et al*, 2002). The other end of the 100 nm long spectrin dimer forms a junctional complex with actin and protein 4.1R (Ungewickell *et al*, 1979; Karinch *et al*, 1990; An *et al*, 2005). The length of the actin filament in the red cell membrane is tightly regulated by tropomyosin and is made up of 14–16 actin monomers (Fowler, 1996). While actin interacts weakly with the N-terminus of β-spectrin; the interaction is significantly enhanced by protein 4.1R (Ohanian *et al*, 1984). The ternary complex of sprectrin-actin-protein 4.1R is a critical regulator of membrane mechanical integrity.

Based on detailed analysis of normal and variously modified red cells, the following concepts regarding the structural basis for membrane material properties have evolved. The unfolding and refolding of distinct spectrin repeats accounts for the remarkable elasticity of the normal red cell membrane and hindrance of these unfolding results in increased membrane rigidity. The vertical linkages between bilayer and membrane skeleton play a critical role in maintaining membrane cohesion while the lateral linkages between spectrin dimers and between spectrin-actin-protein 4.1R are the dominant regulators of membrane mechanical stability. Maintenance of both membrane cohesion and membrane mechanical stability is critical for the red cell to maintain its redundant surface area that is critical for it to undergo extensive deformations. Mutations in various membrane and skeletal proteins that result in either decreased membrane cohesion or membrane mechanical stability lead to membrane surface area loss, decreased red cell life span and resultant anaemia in a variety of inherited red cell membrane disorders.

Hereditary spherocytosis

Hereditary spherocytosis (HS) is a common inherited haemolytic anaemia that occurs in all racial groups and is particularly common in individuals of northern European ancestry, affecting approximately one person in 3000 (Gallagher & Lux, 2003; Eber & Lux, 2004; Gallagher & Jarolim, 2005). The clinical manifestations of HS vary widely. ''Typical'' HS consists of evidence of haemolysis with anaemia, jaundice, reticulocytosis, gallstones, splenomegaly as well as spherocytes with reduced membrane surface area on peripheral blood smear. HS is most commonly associated with dominant inheritance (75%), although non-dominant and recessive inheritance (25%) have been described. Disease severity of HS is classified as mild, moderate, moderately severe and severe according to a few common haematological parameters including haemoglobin and reticulocyte counts. About 20% of HS patients have mild HS with compensated haemolysis, minimal spherocytosis, near normal haemoglobin levels, slight reticulocytosis (<6%), and mild splemenomegaly. Many of these individuals escape detection until adulthood when complications related to chronic haemolysis, such as gallstones, occur. Moderate HS is the largest group of HS patients comprising about 60% of cases. The haemoglobin level in this

Table I. Mutations in inherited human red cell membrane disorders.

	%
Spherocytosis	
Ankyrin	50–60
Spectrin	20
Band 3	15–20
Protein 4.2	<5
Rh complex	<1
No defect identified	10
Elliptocytosis	
α-spectrin	65
β-spectrin	30
Protein 4.1	5
Ovalocytosis	
Band 3	100

group is between 80–110 g/l and reticulocytes in most cases are >8%. The incidence of palpable splenomegaly varies, from about 50% in young children to 75–95% in older children and adults. A small group of patients (approximately 10%) have moderately severe HS with low haemoglobin values (60–80 g/l), reticulocytosis (>15%) and intermittent need for transfusions. Approximately 3–5% have severe HS with life-threatening anaemia requiring regular transfusions. They almost always have recessive HS.

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. 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 the disease is directly related to extent of decrease in membrane surface area. Since splenic sequestration is the dominant mechanism responsible for reduced life span of spherocytes with decreased membrane surface area, splenectomy significantly reduces the severity of anaemia by increasing the circulatory life span of spherocytes.

The mechanistic basis for membrane loss in HS is the result of the defective anchoring of the skeletal network to the membrane as a result of defects in several proteins (Table I). Reduced anchoring as a result of deficiencies of transmembrane proteins that link the bilayer to membrane skeleton (band 3 or RhAG), or of anchoring proteins (ankyrin or protein 4.2) or of spectrin leads to decreased membrane cohesion with resultant loss of membrane surface area. Thus, HS is the result of defects in any of the protein components involved in vertical linkages between skeletal network and the membrane (Fig 3).

