Physiology and Pathophysiology of Eryptosis

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Keywords

Erythrocytes · Apoptosis · Anemia · Malaria · Iron deficiency · HUS · Sepsis · Renal insufficiency · Diabetes

Summary

Suicidal erythrocyte death (eryptosis) is characterized by cell shrinkage, cell membrane blebbing, and cell membrane phospholipid scrambling with phosphatidylserine exposure at the cell surface. Eryptotic cells adhere to the vascular wall and are rapidly cleared from circulating blood. Eryptosis is stimulated by an increase in cytosolic Ca^{2+} activity, ceramide, hyperosmotic shock, oxidative stress, energy depletion, hyperthermia, and a wide variety of xenobiotics and endogenous substances. Inhibitors of eryptosis include erythropoietin and nitric oxide. Enhanced eryptosis is observed in diabetes, renal insufficiency, hemolytic uremic syndrome, sepsis, mycoplasma infection, malaria, iron deficiency, sickle cell anemia, beta-thalassemia, glucose-6-phosphate dehydrogenase (G6PD) deficiency, hereditary spherocytosis, paroxysmal nocturnal hemoglobinuria, Wilson's disease, myelodysplastic syndrome, and phosphate depletion. Eryptosis is further enhanced in gene-targeted mice with deficient annexin 7, cGMP-dependent protein kinase type I (cGKI), AMP-activated protein kinase (AMPK), anion exchanger 1 (AE1), adenomatous polyposis coli (APC), and Klotho, as well as in mouse models of sickle cell anemia and thalassemia. Decreased eryptosis is observed in mice with deficient phosphoinositide-dependent kinase 1 (PDK1), platelet activating factor (PAF) receptor, transient receptor potential channel 6 (TRPC6), janus kinase 3 (JAK3), and taurine transporter (TAUT). Eryptosis may be a useful mechanism to remove defective erythrocytes prior to hemolysis. Excessive eryptosis may, however, compromise microcirculation and lead to anemia.

Schlüsselwörter

Erythrozyten · Apoptose · Anämie · Malaria · Eisenmangel · HUS · Sepsis · Niereninsuffizienz · Diabetes

Zusammenfassung

Der suizidale Erythrozytentod (Eryptose) ist durch Zellschrumpfung, Zellmembran-Blebbing und Scrambling der Zellmembranphospholipide mit Exponierung von Phosphatidylserin auf der Zelloberfläche gekennzeichnet. Eryptotische Zellen haften sich an die Gefäßwand und werden schnell aus dem Blutstrom entfernt. Eryptose wird durch erhöhte zytosolische Ca^{2+} -Aktivität, Ceramid, hyperosmotischen Schock, oxidativen Stress, Energie-Depletion, Hyperthermie sowie ein breites Spektrum an Xenobiotika und endogenen Substanzen stimuliert. Zu den Eryptosehemmern zählen Erythropoetin und Stickoxid. Gesteigerte Eryptose wird bei Diabetes, Niereninsuffizienz, hämolytisch-urämischem Syndrom, Sepsis, Mykoplasmeninfektionen, Malaria, Eisenmangel, Sichelzellanämie, Beta-Thalassämie, Glukose-6-Phosphat-Dehydrogenase(G6PD)-Mangel, hereditärer Sphärozytose, paroxysmaler nächtlicher Hämoglobinurie, Morbus Wilson, myelodysplastischem Syndrom und Phosphat-Depletion beobachtet. Des Weiteren ist die Eryptose bei gentechnisch veränderten Mäusen ohne Annexin 7, cGMP-abhängiger Proteinkinase Typ I (cGKI), AMP-aktivierter Proteinkinase (AMPK), Anionenaustauscher (AE1), adenomatöses Polyposis-coli-Protein (APC) und Klotho sowie in Mausmodellen für Sichelzellanämie und Thalassämie gesteigert. Verminderte Eryptose wird bei Mäusen mit fehlendem PDK1(phosphoinositide-dependent kinase 1)-, PAF(platelet activating factor)-Rezeptor, TRPC6 (transient receptor potential channel 6), Januskinase 3 (JAK3) und Taurintransporter (TAUT) beobachtet. Eryptose könnte ein hilfreicher Mechanismus zur Entfernung fehlerhafter Erythrozyten vor Hämolyse sein. Exzessive Eryptose könnte jedoch die Mikrozirkulation beeinträchtigen und zu Anämie führen.

