EDITORIAL

Splenic Remodeling of Red Cell Surfaces

By William H. Crosby

IN THIS ISSUE OF *BLOOD*, Lux and John[1] describe a "high molecular weight red cell membrane protein complex that is normally removed by the spleen." They propose that reticulocyte membranes contain an aggregate of spectrin and other proteins and that splenic "surface remodeling" includes removal of this aggregate, along with much of the normal protein-lipid surface material of maturing reticulocytes. "When the spleen is absent or nonfunctional, the protein complex remains attached to the red cell membrane throughout the life span of the cell. Since the survival of normal red cells is not decreased following splenectomy, the presence of this large protein complex must not be especially harmful to the cell." Thus is written yet another chapter of the intricate relation between red cells and spleens.

The spleen serves different functions in different animals. In the mouse it is erythropoietic, but not in humans. The spleen of a sleeping dog can sequester a third of his red cells; when he awakens the spleen injects them back into the rapid blood stream. The human spleen does not provide this sort of reservoir for red cells. It does, however, show some ability to sequester the youngest of the circulating red cells. Blood squeezed from normal spleens has a reticulocyte count twice that of blood in the open circulation.[2] When the spleen is removed from a patient with hereditary hemolytic anemia of a sort not cured post-splenectomy the reticulocyte count may be doubled, although turnover of red cells remains unchanged.[3] On the basis of these observations, it is tempting to conclude that young red cells move more slowly than their mature counterparts through the stagnant courses of the splenic circulation.

It is known, furthermore, that somehow the spleen accomplishes certain changes in these immature red cells. Removal of the spleen from normal dogs or humans results in the appearance of target cells on the blood smear. The presence of these "leptocytes" indicates that while the erythrocytes formed after splenectomy have a normal mean volume, their surface area remains larger than normal.[4]

During maturation of normal erythrocytes, in addition to the decrease of total surface area there is also a modification of the quality of the surface. Mature cells carry a heavier negative charge with an isoelectric point of 1, versus 3 for reticulocytes.[5] Because the erythrocytes' electrostatic charge is a function of the orientation of the fatty acids at the surface, the pattern as well

---

From the Division of Hematology-Oncology, Scripps Clinic and Research Foundation, La Jolla, Calif.

Invited editorial submitted June 16, 1977; accepted June 20, 1977.

Supported by ERDA Contract E(04-3)899 and NASA Contract NAS 9-14341.

Address for reprint requests: William H. Crosby, M.D., Division of Hematology-Oncology, Scripps Clinic and Research Foundation, 10666 N. Torrey Pines Road, La Jolla, Calif. 92037.

 ISSN 0006-4971.

as the total amount of fatty acids on the reticulocyte surface must be different.[6] This surface charge, repelling all cells of like charge, protects the red cell from mechanical injury during millions of collisions that occur along the 175 km traveled in a 4-mo life span. The mature red cell is extremely unsticky. The reticulocyte, with its softer surface charge, is less so. Does this relative stickiness drag upon the reticulocyte as it bumps its way across the tangles of the splenic filter?

During their extended sojourn in the spleen, how do reticulocytes lose substance? Several mechanisms are known. Red cells lose inclusion bodies during passage from the splenic cords into the splenic sinuses, squeezing through cracks between the cytoplasmic processes of the endothelial cells that form the sinus wall. It is a tight squeeze, but red cells are easily deformable. Inclusion bodies are not; they cannot pass through the tight interstices. In this situation, the migrating red cell ultimately finds itself completely within the sinus lumen except for that portion containing the inclusion. The erythrocyte simply amputates that portion. The liberated cell escapes along the venous channels, its surface reduced by the small amount of membrane surrounding the inclusion. Examples of inclusions that may thus affect reticulocytes are Howell-Jolly bodies, iron-laden mitochondria, other organelles, and even organisms.[7]

