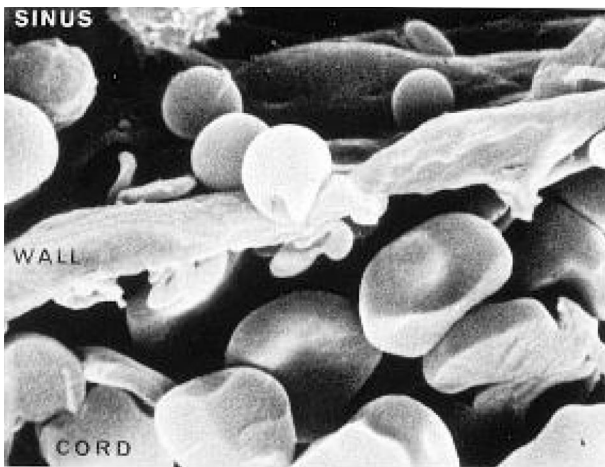


What does the spleen see?

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In this issue of *Blood*, Safeukei et al have come to grips with an important issue in red blood cell (RBC) biology.¹ Their study deals not only with the mechanism of RBC removal in diseases like hereditary spherocytosis (HS) and autoimmune hemolytic anemia, but also with RBC senescence.²



Rat spleen showing a red blood cell leaving the cord and deforming to pass through a slit in the wall of the sinus. Slide provided by Dr Mohandas Narla. Reproduced with permission from: Mohandas Narla. Red cell membrane dynamics and organization. In: UpToDate, Basow, DS (Ed), UpToDate, Waltham, MA 2012. Copyright © 2012 UpToDate, Inc. For more information visit www.uptodate.com.

To understand their contribution, first consider the evolutionary beauty of our biconcave discocytic RBC. A sphere with a volume of approximately 90 femtoliters (the volume/mean corpuscular volume [MCV] of a normal RBC) would have a surface area of 95 square microns. By actual measurement the surface area of an RBC is 140 square microns, giving an excess surface area of 45 square microns—this is the surface area volume (S/V) ratio beloved by red cell hematologists who somehow missed the future belonging to Flt3 and NPM1. It is this excess surface area that allows the RBC with a diameter of 7 to 8 microns to elliptically deform to a length 5 times its width and tank tread its way down 3- to 5-micron-diameter capillaries and through 3-micron slits in the sinus-endothelial wall of the reticulo-endothelial organs (see figure). A tennis ball cannot elliptically deform, but a biconcave discocyte with excess surface area can. But it is not only S/V considerations that determine RBC deformability.

There are two other important determinants: the internal viscosity of the RBC reflected in the mean corpuscular hemoglobin concentration (MCHC), that is, the concentration of densely packed hemoglobin molecules normally approximately 35 g/dL (consider that we worry about serum viscosity in our myeloma patients when the globulin reaches levels of 6 gm/dL); and the intrinsic properties of the proteins and lipids that make up the RBC membrane.

We have long believed that this critical S/V ratio was monitored by the reticulo-endothelial system, particularly in the complex vasculature of the spleen, where the RBCs are first discharged into the cords of Billroth and then have to elliptically deform to pass into the sinuses through slits in the sinus wall (see figure). All this happens while adjacent macrophages are looking on and performing quality control. This belief in the role of the spleen has been fostered by the generally successful clinical use of splenectomy in HS

and somewhat less successful use in autoimmune hemolytic anemia. A recurrent biologic question has always been, “What does the spleen see?” Is it RBC surface charge, or membrane-attached immunoglobulins or complement, or can the spleen actually sense deformability and spheroidicity?

Safeukei and colleagues have cleverly designed a method for perfusing recently removed human spleens with tagged RBCs. Their idea was to test a hypothesis concerning the mechanism of splenic sequestration or splenic entrapment of RBCs that have been altered to reduce their deformability. They used a trick well known to red cell hematologists; that is, incubating RBCs with lysophosphatidylcholine (LPC). The LPC intercalates specifically into the outer half of the RBC membrane phospholipid bilayer, causing it to undergo outward expansion, producing first echinocytosis and then after the spicules fall off, spherocytosis. The amount of LPC and the incubation time can be altered to produce varying degrees of spherocytosis. In these experiments the MCHC was not altered so that increases in internal viscosity could not contribute to the reduction in deformability. Further, there were no alterations in the RBC membrane proteins. Therefore, the reduced deformability³ of these LPC-treated RBCs was directly related only to S/V considerations or spheroidicity. The authors showed that graded RBC surface area loss lead to parallel increases in splenic entrapment. Therefore, the spleen can see loss of S/V and spheroidicity.

Interestingly, Safeukei et al then noted a heterogeneity in removal of apparently equivalently spherocytic RBCs, leading them postulate that deformation may cause a leak of intracellular ions that causes a loss in RBC volume, restoring the S/V. In fact, there are mechanically activated ion channels in RBCs,⁴ so the hypothesis is reasonable.

