ADVANCES IN HEMATOLOGY

Current Developments in the Management of Hematologic Disorders

Section Editor: Craig M. Kessler, MD

The Red Cell Membrane, Part 3: The Role of the Red Cell Membrane in Coagulation and Hypercoagulability

Bas De Laat, PhD
Associate Professor
Department of Biochemistry
Maastricht University
Maastricht, the Netherlands

This is part 3 of a 3-part series on the red cell membrane.

H&O What has been the traditional view of the role the red cell membrane plays in coagulation?

BDL Many studies have shown that erythrocytes contribute to thrombus formation in various ways. Erythrocytes determine the viscosity of the blood and the extent of laminar flow through changes in hematocrit levels, erythrocyte aggregation, and erythrocyte deformability.

An increase in hematocrit levels results in an increase in blood viscosity. Increased viscosity decreases the blood flow and can lead to the development of a thrombus. Furthermore, an elevated hematocrit level promotes the skimming of platelets and coagulation factors toward the vessel wall, thereby enhancing the interaction of platelets with the vasculature.

Erythrocyte aggregation influences several aspects of in vivo hydrodynamics. In large blood vessels, where shear is low, blood behaves as a continuous fluid—enhancing erythrocyte aggregation and increasing blood viscosity. In the microvasculature, increased erythrocyte aggregation induces axial migration of erythrocytes, resulting in skimming of platelets toward the vessel wall, as well as a decreased local viscosity. This reduction in local viscosity and consequent reduction in wall shear stress results in a decrease in local nitric oxide availability. Nitric oxide is an important substance in preventing hyperactivity of endothelial cells and platelets. The decrease in local nitric oxide may promote platelet hyperactivity, thereby promoting thrombus formation.

Erythrocyte deformability is an important characteristic for minimizing resistance to blood flow. A decrease in red blood cell deformability increases the sensitivity for thrombus formation by inhibiting the ability of erythrocytes to squeeze through narrow apertures. Greater rigidity of erythrocytes also is thought to increase the transport of platelets toward the vascular surface.

Coagulation involves a complicated system of enzymes and their inhibitors regulating the balance between bleeding and thrombosis. Coagulation can be divided into 2 pathways: the intrinsic pathway and the extrinsic pathway. The intrinsic pathway, which can be initiated by the exposure of a negatively charged surface, is called the contact pathway. The exposed surface is able to activate factor XII, using a complex reaction that includes prekallikrein and high-molecular-weight kininogen as cofactors. This results in the activation of factor IX, creating factor IXa. Factor IXa in combination with activated factor VIII forms a complex, called tenase, that creates factor Xa. Activated factor X in turn forms a complex with factor V, also known as the prothrombinase complex, that is able to activate prothrombin into thrombin. Thrombin is the final enzyme that is needed to convert fibrinogen into fibrin.

The extrinsic pathway starts with the exposure of tissue factor to the circulating blood, which is the major initiator of coagulation. Tissue factor interacts with and activates factor VII, creating factor VIIa. The tissue factor–factor VIIa complex is able to activate both factor IX and factor X. As mentioned above with the contact pathway, factor VIIIa—in complex with factor IXa—converts factor X to factor Xa. There are several feedback loops