Ankyrin deficiency is the most common cause of HS in Northern European populations accounting for approximately 50–60% of cases but it is found in only 5–10% of HS cases in Japan (Eber *et al*, 1996; Eber & Lux, 2004). Ankyrin mutations cause both dominant and recessive HS and patients with ankyrin defects have prominent spherocytosis with clinical severity ranging from mild to severe depending on the extent of

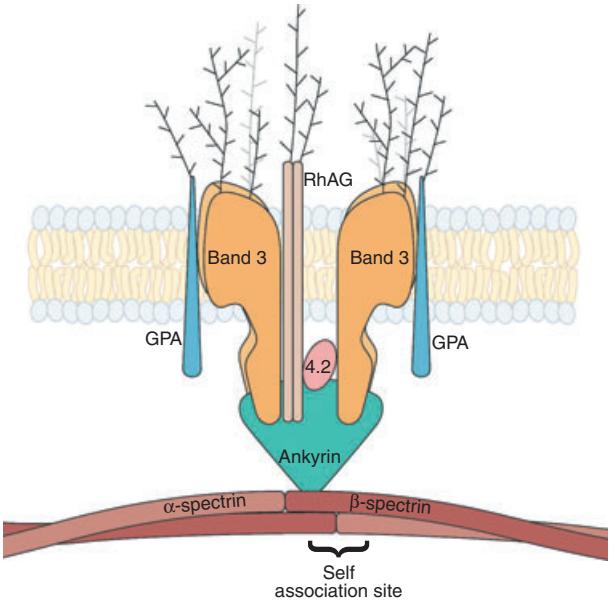

Fig 3. Membrane defects in HS affect the "vertical" interactions anchoring the membrane skeleton to the lipid bilayer. Deficiency in any one of the protein components (Band 3, RhAG, ankyrin, protein 4.2 and spectrin) involved in the anchoring process leads to HS.

membrane loss. Since ankyrin links β-spectrin to band 3, it is not surprising that ankyrin deficiency leads to a proportional and secondary decrease in spectrin assembly on the membrane despite normal spectrin synthesis. Isolated spectrin deficiency due to mutations in either *SPTA1* or *SPTB* account for 20% of HS cases (Eber & Lux, 2004). Patients with β-spectrin defects typically have mild to moderately severe HS while patients with α-spectrin suffer from severe HS. Band 3 deficiency is found in approximately 15–20% of HS patients, presenting with a phenotype of a mild to moderate anaemia although a few cases of severe HS have also been reported (Jarolim *et al*, 1996; Dhermy *et al*, 1997; Tanner, 2002). Band 3 mutations are dominantly inherited. Mushroom-shaped or "pincered" red cells in addition to spherocytes may be seen on peripheral blood smear. Recessive HS due to homozygous mutations in *EPB42* is common in Japan but is rare in other populations accounting for less than 5% of HS cases (Bouhassira *et al*, 1992; Yawata *et al*, 2000). In these cases, an almost complete deficiency of protein 4.2 is noted. Ovalocytes and stomatocytes predominate with rare spherocytes in peripheral blood smears. Rh deficiency associated with absent or markedly reduced RhAG expression is associated with mild to moderate haemolytic anaemia associated with presence of stomatocytes and spherocytes on peripheral blood smear (Ballas *et al*, 1984; Cartron, 1999). Rh deficiency accounts for less than 1% of HS cases. Molecular basis for approximately 10% of HS cases has yet to be defined.

Hereditary elliptocytosis

Hereditary elliptocytosis (HE) is a relatively common, clinically and genetically heterogeneous disorder characterised by

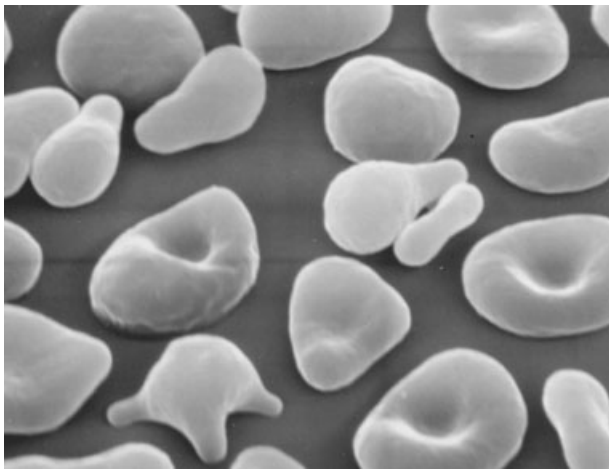

Fig 4. Abnormal red cell morphology in HE with severe anaemia. Note the presence of elliptocytes, poikilocytes and fragmented red cells in peripheral blood. Original magnification ×10 000.

the presence of elliptically-shaped red cells on peripheral blood smear (Gallagher, 2004). 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 clinical presentation of HE is heterogeneous ranging from asymptomatic carrier to severe, life-threatening anaemia with a few reported cases of hydrops fetalis. The overwhelming majority of HE is asymptomatic but approximately 10% of patients have moderate to severe anaemia. Typically, individuals heterozygous for an elliptocytic variant have asymptomatic elliptocytosis while individuals with homozygous or compound heterozygous for HE variants experience mild to severe anaemia. Poikilocytes and fragmented red cells in addition to elliptocytes is a feature of red cell morphology in HE cases with severe anaemia.