Introduction

In the absence of injury, the erythrocyte lifespan approaches 100–120 days [1]. It is ultimately limited by senescence [2] which results from binding of modified hemoglobin to band 3, followed by modification of band 3, binding of autologous immunoglobulin G (IgG), disruption of the band 3-dependent anchorage of the cytoskeleton to the lipid bilayer, and formation of vesicles that expose cell antigens and phosphatidylserine at their surface [2]. The vesicles are subsequently bound to scavenger receptors, engulfed, and thus removed [2]. Erythrocytes may experience survival-threatening injury prior to senescence. In that case, they may enter programmed cell death or eryptosis [3]. Similar to programmed death of nucleated cells or apoptosis, eryptosis is a coordinated suicidal death eventually leading to disposal of defective cells without rupture of the cell membrane and release of intracellular material [3]. In contrast to nucleated cells, however, erythrocytes lack nuclei and mitochondria [1] which actively participate in the machinery underlying apoptosis [4, 5]. Eryptosis thus lacks several hallmarks of apoptosis, such as mitochondrial depolarization and condensation of nuclei. Moreover, the signaling eventually leading to eryptosis [3] is not identical to that underlying apoptosis [4, 5]. Nevertheless, eryptosis shares several features of apoptosis, such as cell shrinkage, cell membrane blebbing, and cell membrane scrambling with phosphatidylserine exposure at the cell surface [3]. Similar to apoptotic cells and particles, eryptotic cells and particles are engulfed and degraded by macrophages [3, 6]. Also similar to apoptosis, eryptosis allows removal of defective, infected, or otherwise potentially harmful cells. The present review briefly describes mechanisms known to trigger and inhibit eryptosis. Due to limitation of space, a previous review [3] is cited instead of earlier original papers.

Triggers and Signaling of Eryptosis

Eryptosis is stimulated by an increase in cytosolic Ca^{2+} activity which may be the consequence of increased Ca^{2+} entry across the erythrocyte cell membrane [3]. Erythrocytes express Ca^{2+} -permeable non-selective cation channels. The molecular identity of those channels has remained ill-defined but somehow involves transient receptor potential channel 6 TRPC6 [3]. Along those lines, Ca^{2+} entry is blunted in erythrocytes drawn from TRPC6 knockout mice [3]. Stimulators of the non-selective cation channels include oxidative stress, Cl^- removal, and hyperosmotic shock [3]. Osmotic shock is in part effective by stimulating the release of prostaglandin E2 which in turn activates the cation channels [3]. An increase in cytosolic Ca^{2+} is followed by activation of Ca^{2+} -sensitive K^+ channels [3] with subsequent exit of K^+ , hyperpolarization of the cell membrane, Cl^- exit, cellular loss of KCl, and exit of osmotically obliged water resulting in cell shrinkage [3]. In addition, Ca^{2+}

activates calpain, a cysteine endopeptidase degrading cytoskeletal proteins and thus fostering cell membrane blebbing [3]. Increased cytosolic Ca^{2+} activity further leads to stimulation of cell membrane scrambling with breakdown of phospholipid asymmetry of the cell membrane and phosphatidylserine exposure at the cell surface [3]. Cell shrinkage augments the Ca^{2+} -induced cell membrane scrambling [3].

