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Review

Mechanisms and pathophysiological significance of eryptosis, the suicidal erythrocyte death

Elisabeth Lang, Florian Lang*

Department of Physiology, University of Tübingen, Gmelinstr. 5, 72076 Tübingen, Germany

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ABSTRACT

Eryptosis, the suicidal erythrocyte death characterized by cell shrinkage and cell membrane scrambling, is stimulated by Ca^{2+} entry through Ca^{2+}-permeable, PGE_2-activated cation channels, by ceramide, caspases, calpain, complement, hyperosmotic shock, energy depletion, oxidative stress, and deranged activity of several kinases (e.g. AMPK, GK, PAK2, CK1α, JAK3, PKC, p38-MAPK). Eryptosis is triggered by intoxication, malignancy, hepatic failure, diabetes, chronic renal insufficiency, hemolytic uremic syndrome, dehydration, phosphate depletion, fever, sepsis, mycoplasma infection, malaria, iron deficiency, sickle cell anemia, thalassemia, glucose 6-phosphate dehydrogenase deficiency, and Wilson's disease. Eryptosis may precede and protect against hemolysis but by the same token result in anemia and deranged microcirculation.

Contents

1. Introduction

The life span of mature circulating erythrocytes is limited by senescence and normally approaches some 100–120 days [1–3]. The senescence involves formation of hemichromes binding to and clustering of the anion exchanger protein band 3 (AE1) with subsequent attachment of complement C3 fragments and anti-band 3 immunoglobulins [4]. Upon injury, erythrocytes may be removed prior to senescence by premature suicidal death or eryptosis, which is characterized by erythrocyte shrinkage and breakdown of the cell membrane asymmetry with translocation of phosphatidylserine from the inner leaflet of the cell membrane to the erythrocyte surface [5,6].

Eryptosis is a physiological mechanism under complex regulation. The present paper describes the cellular mechanisms governing eryptosis and lists the various clinical conditions

Abbreviations: AE1, Band 3 or anion exchanger; AMPK, AMP-activated kinase (AMPK); $[Ca^{2+}]_i$, cytosolic Ca^{2+} concentration; CD36, cluster of differentiation 36; cGK, cGMP-dependent protein kinase; CK1α, casein kinase 1α; CXCL16/SR-PSOX, CXC-Motiv-Chemokin 16/Scavenger receptor for phosphatidylserine and oxidized low density lipoprotein (SR-PSOX)); GSH, reduced glutathion; G6PDH, glucose-6-phosphate dehydrogenase; Hb, hemoglobin; HUS, hemolytic uremic syndrome; JAK3, Janus-activated kinase JAK3; L-NAME, L-NG-nitroarginine methyl ester; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; PAK2, p21-activated kinase PAK2; PGE_2, prostaglandin E_2; ROS, reactive oxygen species; SOD, Cu,Zn-superoxide dismutase; TRPC6, transient receptor potential channel 6; TSP, thrombospondin-1; VLA-4, very late-activating antigen-4.

* Corresponding author at: Physiologisches Institut der Universität Tübingen, Gmelinstr. 5, D 72076 Tübingen, Germany. Tel.: +49 7071 29 72194; fax: +49 7071 29 5618.
E-mail address: florian.lang@uni-tuebingen.de (F. Lang).

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associated with enhanced eryptosis. Several previous reviews have discussed the various aspects of suicidal erythrocyte death [6–12]. Owing to limitation of space, reviews had to be cited at several places instead of original papers.

2. Mechanisms regulating and executing eryptosis

A wide variety of conditions stimulate eryptosis, such as osmotic shock [13], energy depletion [14], oxidative stress [11,15] or increase of temperature [16]. Both cell shrinkage and cell membrane scrambling are triggered by increase of cytosolic Ca^{2+} activity ($[Ca^{2+}]_i$) [6]. Mechanisms increasing $[Ca^{2+}]_i$ include activation of Ca^{2+}-permeable unselective cation channels with subsequent Ca^{2+} entry [6,17]. The channels are permeable to further cations including Na^+ [18]. The cation channels could be activated by prostaglandin E_2 (PGE_2) [19,20] and activation of the channels following osmotic shock is prevented by pharmacological inhibition of cyclooxygenase or phospholipase A_2 [19]. Besides hyperosmotic shock, isoosmotic replacement of NaCl with sorbitol [18], substitution of extracellular Cl^- with gluconate, Br^-, I^- or SCN^- [18], oxidative stress or defects of antioxidative defence [21–24] activate the Ca^{2+}-permeable unselective cation channels thus leading to Ca^{2+} entry and eryptosis [5,18]. The cation channels involve the transient receptor potential channel TRPC6 and are thus inhibited by antibodies against TRPC6 and blunted in erythrocytes drawn from TRPC6 deficient mice [6]. The Ca^{2+}-permeable unselective cation channels and thus eryptosis are inhibited by erythropoietin [25,26].

An increase of $[Ca^{2+}]_i$ following activation of the cation channels leads to activation of Ca^{2+}-sensitive K^+ channels [6,27] followed by cell membrane hyperpolarization and increase of the electrical driving force for Cl^- exit. The cellular loss of KCl with osmotically obliged water thus leads to cell shrinkage [28]. Cl^- exits through Cl^- channels [6], which are similarly activated by oxidative stress [29,30]. The exit of K^+ through Ca^{2+}-sensitive K^+ channels and the entry of Na^+ through unselective cation channels dissipates the respective ion gradients across the cell membrane. The dissipation of the K^+ gradient could eventually lead to cell membrane depolarization and thus to entry of Cl^- and cell swelling [31]. Excessive cell swelling may lead to disruption of the cell membrane and thus to hemolysis [18,31].