A common feature of all forms of HE is a mechanically unstable membrane, which results in progressive transformation of cell shape from discocytes to elliptocytes during circulation and, in severe cases, to membrane fragmentation and generation of cells with reduced membrane surface area and abnormal morphology (Fig 4). Red cells with decreased membrane surface area as result of membrane fragmentation are sequestered and removed from circulation by the spleen. Importantly, the severity of the disease is directly related to the extent of the decrease in membrane mechanical stability. Since splenic sequestration is the dominant mechanism responsible for the reduced life span of fragmented red cells with decreased membrane surface area, splenectomy significantly reduces the severity of the anaemia by increasing the circulatory life span of fragmented red cells.

The mechanistic basis for decreased membrane mechanical stability in HE is weakened lateral linkages in membrane skeleton due to either defective spectrin dimer-dimer interaction or a defective spectrin-actin-protein 4.1R junctional complex (Fig 5). Reduced avidity of lateral interactions due to defects in α-spectrin, β-spectrin or protein 4.1R lead to decreased membrane mechanical stability (Table I). Thus, HE is the result of defects in any of the protein components that are involved in lateral linkages in the skeletal network (Fig 5).

Mutations in α-spectrin are the most common cause of HE accounting for approximately 65% of the cases. Missense mutations in the amino-terminal region of α-spectrin that is involved in spectrin dimer-dimer interaction are the most frequent while mutations in other parts of α-spectrin that are not directly involved in spectrin self-association have also been identified (Delaunay & Dhermy, 1993; Gallagher, 2004). One common missense mutation in African-Americans is Arg28His and patients homozygous for this mutation have severe haemolytic anaemia (Coetzer & Palek, 1986). Mutations in β-spectrin account for 30% of HE cases (Gallagher, 2004). Point mutations as well as truncations in carboxyl-terminus of β-spectrin that impair spectrin self-association have been identified. Heterozygous mutations in this region of β-spectrin are associated with variable clinical severity while in the homozygous state they are fatal or near-fatal. Importantly, in almost all cases of spectrin mutations associated with HE the degree of spectrin self-association disruption correlates with clinical severity – the larger the degree of disruption, the more severe the clinical phenotype. Quantitative deficiency of protein 4.1R as well as qualitative defects in protein 4.1R account for 5% of HE cases (Tchernia *et al*, 1981; Marchesi *et al*, 1990; Gallagher, 2004). In all these instances, the defects in protein 4.1R lead to a weakened

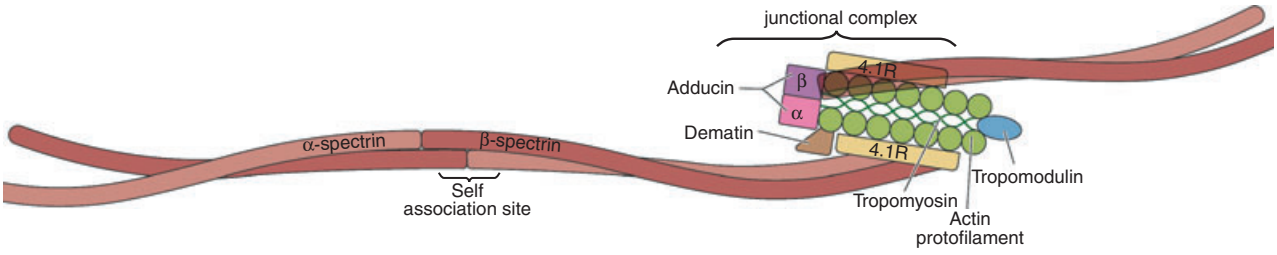

Fig 5. Membrane defects in HE affect the "lateral" interactions in the membrane skeleton. Weakening of lateral interactions due to defects in either α-spectrin or β-spectrin, resulting in reduced avidity to spectrin dimer-dimer interaction or defects in protein 4.1R, leading to reduced avidity of spectrin-actin-protein 4.1R junctional complex lead to HE.

spectrin-actin junctional complex leading to decreased membrane mechanical stability.