The Ca^{2+} sensitivity of erythrocyte cell membrane scrambling is enhanced by ceramide which, similar to cellular Ca^{2+} accumulation, stimulates eryptosis. Ceramide may be generated by acid sphingomyelinase which produces ceramide from membrane sphingomyelin [3]. Stimulators of acid sphingomyelinase include platelet-activating factor (PAF) which is formed by phospholipase-dependent degradation of cell membrane lipids [3]. A stimulator of phospholipase is hyperosmotic shock [3]. Accordingly, eryptosis following osmotic shock is blunted in gene-targeted mice lacking PAF receptors [3].

Erythrocytes express p38 kinase which is activated by hyperosmotic shock [7]. The p38 kinase inhibitors SB203580 and p38 Inh III blunt the eryptosis following osmotic shock [7]. Erythrocytes further express casein kinase 1 (CK1). The CK1 inhibitors D4476 and (R)-DRF053 blunt eryptosis following energy depletion or oxidative stress [8]. Conversely, eryptosis is stimulated by the CK1 α -specific activator pyrinium pamoate [8]. CK1 is effective by activation of Cl^- -sensitive Ca^{2+} -permeable cation channels [8]. On the other hand, pyrinium pamoate inhibits Ca^{2+} -activated K^+ channels and thus counteracts eryptotic cell shrinkage [9].

Erythrocytes further express Janus kinase 3 (JAK3) which is phosphorylated and thus activated following glucose depletion [10]. Erythrocytes from JAK3-deficient mice are slightly smaller than erythrocytes from wild-type mice. Genetic knockout of JAK3 or treatment with JAK3 inhibitors WHI-P131/JANEX-1 (4-(4'-hydroxyphenyl)-amino-6,7-dimethoxyquinazoline) and WHI-P154 (4-(3'-bromo-4'-hydroxyphenyl)-amino-6,7-dimethoxyquinazoline) do not significantly modify cell membrane scrambling in the absence of triggers of eryptosis, but counteract eryptosis following energy depletion [10].

Eryptosis is further stimulated following activation of protein kinase C (PKC) [3]. Similar to JAK3, PKC is activated during energy depletion, a powerful stimulator of eryptosis [3]. Inhibition of PKC blunts eryptosis following energy depletion [3].

Another stimulator of eryptosis is oxidative stress which is at least in part effective by opening of the unspecific cation channels with subsequent Ca^{2+} entry, by activation of erythrocyte oxidation-sensitive Cl^- channels, and by activation of caspases [3]. It is noteworthy, however, that caspases are not required for stimulation of eryptosis following Ca^{2+} entry. The role of CD95, an important apoptosis-inducing receptor, for erythrocyte phosphatidylserine exposure is controversial [11, 12].

The sensitivity of erythrocytes to suicidal death depends on age. Erythrocytes of different age can be separated according

Table 1. Substances stimulating eryptosis

Substance	Reference	Substance	Reference
A23187 (Ca ²⁺ ionophore)	[3, 41]	Hemin	[3]
α-Lipoic acid	[42]	Hemolysin	[3]
Aluminium	[43]	Immunoglobulin A	[55]
Amantadine	[3]	IPA3	[56]
Amiodarone	[3]	Lead	[3]
Amphotericin B	[3]	Leukotriene C ₄	[3]
Amyloid	[3]	Lipopeptides	[3]
Anandamide	[3]	Listeriolysin	[3]
Anti-A IgG	[3]	Lithium	[57]
Apigenin	[44]	Lysophosphatidic acid	[58]
Arsenic	[3]	Mercury	[3]
Azathioprine	[3]	Methyldopa	[3]
Bay 11–7082	[45]	Methylglyoxal	[3]
Bay-Y5884	[3]	Monensin	[59]
Beauvericin	[46]	Oridonin	[60]
Benzethonium	[47]	Paclitaxel	[3]
Bismuth chloride	[3]	PAF	[3]
Cadmium	[3]	Parthenolide	[45]
Ceramide (acylsphingosine)	[3]	Peptidoglycan	[3]
Chlorpromazine	[3]	Phytic acid	[61]
Chromium	[48]	Phorbol-12 myristate-13 acetate	[58]
Ciglitazone	[3]	Polyphyllin	[62]
Cisplatin	[3]	Prostaglandin E ₂	[3]
Copper	[3]	Radiocontrast agents	[3]
Cordycepin	[3]	Retinoic acid	[3]
Curcumin	[3]	Selenium (sodium selenite)	[3]
Cyclosporine	[3]	Silver ions	[3]
CD95/Fas/ligand	[3, 12]	Sphingosine	[63]
Dicoumarol	[49]	Thrombospondin-1 receptor CD47	[3]
Dimethylfumarate	[50]	Thymoquinone	[3]
Enniatin A	[51]	Tin	[3]
FTY720	[52]	Ursolic acid	[64]
Glycation	[26]	Valinomycin	[3]
Glycophorin-C	[3]	Vanadate	[3]
Gold	[53]	Vitamin K(3)	[3]
H ₂ O ₂	[54]	Zinc	[3]