An increase of $[Ca^{2+}]_i$ further triggers cell membrane scrambling with translocation of phosphatidylserine from the inner leaflet of the cell membrane to the erythrocyte surface [32]. The Ca^{2+} sensitivity of cell membrane scrambling is enhanced by ceramide [6]. Hyperosmotic shock activates a phospholipase A_2 with subsequent release of platelet activating factor (PAF). The released PAF activates a sphingomyelinase, which generates ceramide [6].

Erythrocytes express caspases [33,34], which are activated by some stimulators of eryptosis [6,35,36], cleave the anion exchanger AE1 [33] and trigger phosphatidylserine exposure of erythrocytes [37]. For instance caspase activation is involved in the stimulation of eryptosis by leukotrienes [16], α-lipoic acid [6], and oxidative stress [38]. However, most stimulators of eryptosis, including Ca^{2+} entry and Ca^{2+}-dependent cell membrane scrambling, do not require activation of caspases [32,39,40].

Several kinases participate in the regulation of eryptosis. Eryptosis could be stimulated by Janus-activated kinase JAK3 [41]. JAK3 is activated by ATP depletion [41] and participates in the stimulation of cell membrane scrambling following energy depletion [41]. Accordingly, the stimulating effect of energy depletion on cell membrane scrambling is significantly blunted following pharmacologic or genetic knockout of JAK3 [41]. The cell membrane scrambling following energy depletion is further blunted following pharmacological inhibition of protein kinase C (PKC) [14], In contrast, eryptosis is counteracted by the similarly energy sensing AMP-activated kinase (AMPK) [17]. Genetic knockout of AMPKα1 is followed by enhanced spontaneous eryptosis [17] leading to profound anemia and splenomegaly [17]. The AMPK deficiency leads to downregulation of p21-activated kinase 2 (PAK2), a kinase, which may similarly contribute to inhibition of eryptosis [42]. Along those lines pharmacological inhibition of PAK2 with IPA3 leads to cell membrane scrambling and augments the cell membrane scrambling following energy depletion [42].

Eryptosis following exposure to oxidative stress or energy depletion is blunted by pharmacological inhibition of casein kinase 1α (CK1α) [43]. CK1α inhibitors are at least partially effective by blunting the increase of $[Ca^{2+}]_i$ following oxidative stress or energy depletion [43]. Conversely, pharmacological activation of CK1α stimulates the unselective cation channels thus leading to increase of $[Ca^{2+}]_i$ [44].

Exposure of erythrocytes to hyperosmotic shock activates the p38 mitogen activated protein kinase [45] and the eryptosis following osmotic shock is disrupted by p38 protein kinase inhibitors [45]. Eryptosis is triggered by the tyrosine kinase inhibitors sorafenib [46] and sunitinib [47], providing circumstantial evidence that tyrosine kinases counteract stimulation of eryptosis.

Eryptosis is further inhibited by the cGMP-dependent protein kinase (cGKI) [48]. Accordingly, enhanced spontaneous eryptosis is observed in cGKI deficient mice, which suffer from severe anemia and splenomegaly [48]. The cGKI is in part effective by blunting the increase of $[Ca^{2+}]_i$ [48] The cGKI is stimulated by nitric oxide (NO) [49–52], which is stored by erythrocytes in large quantities and released from erythrocytes following deoxygenation of hemoglobin [53–55]. NO is the most powerful inhibitor of eryptosis [56], an effect which can also be observed with NO-donors, such as nitroprusside or papanonoate [56]. Similar to NO, dibutyryl-cGMP inhibits eryptosis [56]. NO inhibits eryptosis at concentrations similar to or even below the concentrations shown to inhibit apoptosis of nucleated cells [57,58]. As NO counteracts eryptosis following exposure to the Ca^{2+} ionophore ionomycin without appreciably blunting the ionomycin-induced increase of $[Ca^{2+}]_i$, it is at least partially effective downstream of Ca^{2+} [56]. In nucleated cells NO is partially effective by inhibiting caspases [59,60], which are, however, not required for the stimulation of eryptosis by increased $[Ca^{2+}]_i$ [6]. Similar to what has been shown in nucleated cells [61–64], NO triggers nitrosylation of enzymes, which are necessary for induction of cell membrane scrambling [56]. Conversely, ionomycin treatment decreases protein S-nitrosylation [56]. Enzymes activated by S-nitrosylation include the antiapoptotic enzyme thioredoxin [56,62], which counteracts oxidative stress [62]. In contrast to low nitroprusside concentrations [56], excessive nitroprusside concentrations stimulate eryptosis, an effect presumably due to oxidative stress [65–67]. The release of NO is particularly fast from fetal hemoglobin HbF, which may thus protect against eryptosis and be particularly effective in triggering vasodilation [68,69]. Increased expression of HbF thus counteracts oxidative stress and presumably eryptosis in sickle cell disease [70].

Erythrocytes from newborns are particularly sensitive to oxidative stress, but are relatively resistant to other stimulators of eryptosis [71–73]. The enhanced sensitivity of fetal erythrocytes to oxidative stress is not relevant for their intrauterine survival but presumably contributes to their rapid removal after birth. After birth, fetal erythrocytes are exposed to oxidative stress in the inflated lung. The removal of the fetal erythrocytes and subsequent replacement by adult erythrocytes is physiologically important, as the high oxygen affinity of HbF is favorable in the oxygen depleted intrauterine environment but not after birth [74].

Aged erythrocytes are particularly sensitive to stimulation of eryptosis by oxidative stress [75], a property reversed by the antioxidant N-acetyl-L-cysteine [75]. The survival of newly formed