Hereditary pyropoikilocytes (HPP), when originally described, was thought to be a distinct entity due to increased thermal sensitivity of the red cells and the unusual morphological features that were similar to those seen in blood smears in severe thermal burns (Zarkowsky *et al*, 1975). However, recent molecular studies have clearly established that HPP is a subset of HE due to either homozygous or compound heterozygous mutations in spectrin leading to severe disruption of spectrin self-association (Gallagher, 2004).

Hereditary ovalocytosis

Southeast Asian Ovalocytosis is very common in malaria endemic areas in Melanesia, Malaysia, Philippines, Indonesia and Southern Thailand. In endemic areas its prevalence ranges from 5 to 25% (Amato & Booth, 1977). Ovalocytosis is characterized by the presence of oval-shaped red cells with one or two transverse ridges or a longitudinal slit on blood smears. Inheritance of ovalocytosis is autosomal dominant and, to date, only heterozygotes have been identified in high prevalent regions, implying that homozygosity may lead to embryonic or fetal lethality. Hereditary ovalocytosis appears to provide some protection against all forms of malaria.

A distinguishing feature of ovalocytes is that their red cell membrane is very rigid and mechanically more stable (Mohandas *et al*, 1992). In terms of clinical manifestations, it is important to note that, in spite of a marked increase in red cell membrane rigidity, most affected individuals experience no or minimal haemolysis. It has been suggested that the increased rigidity of the membrane could impede the ability of the malarial parasite to effectively invade these red cells (Mohandas *et al*, 1984) and thereby decrease parasitaemia and clinical severity of malarial infections. However, it should be noted that the precise basis for protection against malaria has yet to be defined (Gallagher, 2004).

In all cases of ovalocytosis studied to date, only one mutation has been identified – a genomic deletion of 27 bp encoding amino acids 400–408 located at the boundary of the cytoplasmic and first transmembrane domain of band 3 (Jarolim *et al*, 1991; Mohandas *et al*, 1992; Schofield *et al*, 1992). Thus, hereditary ovalocytosis is unique among red cell membrane disorders in that the identical mutation in a single gene is responsible for the morphological phenotype (Table I). A number of hypotheses have been proposed regarding how mutation in band 3 could lead to a marked increase in membrane rigidity. These include the hypothesis that as a result of a conformational change induced by the mutation, the cytoplasmic domain of mutant band 3 forms extra linkages with membrane skeleton thereby interfering with structural rearrangements of spectrin network necessary for membrane deformation. However, the precise mechanistic basis for increased membrane rigidity of ovalocytes has yet to be established.

Red cell membrane transport defects: hereditary stomatocytoses

In addition to playing a critical role in regulating the key membrane material properties of deformability and membrane integrity, red cell proteins play a crucial role in regulating cell volume homeostasis. As with requirement for maintenance of normal membrane surface area for optimal cell function, there is a similar requirement for maintenance of normal cell volume. The reason for this requirement is that a normal red cell with a surface area of 140 μm^2 and a volume of 90 fL has sufficient redundant surface area to undergo extensive membrane deformations while maintaining cell haemoglobin concentration of 330 g/l and cytoplasmic viscosity at 6–8 cp (viscosity of water being 1 cp) to minimize viscous dissipation during deformation. A large number of membrane proteins play an important role in cation homeostasis and hence normal cell water content of red cells (Table II) (Brugnara, 1997). These include gradient-driven, passive transporters Na^+-K^+-Cl^- co-transporter, Na^+-Cl^- co-transporter, Band 3 anion exchanger, Na^+-K^+ co-transporter as well as active transporters, Na^+-K^+ ATPase and Ca^{2+} ATPase. In addition, the SK1-Gardos channel and AQP1 water channel also play a role in cell volume regulation.

Loss of the ability of the red cell to regulate its volume compromises its ability to optimally perform its function as well as reduce its life span. An increase in net cation content with accompanying increase in cell water content leads to increased cell volume while a decrease in net cation content with accompanying loss of cell water leads to decreased cell volume. Increase in cell volume by reducing redundant cell surface area leads to generation of undeformable spherocytic red cells that are sequestered by the spleen whereas decrease in cell volume by increasing cell haemoglobin concentration, and hence cytoplasmic viscosity, compromises the ability of the cell to undergo rapid deformation needed for optimal oxygen delivery.