These experiments were designed with the idea that the alterations in RBCs induced by LPC might mimic those seen in HS. But as Safeukei and colleagues point out, the mimicry is not exact because HS RBCs frequently have an increased MCHC, the cause of which is not clear. In addition, we know from use of the eosin-maleimide diagnostic test that there are further mysteries in the patho-biology of HS.⁵ The eosin-maleimide test seems to have increased diagnostic accuracy because it not only detects S/V reduction but also the

reduced access of eosin-maleimide to CD 47, band 3, and Rh protein. Why this should occur in HS is not clear.

We look forward to see use of this experimental design in studying splenic RBC removal in autoimmune hemolytic anemia.

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● ● ● THROMBOSIS & HEMOSTASIS

Comment on Lotta et al, page 440

Forecasting the future for patients with hereditary TTP

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Hereditary thrombotic thrombocytopenic purpura (TTP) may be rare, but it is forever. What is the future for our patients?

Hereditary TTP, caused by *ADAMTS13* mutations resulting in a severe deficiency of ADAMTS13 activity, is also known as Upshaw-Schulman syndrome. In 1960, Schulman et al reported an 8-year-old girl who had repeated episodes of thrombocytopenia that responded to plasma infusion; they concluded that she had a “congenital deficiency of a platelet-stimulating factor.”¹ In 1978, Upshaw et al reported a 29-year-old woman who had repeated episodes of TTP.² These 2 patients were among the 4 patients with chronic relapsing TTP studied by Moake et al in 1982, demonstrating the presence of unusually large von Willebrand factor (VWF) multimers and postulating a missing factor required for VWF cleavage.³ The missing factor was initially identified as a VWF-cleaving protease in 1997 in a patient with hereditary TTP⁴ and subsequently characterized in 2001 as ADAMTS13 in a study of 4 families with hereditary TTP.⁵ This is a wonderful scientific story, but then the next question is, what happens to people with a lifetime deficiency of ADAMTS13?

The experience of Fujimura et al with 43 patients from Japan documents the heterogeneity of hereditary TTP.⁶ Eighteen (42%) had severe neonatal hemolysis requiring exchange transfusion; 25 (58%) were diagnosed as children; 15 (35%) were 15 to 45 years old

when they were diagnosed, 7 in association with pregnancy; 3 (7%) were 51 to 63 years old when TTP suddenly developed. Fujimura et al postulated that some ADAMTS13 mutations may result in a mild, late-onset phenotype, without manifestations of overt TTP unless additional factors, such as pregnancy or infection, are present.⁶

These observations are extended by Lotta et al in this issue of *Blood*.⁷ They describe 29 patients with hereditary TTP in 25 families from 4 centers across Europe. Using an assay with sensitivity to 0.5%, 26 patients had measurable ADAMTS13 activity (range, 0.5%-6.8%); 31 different *ADAMTS13* mutations were identified. The severity of the deficiency of ADAMTS13 activity correlated with younger age of the first TTP episode, a higher frequency of TTP episodes, and the use of regular plasma prophylaxis. *ADAMTS13* mutations affecting the highly conserved N-terminal domains of ADAMTS13 were associated with lower residual ADAMTS13 activity and more severe clinical features. These data provide a basis for understanding the genetic heterogeneity of hereditary TTP, similar to the heterogeneity of hemophilia related to residual factor VIII or IX activity. However, Lotta et al also observed exceptions; some patients with higher ADAMTS13 activ-

ity had early onset and frequent recurrences of acute episodes. These observations emphasize that multiple additional factors are important for triggering acute episodes of microvascular thrombosis, similar to other thrombotic disorders. Pregnancy appears to be a particular hazard for women with hereditary TTP.^{6,8}

Until 3 months ago I thought that hereditary TTP was too rare for clinicians to care about. I had not recognized a patient with hereditary TTP since 1974.⁹ Then we diagnosed hereditary TTP in a teenage girl and subsequently in both of her younger teenage sisters. All 3 girls had required exchange transfusion at birth for severe hemolysis and thrombocytopenia; the oldest sister had a focal seizure and small cerebral thrombosis on her first day of life that promptly resolved. At that time, these episodes were attributed (with some uncertainty) to their other clinically apparent hereditary disorder, elliptocytosis. Subsequently all 3 girls have had excellent health except for 3 episodes of transient, asymptomatic thrombocytopenia: 2 in the youngest sister, 1 in the oldest. They are all outstanding students and athletes. My conversations with this family around their kitchen table have been all about forecasting the future. We've discussed potential problems and developed management plans for the near future, but what about the next 75 years? Other than for pregnancies, should plasma infusions be given only when there is evidence of TTP? What are the relative benefits and risks of regular prophylactic plasma infusions?¹⁰ What about the girls' risks for kidney disease, hypertension, and cardiovascular disease; should these be anticipated and can they be prevented? The patient reported by Schulman et al¹ developed renal failure and became dialysis dependent.¹⁰ Experimental data suggest that ADAMTS13 deficiency can accelerate the development of atherosclerosis.¹¹ These observations emphasize the potential dangers in the decades ahead for my patients.

To answer these questions about the future of patients with hereditary TTP, systematic lifetime follow-up of many patients will be required. To achieve this, an international Hereditary TTP Registry (www.ttpregistry.net; NCT 01257269) was established in 2009; 83 patients from 74 families in 18 countries have been enrolled. Genetic data and detailed clinical observations from long-term follow-up will determine the risk for renal, cardiac, or cognitive problems in patients with