to density which increases with age. Utilizing this method, a positive correlation was found between age and the percentage of erythrocytes undergoing spontaneous cell membrane scrambling [13]. The increase of cell membrane scrambling in aged erythrocytes is reversed in addition of the antioxidant N-acetyl-L-cysteine which increases the in vivo half-life of circulating mouse erythrocytes [13]. Accordingly, the increased susceptibility of aged erythrocytes to eryptosis is at least partially due to increasing sensitivity to oxidative stress.

On the other hand, preferential suicidal death of young erythrocytes or ‘neocytolysis’ has been observed in individuals returning from high altitude or space flight [14]. It has been speculated that the enhanced erythropoietin concentrations as a consequence of high altitude or space flight confer survival of progenitor cells otherwise prone to undergo apoptosis, thus generating erythrocytes particularly sensitive to eryptosis [3]. Those erythrocytes survive at high erythropoietin concentrations but die as soon as erythropoietin concen-

trations decline. Eryptosis following decreasing erythropoietin concentrations upon return from hypoxic conditions may rapidly readjust the erythrocyte number to the requirements under normoxic conditions. Thus, an otherwise extremely slow negative feedback loop is accelerated. Additional experimental effort is needed to prove or disprove this speculation.

Eryptosis is further stimulated by a myriad of xenobiotics and a wide variety of endogenous substances and challenges (table 1).

Inhibitors of Eryptosis

The unselective Ca²⁺-permeable cation channels are inhibited by erythropoietin which thus may counteract Ca²⁺ entry and eryptosis [3]. Administration of erythropoietin to patients on hemodialysis thus decreases the percentage of circulating eryptotic erythrocytes [3]. The effect of erythropoietin is rapid and

reversible. Ca^{2+} entry and subsequent eryptosis following energy depletion are further counteracted by endothelin 1 and the endothelin B (ETB) receptor agonist sarafotoxin, while endothelin 2 or endothelin 3 do not appreciably modify eryptosis [15]. Mice lacking the ETB receptor suffer from increased eryptosis leading to splenomegaly and compensatory stimulation of erythropoiesis with reticulocytosis [15].

Eryptosis is inhibited by the AMP-activated kinase (AMPK) which is activated by energy depletion [3]. Similar to ETB receptor knockout mice, AMPK1 α -deficient mice suffer from excessive eryptosis with splenomegaly and reticulocytosis [3]. Activation of AMPK is able to blunt but not abrogate eryptosis upon energy depletion.

