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TRANSFUSION MEDICINE

Comment on Roussel et al, page 2285

In vivo clearance of stored red blood cells

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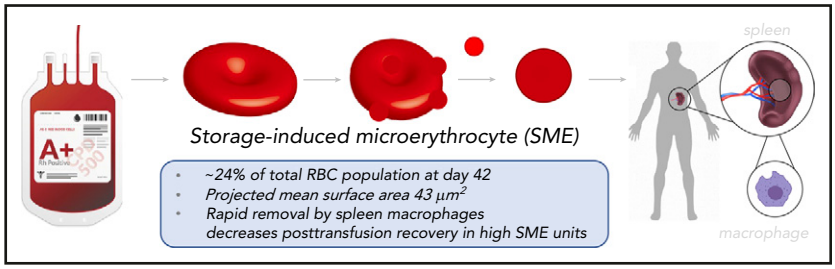
In this issue of *Blood*, Roussel et al focus on the morphologic alterations of stored red blood cells (RBCs) and their impact on the ability of cells that have been damaged by storage to circulate after transfusion, which is the minimum requirement for the physiological function of transfused RBCs regarding oxygen transport.[1] Specifically, they describe the progressive accumulation in stored units of a population of RBCs with reduced size (mean projected surface area <43 μm^2), which they define as storage-induced microerythrocytes (SMEs). They thus combine elegant murine models and ex vivo perfusion of human spleens to demonstrate that SMEs are more rapidly removed than RBCs with normal morphology from the bloodstream of the recipient upon transfusion.

Blood storage is a logistic necessity for providing >100 million units annually for transfusion worldwide. Unfortunately, unlike fine wine, blood quality does not improve with age. As refrigerated RBCs age in the blood bank, they accumulate multiple molecular alterations, collectively denoted as the "storage lesion."[2] Under refrigerated storage conditions, RBC proton pumps fail, energy metabolism progressively deteriorates, and oxidant stress increases, thereby overwhelming intracellular redox systems and ultimately resulting in the accumulation of irreversibly oxidized proteins, metabolites, and lipids.[2] Mechanisms in mature RBCs eliminate irreversibly oxidized components in vesicles, which negatively affects RBC morphology via a progression from the classical discocyte to echinocyte, spheroechinocyte, and—when membrane shedding is extreme—spherocyte.[2] Although the timeline of these phenomena is well established, their clinical relevance remains unclear.

Roussel et al report that SMEs comprising type III echinocytes, spheroechinocytes, and spherocytes (Bessis classification) represent ~24% of the entire RBC population in a unit by the end of its shelf life (ie, 42 days in the United States). These numbers almost perfectly overlap with earlier reports on morphologic alterations of stored RBCs, as determined by scanning electron microscopy.[3] Through a series of elegant studies in murine models of storage and posttransfusion recovery, as well as an innovative model of ex vivo perfusion of human spleens with fresh and stored blood, they demonstrate that SMEs are rapidly sequestered by the spleen where they are phagocytosed by resident macrophages, thereby contributing to extravascular hemolysis (see figure). Indeed, macrophage depletion or splenectomy decreased the untimely clearance of transfused SMEs from the bloodstream of mouse recipients, which confirmed previous results in mice lacking red pulp macrophages.[4]



Roussel et al describe the progressive accumulation in stored units of a population of RBCs with reduced size, which accounts for ~24% of the total RBCs by storage day 42. These SMEs have a mean projected surface area <43 μm^2 and are rapidly removed from the bloodstream of the recipient upon transfusion.

Roussel et al present compelling evidence that suggests a negative correlation between SMEs and posttransfusion recovery. Despite chromium-51 being the gold standard for determining end-of-storage RBC quality (according to US Food and Drug Administration and European Council guidelines), studies of autologous chromium-51 posttransfusion recovery in healthy donor volunteers have several limitations.[5] Future studies could borrow some of the elegant tools and workflows developed by Roussel et al to address the question of whether accumulation of SMEs also reduces the efficacy of transfused units, which can be determined by clinically relevant parameters such as posttransfusion hemoglobin increment,[6] production of non–transferrin-bound iron,[5] and correction of hypoxemia in patients.

