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REVIEW

The molecular basis of hereditary red cell membrane disorders

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Summary The red cell membrane is one of the best known membranes in terms of structure, function and genetic disorders. As any plasma membrane it mediates transport functions. It also provides the erythrocytes with their resilience and deformability. Many of the proteins and the genes performing these functions are known in great detail, although some disease-responsible genes are yet to be elucidated. Basic knowledge has shed light on important groups of genetic disorders. The latter include (i) the disorders of the red cell mechanics: hereditary spherocytosis, hereditary elliptocytosis and poikilocytosis, and (ii) the disorders of the passive flux of the monovalent cations across the membrane: the stomacytoses and allied conditions. Reciprocally, many information have come from genetics abnormalities. We will review the mutation-disease relationship. A number of points will be underscored: widespread weak alleles modulate the expression of the *SPTA1* gene, encoding the α-chain of spectrin; mutations in the anion exchanger can give rise to an array of distinct nosological entities, including a renal condition; splenectomy is banned in the stomatocytoses; a variety of stomatocyosis is part of a pleiotropic syndrome that may includes perinatal fetal liquid effusions. The diagnosis, follow-up and treatment of the involved diseases have gradually improved.

Introduction

The red cell membrane is the only membrane left in the circulating red cell. In a strict sense, it is comprised of a lipid bilayer. The lipid bilayer is studded with transmembrane proteins. Covalently linked to external segments of most transmembrane proteins, or to lipids, glycans stick out toward the plasma. In a wider sense, which we will refer to, the red cell membrane includes a thick, two-dimensional protein network running beneath the inner surface of the bilayer. This is the membrane skeleton (or red cell skeleton). It provides the erythrocytes with two major mechanical

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properties which seem antinomic: resilience and flexibility. Skeletal proteins establish contacts with transmembrane proteins through linker proteins. Hematologists are often confronted with genetic conditions of the red cell membrane. Although classical manifestations, revolving around an haemolytic anaemia, come into play, they are not usually life-threatening. They nonetheless pose diagnostic and therapeutic questions which are not easily coped with. The main conditions are those affecting the mechanical properties of the erythrocyte (spherocytosis, ellipocytosis, poikilocytosis), and those disturbing the passive flux of monovalent cations across the membrane (the stomatocytoses and allied conditions). These two groups share a common, still ill-defined border. Knowledge of many of the responsible genes has opened vistas on the molecular mechanisms involved under normal and abnormal conditions. Diagnosis and treatment have benefited from new methods based on long follow-ups. This review will not deal with blood groups molecules and the corresponding genes. The readers interested in this topic are kindly invited to refer to a recent review.[1]

The clinical signs and laboratory data

Most conditions share a core of similar symptoms : hyperhaemolysis and anaemia, icterus, splenomegaly. The symptoms may widely vary in intensity. For example, anaemia may require *in utero* blood transfusions or remain well compensated during the whole life. Common complications are gallstones and iron overload. The latter is a serious concern in the present chapter, even in the absence of transfusions. A point which has not been studied in a systematic manner is the incidence of the mutation ($A(TA)_6TAA \rightarrow A(TA)_7TAA$)[2] in the promoter of the bilirubin UDP-glucuronosyltransferase 1 (*UTGA* gene), as observed in Gilbert syndrome, on the development of biliary stones. Nor has been investigated the impact of the different mutations in the HFE gene,[3] predisposing to hemochromatosis, on the intensity of iron overload. Allo- and auto-antibodies will be searched for in order to rule out an immunological disorder. More specific signs which are associated with particular conditions will be mentioned with the latter.

Routine laboratory data include the following determination : red cell indices (macrocytosis ?), erythrocyte morphology, bilirubinaemia (unconjugated) and haptoglobinaemia, the iron status, particularly ferritinaemia. Many tests exist as to measure the erythrocyte osmotic resistance, but are not always reliable. Osmotic gradient ektacytometry is the key test here and applies to nearly all the disorders. However only a very few laboratories possess an ektacytometer and more recent versions of the apparatus are awaited. In a large proportion of cases, SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of membrane proteins allows to detect a protein change, however slim, and indirectly to point to the responsible gene. The temperature dependence of the passive flux of monovalent cations is also highly discriminating, in stomatocytosis and allied disorders, but is performed in one laboratory only. Molecular genetics has revolutionized the diagnosis of the red cell membrane conditions. Driven by a scientific interest, research has known a peak of excitement in the late 80's and in the 90's. Scores of mutations have then been identified, and have refined the classification of the conditions. They brought about much insight on the genotype-phenotype correlation, as well as on the mechanism of expression of genes (splicing, in particular) or the structure-function relationship within proteins. Later, mutation research abated. Currently, tests are restricted to the straightforward screening, based on PCR, of some common mutations or polymorphisms. Otherwise, the systematic elucidation of mutations is too costly, partly because many genes are large. Besides, knowledge of the mutation is not strictly necessary for the treatment and a smaller number of mutations are expected to show important biological significance. With this policy, we go past the critical information that disease has previously cast on basic physiology. The last mutation surveys[4–6] have not been updated. New mutations are usually not reported. It is out of the scope of this review, nonetheless, to list all the new mutations, especially those unpublished. Only the most remarkable mutations will be dwelt upon.

The lipid bilayer

The red cell membrane is comprised of a lipid bilayer. Phospholipids (PL) are its main components. They allow the monolayers to coalesce in such a way that their fatty acid chains face one another and constitute the hydrophobic core of the bilayer. Their polar heads look toward the plasma (outer leaflet) or the cytoplasm (inner leaflet). The red cell membrane is also exceptionally rich in cholesterol.