The inability to regulate cell volume has long been recognized to be a feature of a number of haemoglobinopathies including sickle cell disease, Hb CC disease and thalassaemias (Fabry *et al*, 1981; Embury *et al*, 1984; Schrier *et al*, 1989). Interestingly, increased cell volume is a feature of HbH (α-thalassaemia) while cell dehydration is a feature of β-thalassaemia intermedia and major. Although SK1-Gardos channel and Na^--K^- co-transporter have been implicated in

Table II. Principal ion transport pathways of the human erythrocyte.

Gradient-driven, passive transporters	Active transporters	Channels
Na^+-K^+-Cl^- co-transporter	Na^+-K^+-ATPase	AQP1 water channel
Na^+-Cl^- co-transporter	Ca^{2+}-ATPase	SK1-Gardos channel
Band 3 anion exchanger		
Na^+-K^+ co-transporter		

dehydration of sickle red cells, the mechanistic understanding of disordered volume regulation in various haemoglobinopathies is far from complete.

In terms of inherited red cell membrane disorders with membrane transport defects, two distinct phenotypes have been identified: dehydrated hereditary stomatocytosis (xerocytosis) (DHS) and overhydrated hereditary stomatocytosis (OHS) (Delaunay, 2004). DHS may appear alone or in conjuction with other clinical manifestations including pseudohyperkalaemia and/or perinatal fluid effusions. The inheritance pattern of DHS is autosomal dominant. DHS alone is associated with well-compensated anaemia with borderline macrocytosis and a mild to moderately enlarged spleen. Blood smears show stomatocytosis but usually less than 10%. The distinctive feature of DHS is cell dehydration with a resultant increase in mean corpuscular haemoglobin concentration (MCHC) and decreased osmotic resistance.

Overhydrated hereditary stomatocytosis is associated with uncompensated haemolytic anaemia with frank macrocytosis and reticulocytosis. In contrast to DHA, stomatocytes are a major feature of red cell morphology on blood smears. The distinctive feature of OHS is increased cell hydration with resultant increase in mean corpuscular volume, a decreased MCHC and an increased osmotic fragility. In contrast to HS, the increased osmotic fragility is not the result of reduced cell surface area but increased cell volume with normal surface area. The inheritance pattern of OHS is autosomal dominant.

While splenectomy is highly beneficial in the management of HS and HE patents with moderately severe to severe anaemia, it is contraindicated in hereditary stomatocytosis due to membrane transport defects since venous thromboembolic complications occur following splenectomy (Stewart *et al*, 1996; Jais *et al*, 2003). The mechanistic basis for this complication has yet to be defined.

In contrast to our detailed understanding of the molecular basis for HS, HE and Hereditary ovalocytosis, the molecular basis for HHS and OHS has not yet been fully defined (Iolascon *et al*, 2003; Bruce *et al*, 2005). The rarity and heterogeneity of red cell membrane disorders involving disordered cation homeostasis have hindered identification of the gene(s) responsible for DHS and OHS and thus developing a more comprehensive understanding of the underlying pathophysiology.

Conclusion

Extensive studies during the last three decades on red cells from normal individuals as well from individuals with various inherited red cell membrane disorders using biochemical, biophysical and molecular biological approaches have enabled the development of detailed molecular insights into structural basis for normal red cell membrane function and for altered function in various inherited red cell membrane disorders. Our current understanding of the molecular basis for inherited red cell disorders involving disordered membrane structural organization (hereditary spherocytosis, hereditary elliptocytosis and hereditary ovalocytosis) is comprehensive. Mutations in a number of distinct genes account for hereditary spherocytosis and elliptocytosis, while a single genetic defect accounts for all cases of hereditary ovalocytosis. Loss of vertical linkage between membrane skeleton and lipid bilayer leads to membrane loss in hereditary spherocytosis, while weakening of lateral linkages between skeletal proteins leads to membrane fragmentation and surface area loss in hereditary elliptocytosis. Importantly the severity of anaemia in both these disorders is directly related to extent of membrane surface area loss. Splenectomy results in amelioration of anaemia.

The molecular basis for inherited red cell membrane disorders due to membrane transport defects, dehydrated hereditary stomatocytosis and overhydrated hereditary stomatocytosis has yet to be defined and remains a continuing challenge. While anaemia is mild in dehydrated hereditary stomatocytosis, it is severe in overhydrated hereditary stomatocytosis. While splenectomy is highly beneficial in the management of HS ad HE, it is contraindicated in hereditary stomatocytosis since venous thromboembolic complications occur following splenectomy.

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