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Chapter 3

# Red Blood Cells and Relation to Thrombosis

Anil Tombak

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**Abstract**

Blood coagulation and thrombin generation are primarily a function of platelets, coagulation factors, and endothelial cells. Red blood cells (RBCs) have generally been viewed as innocent bystanders in the clotting process. However, there has been a steadily growing clinical data revealing the active roles of erythrocytes in hemostasis. RBCs may contribute to thrombosis in several ways. In polycythemia, RBCs increase blood viscosity and marginate platelets toward the endothelium. The increased incidence of thrombosis is also associated with hemolytic anemia, especially with sickle cell disease and paroxysmal nocturnal hemoglobinuria. RBCs express phosphatidylserine and microparticles, supporting thrombin generation. They interact with platelets, endothelial cells, and fibrinogen, and these interactions lead their incorporation into the thrombi. The presence of RBCs in clots suppresses plasmin generation and reduces clot dissolution. Decreasing thrombus RBC content would accelerate thrombus resolution. In conclusion, RBCs are important complements of the complex reactions of clot formation.

**Keywords:** thrombin, red blood cells, blood coagulation, thrombophilia

## 1. Introduction

Generation of thrombin is a dynamic process that begins with endothelial injury. Endothelial cells, factors in coagulation cascade, platelets, antithrombotic control mechanisms, and fibrinolytic enzymes play major role in this hemostatic process. In addition, various mechanical factors, including blood flow and intercellular molecular bridges, are also involved in the regulation of primary thrombus formation [1]. Red blood cells (RBCs) are the most abundant blood cells, compromising 35–45% of the blood volume. Their plasma membrane has a unique discoid shape, which provides biological and mechanical properties to RBCs necessary to perform their functions [2]. While the major function of RBCs is hemoglobin-mediated oxygen transport through the body, they also actively participate in both arterial and venous thrombosis.

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## 2. Evidences and mechanisms for erythrocyte participation in thrombus formation

There has been a steadily growing clinical data revealing the active roles of RBCs in hemostasis. First clinical observation about the role of RBCs in coagulation was published in 1910. In this article, Duke noted that thrombocytopenic patients showed an improvement in bleeding times after transfusion, even though their platelet counts remained low [3]. Fifty years later, Hellem et al. reported decrease in bleeding time upon transfusion of washed RBCs in anemic patients with bleeding defects [4]. The causal factor was again assumed to be the erythrocyte. Ho et al. showed the improved bleeding times after RBC transfusions in patients with anemia and thrombocytopenia [5]. Ho et al. also reported the shortening bleeding time in patients with iron deficiency anemia as their hematocrit increases after iron administration [6]. Anemia increases the risk of bleeding, whereas erythrocytosis increases the risk of thrombosis. When the hematocrit reduced, platelets travel closer to center of the vascular lumen and are thus less likely to interact with the subendothelium [7, 8]. Hemoglobin also scavenges nitric oxide (NO) and therefore a reduced hematocrit would be associated with enhanced NO activity and promoting platelet inhibition and vasodilatation [8]. In addition, red blood cells release adenosine diphosphate (ADP) and thromboxane A2 (TXA2) which enhances platelet aggregation [8]. Weiss et al. corrected a platelet adhesion defect present in patients with a platelet storage pool deficiency by RBC transfusion and concluded about the possible role of ADP [9].