Eryptosis is further inhibited by cGMP-dependent protein kinase type I (cGKI) [3]. Gene-targeted mice lacking cGKI thus suffer from excessive eryptosis [3]. Dibutyl- cGMP blunts the Ca^{2+} -induced, but not the ceramide-induced, stimulation of cell membrane scrambling [16]. cGKI is activated by nitric oxide (NO), a powerful inhibitor of erythrocyte cell membrane scrambling [3]. The NO donors nitroprusside and papanoate abrogate the cell membrane scrambling following treatment with the Ca^{2+} ionophore ionomycin without preventing the increase in cytosolic Ca^{2+} activity [3]. Accordingly, NO disrupts at least in part the effect of Ca^{2+} on cell membrane scrambling. Excessive nitroprusside concentrations, however, trigger eryptosis [3]. Ionomycin decreases thioredoxin activity and abundance of nitrosylated protein, effects reversed by the NO donor papanoate [3].

Eryptosis is also inhibited by adenosine [3], amitriptyline [3], blebbistatin [17], caffeine [3], catecholamines such as isoproterenol [3], chloride [3], EIPA [3], endothelin 1 (see above), erythropoietin [3], flufenamic acid [3], NBQX/CNQX [3], niflumic acid [3], NO (nitroprusside, see above), NPPB [3], phlorhizin [18], resveratrol [3], sarafotoxin 6c (see above), staurosporine [3], thymol [3], urea [3], WHI-P131/JANEX-1 [10], WHI-P154 [10], and xanthohumol [3].

Physiological Significance of Eryptosis

Suicidal erythrocyte death accomplishes the clearance of defective erythrocytes prior to hemolysis. The phosphatidylserine at the surface fosters adherence of eryptotic cells to endothelial cells and macrophages with subsequent engulfment and intracellular degradation. Without removal, the defective erythrocytes may undergo hemolysis. Compromised Na^+/K^+ -ATPase activity (e.g. in energy depletion) or increased cell membrane leakiness (e.g. following exposure to toxins) result in cellular gain of Na^+ and Cl^- together with osmotically obliged water and thus lead to cell swelling [3]. Excessive cell swelling is followed by rupture of the cell membrane with release of cellular hemoglobin which may be filtered in the renal glomerula and occlude renal tubules [19]. Activation of Ca^{2+} -sensitive K^+ channels during eryptosis hyperpolarizes the cell

membrane, thus enhancing the electrical driving force for Cl^- exit. Cellular loss of KCl counteracts cell swelling and delays hemolysis, thus providing some additional time for the clearance of the defective erythrocytes.

Suicidal erythrocyte death further accomplishes the clearance of infected erythrocytes [3]. A major pathogen entering erythrocytes is the malaria parasite *Plasmodium* [20]. The intracellular pathogen generates oxidative stress resulting in activation of several channels including the unselective cation channels [20]. Pathogen survival depends on Na^+ and Ca^{2+} entry through those channels. However, opening of the channels triggers eryptosis which may eventually lead to clearance of the infected erythrocyte from circulating blood. Accordingly, pathogen-induced eryptosis decreases parasitemia and thus may be an important defense mechanism against *Plasmodium* infection. Accordingly, several diseases or genetic traits predisposing to eryptosis confer some protection against a severe course of malaria, such as sickle cell trait, beta-thalassemia trait, glucose-6-phosphate dehydrogenase (G6PD) deficiency, and iron deficiency. Moreover, several eryptosis-inducing drugs delay the development of parasitemia and ameliorate the clinical course of malaria, such as paclitaxel, chlorpromazine, cyclosporine, curcumin, PGE_2 , and lead.

Pathophysiological Significance of Eryptosis

The clearance of eryptotic cells from circulating blood may result in anemia [3], if accelerated eryptosis cannot be compensated by similarly enhanced formation of new erythrocytes. Prior to anemia accelerated eryptosis may be apparent from the enhanced appearance of reticulocytes [3].

Eryptotic cells adhere to endothelial CXCL16/SR-PSO [21] and presumably further, as yet undefined, molecular binding partners. The phosphatidylserine-binding glycoprotein lactadherin confers adhesion of apoptotic lymphocytes but is apparently not important for the clearance of phosphatidylserine-exposing erythrocytes [22]. The adherence of phosphatidylserine-exposing erythrocytes to the vascular wall may impede microcirculation [3]. Phosphatidylserine-exposing erythrocytes further stimulate blood clotting [23]. Thus, excessive eryptosis may foster thrombosis.

