These data also raise the question of how best to target anergy in C.L.L. One intriguing possibility is combination with other targeted C.L.L. therapies. Currently, much attention is focused on the treatment of C.L.L. with inhibitors of the BCR signaling pathway, especially the phosphatidylinositol-3-kinase inhibitor GS-1101 and the Bruton tyrosine kinase inhibitor ibrutinib. Preliminary data with ibrutinib suggest that patients with IgVH-unmutated disease, associated with more active BCR signaling, respond earlier than patients with IgVH-mutated disease. Apollonio et al show that inhibitors of markers of anergy restore BCR sensitivity—perhaps inhibition of anergy before BCR pathway inhibition might improve response. Alternatively, the concept of anergy maintenance through chronic antigen stimulation opens another avenue to target these cells. Biased immunoglobulin gene usage in C.L.L. suggests that in at least a subset of patients’ C.L.L. pathogenesis is antigen driven. If continued antigen stimulation is required for maintenance of anergy, as suggested by these data, inhibition of antigen binding may be another therapeutic strategy to promote apoptosis.

As we move toward more distinct molecular classification of C.L.L., Apollonio et al identify a subset of C.L.L. patients with a phenotype that could potentially be targeted therapeutically. These data, building on previous work from this group, enhance our knowledge of the biology of this heterogeneous disease and continue to move us forward toward the goal of individualizing targeted therapies for our patients.

Conflict-of-interest disclosure: The author declares no competing financial interests.

REFERENCES

To shrink or not to shrink

Narla Mohandas

In this issue of Blood, Andolfi and colleagues show that dehydrated hereditary stomatocytosis (DHS), an inherited red cell disorder, is associated with a number of distinct germline mutations in PIEZO1, a stretch activated cation channel, in 26 affected individuals from 7 families.

The shrinking cell has been an object of curiosity and some bafflement to physiologists and hematologists since it was first seen some 40 years ago. We know that regulation of its volume within narrow limits through a tightly controlled intracellular cation concentration is critical for optimal functioning and survival of the red cell. An autosomal dominant hemolytic anemia characterized by primary red cell dehydration due to decreased cation content was first described by Miller and colleagues in 1971 and is currently designated as hereditary xerocytosis (HX) or DHS. These patients typically exhibit mild to moderately compensated hemolytic anemia and the red cells are characterized by increased mean corpuscular hemoglobin concentration and decreased osmotic fragility, both reflecting cellular dehydration. In addition to anemia, a subset of the patients exhibit pre- and/or perinatal edema which recedes spontaneously.

The molecular basis of the disorder, which has been under intense scrutiny for decades, was recently resolved thanks to the identification of mutations in the gene encoding PIEZO1. These came to light in 2 large kindreds studied by Zarychanski and colleagues last year and in 7 additional families described in the present study. PIEZO1 was identified as a protein involved in mechanosensation and stretch-activated cation channel regulation in 2010, and it adds to the impact of that work that, 2 years later, a red cell disorder has been identified as the first human disease stemming from mutations in this gene.

Although the identification of mutations in PIEZO1 leading to red cell dehydration in HX by 2 independent groups is a cause for
satisfaction and opens new avenues of research toward a further understanding of red cell volume regulation, a number of questions remain unanswered. Whereas the finding that PIEZO1 protein is expressed in erythroblasts and is present in the membrane of the mature cell could account for dehydration of reticulocytes, as well as of red cells in HX, the mechanism of cell dehydration of erythroid cells remains to be defined. Furthermore, because the newly recognized function of PIEZO1 as a stretch-activated cation channel can be rationalized as an essential element in red cell volume regulation during repeated cycles of membrane deformation during passage through the microvasculature (panel A), it is not clear how its function is regulated in erythroblasts in the relatively static bone marrow environment.

Another unresolved question is how the various identified mutations in PIEZO1 account for the large phenotypic variability in the clinical expression between patients. Similarly, while the documented expression of PIEZO1 in fetal lymphatic vessel endothelium suggests a potential causative role for the protein in the pathogenesis of perinatal effusions, it not clear only why only a subset of HX patients with mutations in PIEZO1 exhibits this clinical syndrome.

What then are the implications of these findings? One is that PIEZO1, as the newest member of transport proteins responsible for regulating cation content of red cells, will advance our mechanistic understanding of disordered volume regulation, not only in HX but also in a number of other red cell disorders, including sickle cells.7,8 It will indeed be exciting if PIEZO1 can be shown to play a key role in the well-documented deoxygenation-induced increase in cation permeability of sickle red cells; for this phenomenon is responsible for pathogenic cell dehydration, contingent on membrane deformation (panel B).7 The study by Adolfo and colleagues1 represents the first step in what should prove a long and productive journey toward a comprehensive understanding of red cell hydration pathways in health and disease.

**Conflict-of-interest disclosure:** The author declares no competing financial interests.

**REFERENCES**


Comment on Carcao et al, page 3946

**F8 gene and phenotype: single player in a team?**

**Anna Pavlova**

In this issue of Blood, Carcao et al demonstrate that the phenotypic variability in patients with severe hemophilia A correlates with the F8 mutation, where patients with non-null mutations exhibit a milder bleeding phenotype compared with those with null mutations, although this difference is not likely to influence the treatment decision making.1

D eficiency of blood coagulation FVIII results in hemophilia A, a serious bleeding disorder. The classification of hemophilia is based on the residual factor VIII activity; thus, the clinical presentation of the disease is primarily dependent on the severity of deficiency. In most cases, the bleeding tendency correlates well with the degree of factor deficiency. However, within the different groups of severity, considerable heterogeneity has been observed. A subset of 10% to 15% of patients with severe hemophilia A exhibit a mitigated disease phenotype, with significantly reduced frequencies of spontaneous bleeding, delay in the onset of the first bleeding and further in development of arthropathy, and lower consumption of factor concentrates.2-4

If it is possible to predict a patient’s specific bleeding risk, individualized treatment regimens could be possible, minimizing the unnecessary burden and costs of prophylaxis in patients having a mild bleeding tendency.

Here, Carcao et al performed a detailed analysis on the correlation between phenotypic expression of severe hemophilia A and F8 mutation in 621 previously untreated patients. Since the introduction of prophylactic treatment, several parameters (bleeding frequency and concentrate consumption) characterizing the clinical phenotype of hemophilia are no longer applicable. Therefore, the authors postulate that the time from birth to the start of prophylaxis, and especially ages at first bleed and ages at first and second joint bleed, may represent the most suitable variables defining the bleeding phenotype of patients with severe hemophilia. They provide evidence that the type of F8 mutation can serve as a determinant of bleeding tendency in severe hemophilia.

Patients bearing non-null mutations exhibit a milder clinical phenotype, a later age of disease diagnosis, and a later age of the first bleed, first joint bleed, and second joint bleed compared with those with null mutations. A review of literature shows limited data, and only a few studies demonstrate that small deletions/insertions within poly-A runs of exon 14 of F8, nonconserved splice-site mutations, and some missense mutations have a mitigating effect on the bleeding phenotype (see figure).5-7
To shrink or not to shrink

Narla Mohandas