In contrast to patients with low hematocrits, abnormally high RBC counts as in polycythemia vera patients predispose to thrombotic disease [10, 11]. An increase in hematocrit is also associated with cerebral infarction and internal carotid atherosclerosis [12, 13]. In addition, diseases which secondarily alter RBC membrane properties can lead to thrombosis; an increase in RBC aggregation has been associated with thrombosis in retinal venous occlusion, leg vein thrombosis, and coronary heart disease [10, 14–16]. In these disorders, thrombus formation was associated with RBC aggregation that blocks microvascular blood flow. An increase in hematocrit leads to an increase in blood viscosity, an increase in RBC aggregation, and/or a decrease in RBC deformability [10, 17]. Increasing hematocrit promotes the transport of platelets and coagulation factors toward the vessel wall, thereby increasing collisions of platelets with the activated endothelium and with themselves (**Figure 1**) [10, 18, 19]. A decrease in RBC deformability may encourage thrombosis by rendering the erythrocyte less capable of squeezing through narrow apertures [10, 17, 20]. In addition, RBCs have been shown to release adenosine triphosphate (ATP) addition to ADP in response to mechanical deformation, as well [21, 22]. Sickle cell disease (SCD) is a well-known hemoglobinopathy in which the deformability of RBCs decreased, thrombin generation and platelet activation increased. Arterial-venous thrombosis can occur during the vaso-occlusive crisis of SCD. RBC membrane proteins can also promote thrombotic episodes and again SCD is a good example for this; microparticles (MPs) are small membrane vesicles that play important roles on coagulation. RBC and platelet-derived MPs can initiate thrombin generation through factor XIIa, presumably via a phosphatidylserine-mediated process (**Figure 1**) [23]. And sickled RBCs not only shed MPs but also there is an abnormal phosphatidylserine (PS) exposure on RBCs as a result of repeated sickling and unsickling processes [24]. An increase

in RBC aggregation and abnormal PS exposure on RBCs have been implicated as possible causative factors of thrombotic complications in beta-thalassemia major cases, as well [10, 25–27]. In addition, under conditions of low $pO_2$ and low pH, which can occur in diseases like hemoglobinopathies, again ATP is secreted by RBCs [28].

Activated platelets express PS on their surfaces which localize the coagulation complexes (intrinsic factor tenase and prothrombinase) to the site of vascular injury and have been viewed as the primary surfaces upon which coagulation occurs [2, 29]. However, normally, a subfraction of RBCs (0.5%) also express PS on their surfaces. With an average RBC count of ~$4 \times 10^9$ $mL^{-1}$, this corresponds to approximately $2.5 \times 10^7$ $mL^{-1}$of PS-expressing RBCs, which is 20% of the average platelet count [2]. So, even a small proportion of PS-positive RBCs could significantly affect thrombin generation and promote fibrin deposition during venous thrombosis [2, 30, 31]. Kawakami et al. identified RBCs as having the most active membrane surface among blood cells and endothelial cells in catalyzing the coagulation process in their *in vitro* study, as well [32].

Horne MK et al. also explored the effect of RBC on thrombin generation in clotting whole blood [33]. They not only found that thrombin concentrations increased as the hematocrit increased from 10 to 40% but also found that maximal thrombin concentration increased when red cell lysate mixed with intact red cells or with platelet. The latter effect was lost by filtering the lysate. The authors concluded that it was due to MPs derived from RBCs, and the effect of intact red cells and MPs derived from RBCs on thrombin generation is probably due to the presence of exposed PS on their membranes [33].

Thrombosis is a well-known complication of paroxysmal nocturnal hemoglobinuria (PNH) and has been suggested due to several pathophysiological sates: a suppressed fibrinolytic