Pinching off of bits of red cell cytoplasm may also occur within the splenic cords. In a process reminiscent of the cytoplasm's participation in mitosis, the red cell becomes a figure-eight and then, at its narrow waist, divides in two. The fragments are unequal; often one of them is quite tiny. These tiny fragments may represent the bits of red cell "dust" found in plasma after erythrocytes have sedimented, or they may be seen on smears as tiny schistocytes. This pinching-off phenomenon has been described in diseases of red cells[8,9] and may *not* be a mechanism of normal remodeling of the red cell surface. On the other hand, the pinched-off fragments may contain abnormal areas of red cell surface to be got rid of as one aspect of surface remodeling. Thus may be removed the high molecular weight membrane protein aggregate of Lux and John.

Yet another means of explaining postsplenectomy phenomena among circulating erythrocytes involves the destruction of entire red cells in the spleen. The "culling" function requires that the spleen recognize and remove viable cells that are in some way deficient. Heinz body–damaged erythrocytes[10] and hereditary spherocytes are examples of abnormal cells surviving longer after splenectomy. The red cell constituent or deformity that provokes the spleen can now persist in the circulating blood. If some cells possess the flaw and others do not, the flaw remains, although diluted by the normal cells. The presence of acanthocytes[11] in postsplenectomy blood smears of normal people provides evidence for the culling function of the spleen.

The processes described above whereby the spleen remodels red cell surfaces all involve a mechanical disposal of defective red cells or portions of red cells. It must be mentioned that some portion of red cell remodeling might occur through metabolic attrition. Is the protein aggregate of Lux and John present as beads attached to the inner layer of the red cell membrane, or is it a functional membrane constituent of erythroblasts and reticulocytes? If it is in beads,

then mechanical removal is feasible. Smoothly distributed (essential, perhaps, for the ameboid movement of young red cells), it could not be pinched off as an inclusion body. If this were the case, the metabolic removal or disaggregation of the aggregate must somehow require the mysterious ambience of the microcirculation of the spleen.

Whatever the splenic mechanism for its removal, the fascinating, newly discovered phenomenon is that the high molecular weight protein complex of Lux and John remains in the red cell population postsplenectomy. This finding does not necessarily mean, as they suggest, that it is not harmful. Its existence may result in splenic destruction of any cell that possesses it. If, however, it is present in only a few of the circulating cells, their premature demise would not result in discernible hemolytic disease. It remains to be learned if the spleen destroys entire red cells to remove the protein aggregate or if it removes only offending portions, permitting the remodeled red cells to survive.

REFERENCES

1. Lux SE, John KM: Isolation and partial characterization of a high molecular weight red cell membrane protein complex which is normally removed by the spleen. Blood 50:625–641, 1977

2. Berendes M: The proportion of reticulocytes in the erythrocytes of the spleen. Blood 14:558–563, 1959

3. Crosby WH: Hereditary nonspherocytic hemolytic anemia. Blood 5:233–253, 1950

4. Crosby WH: The pathogenesis of spherocytes and leptocytes (target cells). Blood 7: 261–274, 1952

5. Ponder E, Ponder RV: Electrophoretic mobility of red cells and their ghosts as observed with improved apparatus. J Exp Biol 32: 175–182, 1955

6. Munn JI: Studies of lipids in human red cells. Br J Haematol 4:344–349, 1958

7. Nathan DG: Rubbish in the red cell. N Engl J Med 281:558–559, 1969

8. Weed RI, Weiss L: The relationship of red cell fragmentation occurring within the spleen to cell destruction. Trans Assoc Am Physicians 79:426–438, 1966

9. Matsumoto N, Ishihara T, Miwa S, Uchino F: The mechanism of mitochondrial extrusion from reticulocytes in the spleen from patients with erythrocyte pyruvate kinase (PK) deficiency. Acta Haematol Jpn 37:25–31, 1974

10. Selwyn JG: Heinz bodies in red cells after splenectomy and after phenacetin administration. Br J Haematol 1:173–183, 1955

11. Dean HM: Acanthocytes after splenectomy. N Engl J Med 279:947, 1968