

The human erythrocyte has provided the basis for much of our basic understanding of plasma membrane structure, beginning with elucidation of the bilayer organization of phospholipids by Gorter and Grendel, and more recently with resolution of the detailed structure of the spectrin-based membrane skeleton. The spectrin skeleton is an assembly of proteins localized on the cytoplasmic surface of the plasma membrane and is responsible for stability of erythrocytes in the high shear environment of the vascular system. The spectrin skeleton of human erythrocytes has been resolved in terms of the major interacting proteins, their sites of recognition and domain organization, as well as their primary structure. Building on this foundation, understanding of the molecular basis for abnormal erythrocyte membranes has undergone a paradigm shift in the past decade (see *Seminars in Hematology* volume 30 for a series of reviews). Two papers in this issue of *The Journal* from the laboratories of Bernard Forget (1) and Jiri Palek (2) exemplify this revolution and present two new molecular defects in proteins of the spectrin skeleton. These papers are significant in terms of their contribution to hematology and also provide valuable insight into structure–function relationships of skeletal proteins. In a broader sense, the detailed dissection of defects in the spectrin skeleton of erythrocytes provides a model for understanding other diseases involving multiprotein structures.

Gallagher and colleagues (1) describe a mutation in β spectrin which, in homozygotes, results in fetal death in the third trimester with a presentation of hydrops fetalis. Using a mutant β -spectrin polypeptide, the authors were able to demonstrate that the mutation of spectrin results in impaired association with the α spectrin and presumably reduced levels of spectrin tetramer in erythrocytes. The lethal consequences of spectrin mutations in utero are in contrast to the general view that hereditary hemolytic anemias are relatively mild disorders with a dominant or sporadic inheritance. Of interest, the results of Gallagher et al. (1) were foreshadowed by studies with anemic mice discovered by Bernstein and his colleagues at the Jackson Laboratories. Four strains of mice have been characterized with recessively inherited mutations resulting in severe, life-threatening anemia and abnormally shaped erythrocytes. Erythrocytes from the mutant mice exhibit deficiency of spectrin as a common feature, and the extent of spectrin deficiency correlates with severity of the anemia (3, 4). The possibility that the severe anemias in mice were related to hereditary spherocytosis and elliptocytosis in humans was initially dismissed. However, a connection between diseases of mice and humans was established when Agre located a family with recessive spherocytosis and spectrin deficiency resulting in transfusion-dependent anemia in early childhood (5).

Jarolim and colleagues (2) present another route to abnormally fragile erythrocytes and spectrin deficiency, involving a mutation in the gene encoding ankyrin, a protein that links spectrin to the plasma membrane. A single base change in an

exon encoding a regulatory domain of ankyrin apparently results in an unstable mRNA for the major spliceform of ankyrin but spares alternatively spliced variants missing this exon. The consequences of reduction of levels of the major ankyrin spliceform are spectrin deficiency and a phenotype of spherocytosis. These findings in humans were anticipated by the normoblastosis (nb/nb) mouse which has a mutation in the ankyrin gene resulting primarily in reduction in levels of ankyrin and secondarily of spectrin (6). Presumably the relatively mild disease of human heterozygotes would be more severe in homozygotes and could either resemble the nb/nb mouse or result in fetal death.

The same genes encoding erythrocyte β spectrin, ankyrin, and many of the components of the spectrin skeleton are expressed in other tissues and are especially abundant in the nervous system (7). Moreover, the genes for erythrocyte proteins are members of families of closely related genes which are ancient and expressed in most metazoans. These considerations raise the question of whether spectrin or associated proteins are involved in diseases other than anemia. Mutant mice may once more provide clues for future work in humans. nb/nb mice have a deficiency of ankyrin in brain as well as erythrocytes (8, 9). Even though two other ankyrin genes are expressed normally, nb/nb mice experience degeneration of a population of Purkinje cell neurons within a few months of birth and develop ataxia and signs of cerebellar dysfunction (8). These observations suggest the possibility that some forms of neuronal degeneration in humans may also accompany anemia and result from defects in erythrocyte skeletal proteins. In addition, diseases would also be predicted to involve members of gene families encoding proteins related to components of the erythrocyte spectrin-based skeleton. One recent example is neurofibromatosis type 2, which is caused by defects in a protein related to protein 4.1 of erythrocytes (10).

Immediate clinical implications of these developments include the importance of genetic counseling and thorough hematologic workup for couples with even mild forms of hereditary anemia. Moreover, neurologists and hematologists should both be alerted to the possibility of neurological involvement in patients with hereditary anemias. Future investigation will certainly reveal additional molecular defects in currently known erythrocyte membrane skeletal proteins. Discovery of new erythrocyte components, especially those involved in regulation and assembly, also can be anticipated. Especially promising is the possibility of directly extending insights from diseases that at one point were viewed as relatively simple disorders of erythrocytes to more complex pathological processes.

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