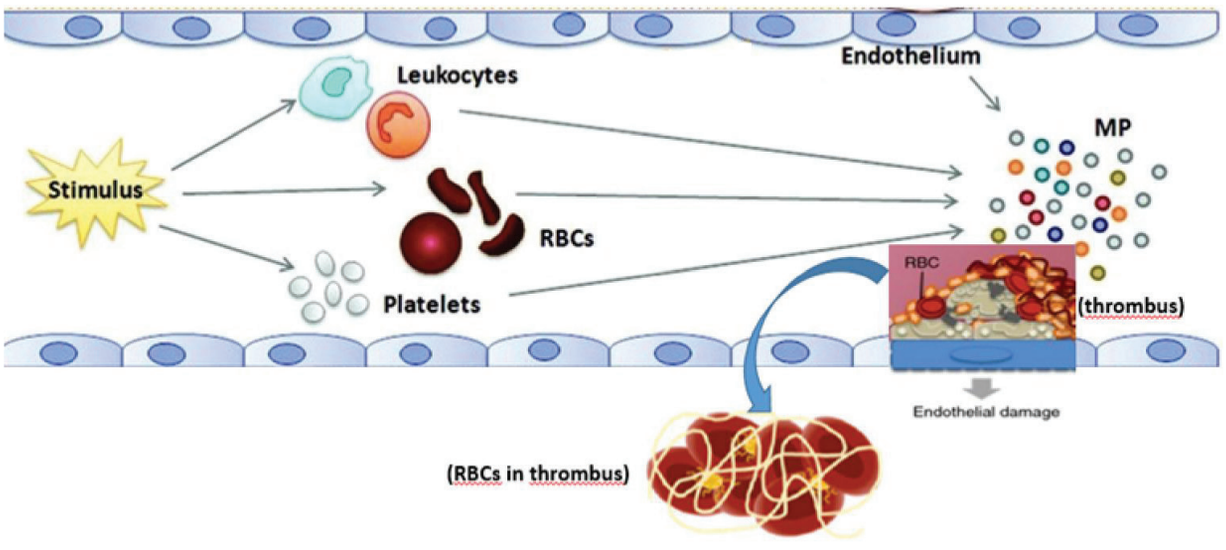


**Figure 1.** In normal conditions, erythrocytes travel in the center of blood flow and platelets travel closer to the endothelial cells. When the hematocrit reduced, platelets travel closer to center of the vascular lumen and are thus less likely to interact with the subendothelium. MPs are small membrane vesicles, which play important roles on coagulation. RBC and platelet-derived MPs can initiate thrombin generation. After formation of the fibrin plaque, RBCs become intertwined within the thrombus to stabilize and strengthen its structure (RBC: red blood cell, MP: microparticle).

system, increased leucocyte-derived tissue factor, complement-mediated damage to platelets and endothelia, and increased platelet derived MPs [34]. Hemolytic attack is often accompanied by thrombosis in PNH and the increased levels of circulating procoagulant MPs derived from hemolyzed RBCs can also contribute thrombophilia by providing the catalytic surface necessary for the assembly of procoagulant, prothrombinase, and tenase enzyme complexes [34]. NO plays an important role in normal platelet functions through the downregulation of platelet aggregation and adhesion. Therefore, NO reduction due to intravascular hemolysis also contributes to thrombogenesis in PNH [34, 35].

Besides all these data about the roles of PS and MPs in thrombogenesis, the erythrocytes do not normally present PS in their outer membrane [10, 36]. For this reason, phospholipid scramblase is required to move the specific aminophospholipids (PS) to an external location. An ATP-requiring mechanism is responsible for this translocation [37] and an increase of the intracellular $Ca^{++}$ concentration in RBC is known to activate the scrambling of membrane phospholipids [37–39]. Phospholipid scrambling plays a stimulatory role in MP generation, as well [40]. Protein kinase C in RBCs mediates the phosphorylation of cytoskeletal proteins and also plays role in $Ca^{++}$ entry into RBCs and subsequent PS exposure on RBC [34, 41, 42].

During clot formation, erythrocytes communicate with platelets as well, and erythrocytes enhance the aggregation of platelets. In the presence of RBCs, greater quantities of free fatty acids and eicosanoid metabolites were generated during platelet activation, rather than in the absence of RBCs [43, 44]. Addition of erythrocytes also enhances platelet degranulation (ADP, serotonin, and beta-thromboglobulin) and aggregation during collagen or thrombin stimulation of platelet-rich plasma [43–48].

RBCs are also incorporated into thrombi via specific interactions during thrombogenesis. RBCs interact with activated endothelial cells (**Figure 2**) and this interaction is demonstrated in a study of arterial thrombosis in which RBCs were the first cells to adhere to a $FeCl_3$-treated intact endothelium, prior to arrival of platelets, and mediate platelet adhesion to the intact endothelial surface [49]. Integrin-mediated interactions between RBCs and leukocytes and platelets may also lead erythrocyte incorporation into thrombi [50]. RBCs bind to platelet $\alpha$IIb$\beta$3 receptor with their intracellular adhesion molecule-4 (ICAM-4) ligand (LW [Landsteiner and Wiener] blood group antigen) and this interaction depends on the platelet activation state [51]. RBC ICAM-4 also interacts with leucocyte $\beta$1 and $\beta$2 integrins [52]. RBCs and fibrinogen also directly interact specifically with each other. Two potential receptors on RBCs have been implicated in fibrinogen-RBC interactions: $\beta$3 or a $\beta$3-like molecule and the integrin-associated protein CD47 [53, 54]. Fibrinogen-mediated transport of factor XIIIa to the clot is necessary for RBC retention in thrombi, as well [55, 56]. Compared to wild-type mice, mice with reduced or delayed factor XIIIa activation produce smaller venous thrombi with reduced RBC content [55]. RBCs affect the structural and mechanical properties of fibrin clots [57]. The interaction of RBCs with fibrin clots (red thrombi) was revealed to be associated with lytic resistance of thrombi due to an increased mechanical strength as compared to clots constituted to plasma only (white thrombi) [58, 59]. In an experimental cerebral ischemia study, it was shown that RBCs within a thrombus transformed from normal discoid shape to form projections which allowed them to interact both with each other and with fibrin fibers. And the authors concluded that through the extension projections, RBCs become intertwined within a thrombus to stabilize and strengthen its structure (**Figure 1**) [57].