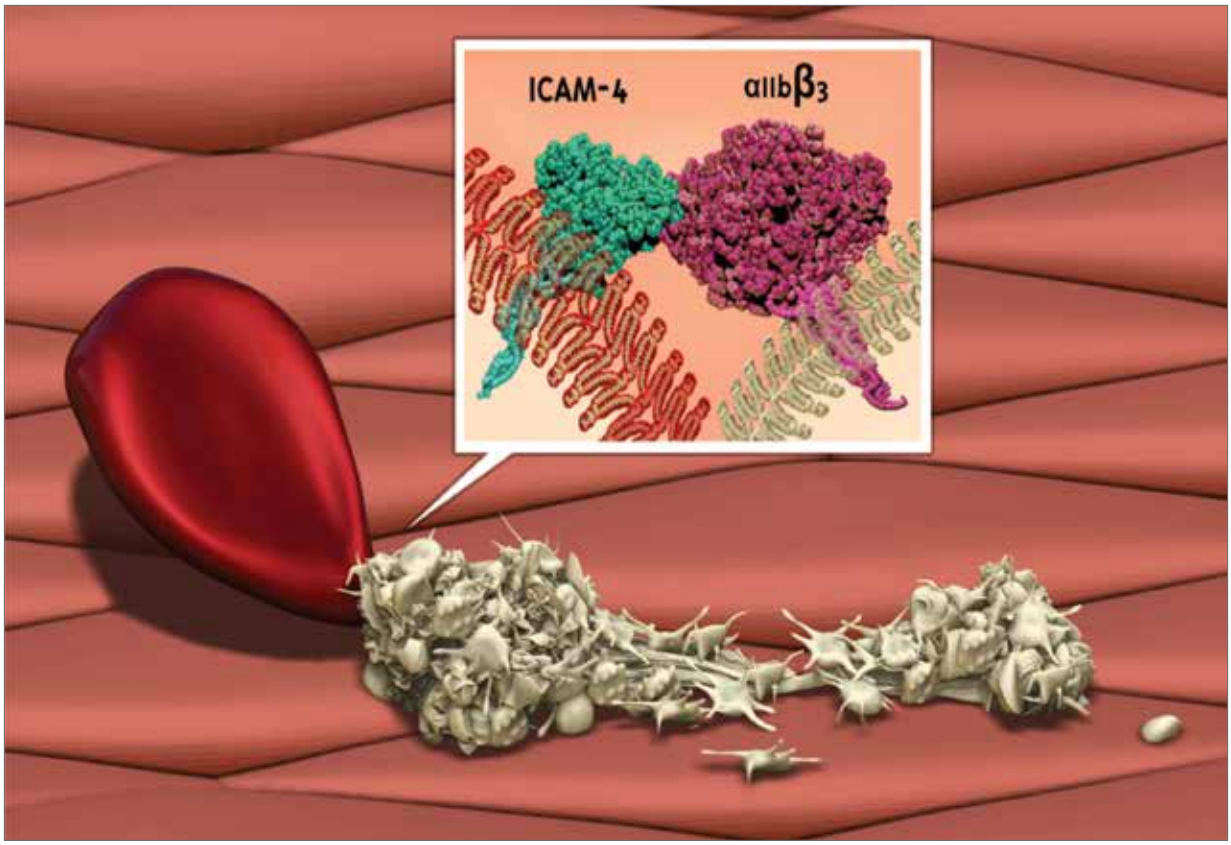

Figure. Schematic illustration of erythrocyte binding to platelets mediated via intercellular adhesion molecule 4 (ICAM-4) and integrin $\alpha_{IIb}\beta_3$.

Reprinted with permission from Du VX, Huskens D, Maas C, Al Dieri R, de Groot PG, de Laat B. New insights into the role of erythrocytes in thrombus formation. *Semin Thromb Hemost*. 2014;40(1):72-80.

that reinforce the coagulation cascade, resulting in large amounts of thrombin. This process is dependent on the presence of procoagulant surfaces of cells expressing negatively charged phospholipids—which include phosphatidylserine (PS)—on their outer membrane. PS-bearing surfaces are able to increase the efficiency of the reactions by concentrating and colocalizing coagulation factors.

H&O What recent changes have there been in our understanding of the relationship between the red cell membrane and coagulation?

BDL Whelihan and colleagues published some interesting findings in 2012 that are directly related to coagulation. It is thought that platelets are the main providers of PS to support coagulation, but recent studies have shown that in red thrombi—which contain a significant amount of erythrocytes—the outer membranes of erythrocytes are also capable of providing sufficient PS to support and enhance coagulation. However, the mechanism by which prothrombin is activated in erythrocyte membranes is still not entirely clear.

Approximately 0.5% of the total erythrocyte population in the human body is PS-positive. The study from Whelihan and colleagues showed that this PS-expressing population enhances coagulation. More interestingly, the researchers showed that after stimulation with tissue factor, prothrombin activation can occur on the erythrocyte membrane through a different route, namely via meizothrombin. Meizothrombin is an intermediate product formed during the conversion of prothrombin into thrombin. It can be measured in clotting blood and is rapidly cleaved into α-thrombin. Meizothrombin together with surface-bound thrombomodulin can activate the protein C pathway and thus exert anticoagulant activity. Erythrocytes are seen as the major surface supporting the formation of meizothrombin. Therefore, erythrocytes can actively participate as procoagulant and anticoagulant agents.

Cines and coauthors recently published an article in *Blood* on the involvement of the erythrocyte membrane in coagulation. They showed that erythrocytes can develop a hedra-like shape, thereby closing the gaps between the erythrocytes and preventing excessive leakage of fluids when damage occurs to the vasculature. This adaptation

of the erythrocyte configuration is probably the result of clot retraction by platelets squeezing the erythrocytes into the most favorable shape.

