Eryptotic red blood cell adhesion to vascular endothelium: CXCL16/SR-PSOX, a pathological amplifier. Focus on “Dynamic adhesion of eryptotic erythrocytes to endothelial cells via CXCL16/SR-PSOX”

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The study by Lang and colleagues (2), published in this issue, shows that red blood cells (RBCs), undergoing suicidal death or “eryptosis,” attach to human umbilical vascular endothelial cells (HUVECs) under experimental flow conditions via their CXC ligand 16 (CXCL16)/SR-PSOX transmembrane ligands with the potential to greatly impact the microvascular circulation. CXCL16, a chemokine with a cysteine-X-cysteine motif, occurs either in soluble form or as a transmembrane receptor (16) that can be tyrosine phosphorylated and bind Src homology 2-containing proteins; it is synonymous with SR-PSOX, the scavenger receptor for phosphatidylserine (PS) and oxidized low-density lipoproteins (oxLDL) (19). Here, I review developments that underlie this discovery (2), emphasizing the complex cellular mechanisms of erythrocyte-augmented/induced vascular occlusion in conjunction with their suicidal death (eryptosis).

After ~120 days in the circulation, ~3 * 10^9 or one-third milliliter human RBCs/day undergo demise within the reticuloendothelial system by macrophage-mediated phagocytosis. Macrophages, which trap and digest old (but not intact mature) erythrocytes, recognize “senescent” antigens (SCA) of ~62 kDa suggested to be related to band-3 proteins involved in Cl/HCO_3 exchange (7). An alternate hypothesis considers autoantibodies formed against SCA epitopes derived from band-3 aggregation (13). Simultaneously, another concept emerged: reduction of the number of sialic acid residues (~10^9/cell) would uncover the presence of Thomsen (T)-Friedenreich autoantigens binding T-antibodies and complement, thereby leading to rapid hemolysis and RBC removal (10). More recently, intercellular adhesion molecules (ICAMs) and CD47 or integrin-associated protein, both belonging to the Rhesus complex involved in NH_3/NH_4 and CO_2 transport (reviewed in Ref. 11), have been associated with modulation of phagocytosis.

It later became apparent that the loss of membrane lipid asymmetry (15), as a consequence of red cell aging or oxidative stress involving radical oxygen and nitrogen species (RONS) and other insults during circulation (17), could lead to externalization of anionic phospholipids, especially PS, into the outer membrane leaflet—the signal by which monocytes (18) recognize senescent RBCs in vivo (8). Thus, while early studies focused on the physiological removal of circulating RBCs, their potential with external PS as microcirculatory “troublemakers” became evident in clinical syndromes that have greater RBC destruction, such as in hemoglobin disorders (e.g., HbSS disease and β thalassemia), spherocytic hemolytic anemias, hemolytic uremic syndrome, chronic hypercoagulable states, and thrombotic episodes (14). The contribution of “damaged” RBCs with externalized PS to vascular occlusion and crises in hemoglobin disorders and artherosclerotic diseases has been a topic of considerable interest (14).

By forcing RBCs to have PS in the external membrane leaflet, physiologists unraveled the mechanism for its externalization. This work was preceded by and coincided with increased understanding of an ATP-depletion-induced Ca-dependent potassium (K) loss in human RBCs as first described by Gardos (5). The molecular demonstration (6) of a Ca-activated intermediate K conducting channel (K_Ca,3.1, IK, also known as SK4, KCNN4, or the Gardos channel) was an important step and achieved to explain the shrinkage of RBCs that had externalized PS. Thus, simulating RBC aging, accelerated breakdown in disease or challenging the RBC steady state by increasing osmolarity, oxidative stress due to RONS, Ca-ionophores, energy depletion, bacterial hemolysins, heavy metals, flavone-derivatives, and potentially homocysteine appear to be associated with the following cellular events:

1) Activation of cation-nonselective channels, such as transient receptor potential (TRP) type channels, L-type Ca channels (12, 21), and other membrane pathways (reviewed in Ref. 11).
2) A rise of intracellular Ca that overwhams the Ca pump depleting ATP (reviewed in Ref. 11).
3) Ca-dependent inactivation of membrane flippases that return PS to the inside (4).
4) Activation of a phospholipid “scramblase,” one of the “Tubby”-like signaling proteins related to membrane-tethered transcription factors proposed to dissipate the membrane lipid asymmetry and permit PS to appear at the external membrane surface (1).
5) Ca/calmodulin-dependent activation of K efflux through IK channels, which, by membrane hyperpolarization, cause Cl ions to follow and loss of cell water, and hence cell shrinkage (6, 9).
6) Stimulation of RBC membrane sphingomyelininas that form ceramides that further enhance loss of lipid asymmetry (20).

Since cellular K loss occurs with caspase activation in nucleated cells (3), the demise of the erythrocyte has been compared to apoptosis. However, during maturation in the bone marrow, RBCs shed mitochondria (and nuclei) required for intrinsic apoptosis. Lang and colleagues thus ingeniously summarized: “Erythrocyte shrinkage and PS exposure (‘eryptosis’) mimic features of apoptosis which, however, involves several mechanisms lacking in erythrocytes” (9).
Energy depletion of RBCs (for example with deoxy-D-glucose) activates the redox-dependent K-Cl cotransport (KCC) due to kinase inhibition, but repletion of ATP by glucose feeding restores the normally low KCC activity (11). In contrast, eryptosis cannot be reversed once set in motion by 48 h incubation in the absence of glucose (2). Herein lies the amplifying impact of adhesion of eryptotic RBCs to the vascular endothelium.

The molecular link between the doomed, eryptotic RBCs and the vascular endothelium appears to be the availability of macromolecules in the circulation or produced by lymphocytes and vascular endothelial cells (VECs), such as immunoglobulins, thrombospondin, C-reactive protein, fibrinogen, and von Willebrand factor (22). However, Lang and colleagues (2) have advanced our understanding of the interactions between RBCs and VECs by showing that CXCL16/SR-PSOX, a class G scavenger receptor for PS and oxLDL, also binds eryptotic RBCs that display PS on their external leaflet. By simulating flow conditions, the authors show that binding of PS-exposing ATP-depleted RBCs to human VECs can be attenuated or prevented by 1) an antibody directed against CXCL16 on HUVECs, 2) binding of annexin-V to PS-bearing RBCs, and 3) small interfering RNA-mediated knockdown of CXCL16 expression in HUVECs. Thus, in addition to proteins of the coagulation pathway, and to the adhesive role of ICAM-4 to VEC α3/β3 integrins or Lutheran blood group/basal cell adhesion molecule (Lu/BCAM), CXCL16/SR-PSOX is another important link of RBCs and VECs. Moreover, since CXCL16/SR-PSOX is present in macrophages and widely expressed throughout tissues, it likely plays a role in their removal from the circulation if eryptotic RBCs come in contact with this receptor.

Chemokines such as CXCL16/SR-PSOX are part of a cellular continuum that participates in VEC secretion of vasoactive compounds. Attachment of eryptotic RBCs via this particular receptor may continuously modulate vascular response as RBCs enter the microvascular bed, or cause major vascular upheavals in the case of a procoagulant or injured VEC bed and/or with a large increase in the number of PS-exposing cells, as may occur in diseases with systemic vascular participation such as atherosclerosis, hemoglobin disorders, coagulopathies, and others. Therein lies an important amplifier effect of eryptotic RBCs in the pathophysiology of disease, since the findings reported here can perhaps be extrapolated as a general consequence of erythrocyte demise in microcirculatory disorders. Given our increased understanding of the molecular mechanisms described here, it will be interesting to learn whether pharmacological interventions such as IK channel blockers, already used in sickle cell anemia, or SR-PSOX receptor antagonists will ameliorate the acute and chronic microcirculatory events precipitated by eryptotic RBC-VEC interactions.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author.

AUTHOR CONTRIBUTIONS
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REFERENCES