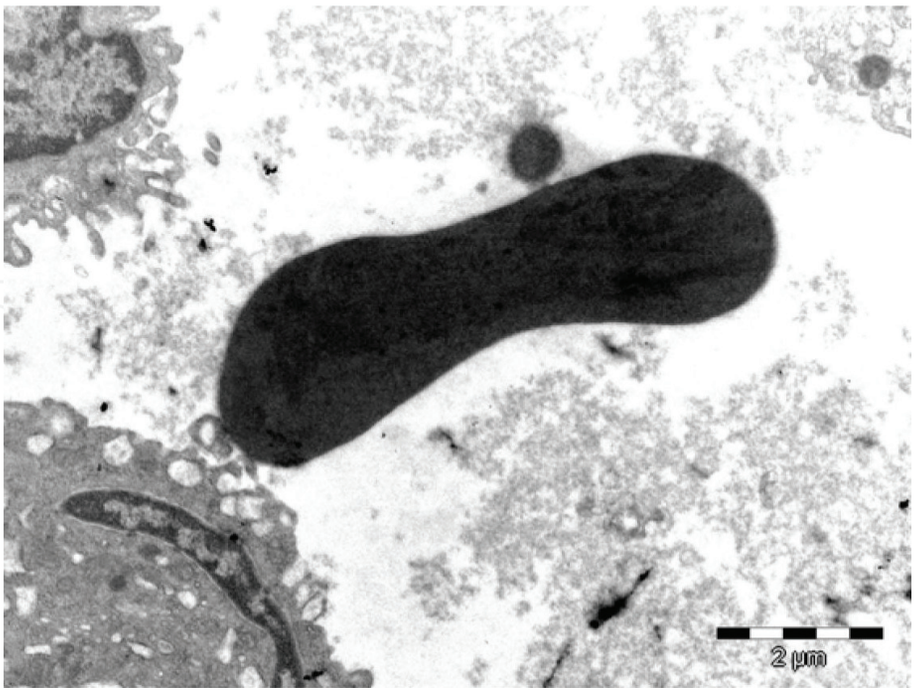


**Figure 2.** Transmission electron microscope of a capillary with a biconcave disk-shaped red blood cell interacting with an endothelial cell (×12,000). By Courtesy of Histology and Embryology Department, Mersin University Medical Faculty.

In summary, RBCs contribute thrombosis by their viscosity effects and by margination of platelets to the vessel wall. However, in addition to these simple viscosity effects of RBC participation in platelet aggregation, RBCs also express PS and MPs, supporting thrombin generation. RBCs interact with platelets, endothelial cells, and fibrinogen, as well and these interactions lead their incorporation into the thrombi. Intertwined RBCs within a thrombus stabilize and strengthens its structure and decrease fibrinolysis. In conclusion, RBCs are important complements of the complex reactions of clot formation.

## Abbreviations

| | |
|---|---|
| RBC | Red blood cell |
| NO | Nitric oxide |
| ADP | Adenosine diphosphate |
| TXA2 | Thromboxane A2 |
| ATP | Adenosine triphosphate |
| SCD | Sickle cell disease |
| MP | Microparticle |
| PNH | Paroxysmal nocturnal hemoglobinuria |
| ICAM-4 | Intracellular adhesion molecule-4 |
| LW | Landsteiner and Wiener |

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