H&O Could you please describe your research in this area?

BDL One of the subjects on which my group is working is the active participation of erythrocytes in the formation of a thrombus. In an article that our team recently published with Dr Vivian Du as the first author, we observed a direct erythrocyte–platelet interaction under conditions of venous flow shear rates. This adhesion of erythrocytes to platelets proved to be dependent on the effect of platelet integrin $\alpha_{IIb}\beta_3$ and intercellular adhesion molecule 4 (ICAM-4) on erythrocytes. In this study, we also showed that ICAM-4 on platelets interacts with activated $\alpha_{IIb}\beta_3$. ICAM-4 is an erythroid-specific membrane receptor that belongs to the immunoglobulin superfamily of proteins. Interaction between ICAM-4 and $\alpha_V\beta_3$ has been shown to be involved in the process of sickle cell adhesion to the endothelium, leading to vascular occlusion in the microcirculation.

H&O What else do we know about the role of the red cell membrane in coagulation?

BDL Erythrocytes are the major cellular components of flowing blood and of thrombi formed under conditions of venous flow. They often are considered to be passive participants in coagulation, sealing off the clot by passive entrapment in a fibrin network. However, it is now also known that erythrocytes play a significant role in thrombus formation by exposure of PS on their outer membrane, which is usually on the inside of the erythrocyte. In nonactivated erythrocytes, this asymmetrical distribution of PS between the inside and the outside of the cell is maintained by constant flippase and floppase activity. However, when activated, the erythrocyte membrane exposes more PS on the outer surface owing to the activation of the enzyme scramblase. PS can facilitate the conversion of factor X to factor Xa and the generation of thrombin from prothrombin, as discussed before. A study led by Dr Eduard van Beers that was published in *Haemotologica* showed that erythrocyte-derived microparticles are also be capable of supporting coagulation in a factor XI–dependent manner, as occurs in sickle cell disease.

H&O Is there anything you would like to add?

BDL People should recognize the elegance of erythrocytes, which are more than simple suppliers of oxygen to the organs of the body or passive components of the hemostatic plug. The mechanisms responsible for thrombus formation are not completely elucidated, and I think that the acknowledged role of erythrocytes will only grow during the process of studying the formation of a thrombus. My group has recently found evidence that further supports the hypothesis that erythrocytes actively participate in thrombus formation by interacting with platelets, thereby increasing the number of erythrocytes in a growing thrombus. Several clinical studies are currently on the way to investigate the clinical significance of our findings.

Suggested Readings

Andrews DA, Low PS. Role of red blood cells in thrombosis. *Curr Opin Hematol*. 1999;6(2):76-82.

Cines DB, Lebedeva T, Nagaswami C, et al. Clot contraction: compression of erythrocytes into tightly packed polyhedra and redistribution of platelets and fibrin. *Blood*. 2014;123(10):1596-1603.

Cokelet GR, Goldsmith HL. Decreased hydrodynamic resistance in the two-phase flow of blood through small vertical tubes at low flow rates. *Circ Res*. 1991;68(1):1-17.

Davie EW, Fujikawa K, Kisiel W. The coagulation cascade: initiation, maintenance, and regulation. *Biochemistry*. 1991;30(43):10363-10370.

Du VX, Huskens D, Maas C, Al Dieri R, de Groot PG, de Laat B. New insights into the role of erythrocytes in thrombus formation. *Semin Thromb Hemost*. 2014;40(1):72-80.

Gailani D, Broze GJ Jr. Factor XI activation in a revised model of blood coagulation. *Science*. 1991;253(5022):909-912.

Schmid-Schönbein H, Wells R, Goldstone J. Influence of deformability of human red cells upon blood viscosity. *Circ Res*. 1969;25(2):131-143.

van Beers EJ, Schaap MC, Berckmans RJ, et al; CURAMA study group. Circulating erythrocyte-derived microparticles are associated with coagulation activation in sickle cell disease. *Haematologica*. 2009;94(11):1513-1519.

Whelihan MF, Zachary V, Orfeo T, Mann KG. Prothrombin activation in blood coagulation: the erythrocyte contribution to thrombin generation. *Blood*. 2012;120(18):3837-3845.

Woon LA, Holland JW, Kable EP, Roufogalis BD. Ca2+ sensitivity of phospholipid scrambling in human red cell ghosts. *Cell Calcium*. 1999;25(4):313-320.