The authors identify high variability among donors in the end-of-storage proportion of SMEs in individual blood units. This agrees with recent studies describing the heterogeneity in molecular markers, especially metabolic markers, of storage quality and hemolytic propensity as a function of donor biology (sex, age, ethnicity, impaired

enzymatic activity [eg, glucose 6-phosphate dehydrogenase deficiency])[7]) and donor habits (eg, physical activity, smoking, consuming alcoholic or caffeinated beverages[8]). Additional factors impact blood product heterogeneity such as xenometabolites from dietary or other exposure (the exposome[8]) and over-the-counter or other drugs that are not grounds for donor deferral. It remains to be determined whether these and other factors such as processing strategies, alkaline additives, pathogen reduction, and hypoxic storage influence the total content of SMEs in end-of-storage units.

Appreciating donor heterogeneity of SME accumulation further fuels the concept that the molecular or metabolic age rather than the chronological age[9] of the blood unit may be clinically relevant. This concept posits that the molecular makeup of a unit is more relevant than the number of storage days that have elapsed from donation, owing to variability in the progression and severity of the storage lesion. These concepts are gaining traction in an attempt to explain the discordance between the enormous body of evidence on storage lesions[2] and the reassuring evidence from clinical trials that shows noninferiority of the current standard of practice when compared with transfusing the freshest available units.

It is worth noting that SME accumulation in murine units recapitulated observations for human units. The RBCs from the mouse strain used by Roussel et al (C57BL/6) stores well compared with erythrocytes from other strains (eg, FVB), which are characterized by significantly higher levels of storage lesion markers in vitro[2] (eg, hypoxanthine, lipid peroxidation) accompanied by poor posttransfusion recovery in vivo[10] and, predictably, higher levels of SMEs. Future studies could leverage the genetic and phenotypic diversity in posttransfusion recovery of these strains to elucidate the mechanisms underlying the etiology of SMEs, which was not the focus of the Roussel et al study. One could postulate, on the basis of the role of oxidant stress in the severity of stored RBC vesiculation, a role of RBC membrane proteins in the etiology of SMEs, including structural or functional components (eg, band 3, ankyrin, spectrin), ion channel and osmolarity regulator (eg, PIEZO1, proton pumps for Ca^{2+}, K^{+}, and Na^{+}), antioxidant enzymes (eg, glucose 6-phosphate dehydrogenase, glutathione peroxidase 4, peroxiredoxins 2 and 6, protein L-isoaspartyl O-methyltransferase), and other regulators of membrane homeostasis and fluidity (eg, sphingosine 1-phosphate, lipid fatty acyl composition as a function of the donor's diet).

Although Roussel et al provide a clear advance for the field of transfusion medicine, the observations and tools they developed are also anticipated to affect the field of hematology in general. For example, future studies can address whether the relationship that "(oxidant) stress promotes SMEs, which are rapidly removed from the bloodstream by splenic macrophages" holds true in the context of other stresses to erythrocytes, such as with hemoglobinopathies (eg, sickle cell disease, beta thalassemia), acute or prolonged exercise (eg, endurance athletes), responses to high-altitude hypoxia or pathological hypoxia (eg, hemorrhagic shock, ischemia, or reperfusion injury), aging and inflammation, and infections (eg, SARS-CoV-2). Understanding the effects of SME phagocytosis on macrophages, (iron) metabolism, and activation state could represent a first step between bridging the concepts of altered erythrocyte physiology and remote organ injury and inflammation.

Conflict-of-interest disclosure: A.D. is a founder of Omix Technologies Inc and Altis Biosciences LLC, is a consultant for Rubius Therapeutics, and is a member of an advisory board for Hemanext Inc and FORMA Therapeutics Inc. ■

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