Several clinical conditions are associated with enhanced eryptosis [3]. Accelerated eryptosis in iron deficiency may result from decreased erythrocyte volume which sensitizes erythrocytes to triggers of eryptosis [3]. Enhanced eryptosis is further observed in diabetes [24, 25]. Exposure of erythrocytes to excessive glucose concentrations stimulates cell membrane scrambling [26]. Moreover, in diabetes, eryptosis may be stimulated by methylglyoxal (see above). Eryptosis is further enhanced in renal insufficiency [3]. Enhanced eryptosis in renal insufficiency may similarly be in part due to methylglyoxal (see above). Eryptosis is further enhanced following phosphate depletion [3].

Eryptosis is stimulated by hyperthermia and may thus be enhanced during fever [27]. Eryptosis in hyperthermia is at least in part the result of enhanced leukotriene formation and could thus be inhibited by the leukotriene receptor CysLT1 antagonist cinalukast [27]. Excessive eryptosis is observed in blood drawn from septic patients who harbor a factor in their plasma triggering formation of ceramide and stimulating Ca^{2+} entry, thus leading to erythrocyte cell membrane scrambling and erythrocyte shrinkage [3]. Some pathogens produce eryptosis-inducing molecules such as bacterial sphingomyelinase which may at least contribute to the eryptosis in septic patients [3]. Excessive eryptosis is further observed in hemolytic uremic syndrome (HUS) [3]. Also, plasma isolated from patients with HUS stimulates erythrocyte cell membrane scrambling. The eryptosis in HUS is probably in part the consequence of complement activation [3]. Eryptosis is further stimulated by bacterial hemolysin [3]. The eryptosis in hemolytic disorders presumably serves to clear defective erythrocytes prior to hemolysis (see above). Possibly, hemolysis is only experienced by those erythrocytes that are not cleared away fast enough by a phagocytosing cell.

Eryptosis is triggered following infection with mycoplasma [28] or, as pointed out above, by the malaria pathogen *Plasmodium* [20, 29–34]. Intraerythrocytic plasmodia induce oxidative stress which in turn activates the Ca^{2+} -permeable cation channels leading to Ca^{2+} entry and eryptosis. The phosphatidylserine-exposing erythrocytes are phagocytosed, which leads to the removal not only of the erythrocytes but also of the pathogen (see above). Accordingly, eryptosis counteracts parasitemia. Thus, at least in mice, several eryptosis-inducing substances favorably influence the clinical course of the disease [3, 29, 30, 32]. Moreover, genetic defects in humans and mice resulting in accelerated eryptosis may confer partial protection against a severe course of malaria [20].

Eryptosis is accelerated in sickle cell anemia [3, 35], thalassemia [36], G6PD deficiency [3, 37], defective anion exchanger 1 (AE1) [3], in a GLUT1 mutation turning this carrier into a Ca^{2+} channel [3], and hereditary spherocytosis [36]. Enhanced eryptosis is further observed in Wilson's disease [3], paroxysmal nocturnal hemoglobinuria [37], and myelodysplastic syndrome [38]. Paroxysmal nocturnal hemoglobinuria and myelodysplastic syndrome affect preferably lighter and thus presumably younger erythrocytes, pointing to enhanced neocytolysis in those disorders [38]. Accelerated eryptosis has been

observed in several gene-targeted mice, such as mice mimicking the defect of sickle cell anemia or thalassemia [3]. Enhanced eryptosis is further observed in annexin 7-deficient mice with enhanced formation of PGE_2 [39] which activates the cation channels and thus triggers eryptosis (see above). Eryptosis is further enhanced in mice lacking cGKI (see above), AMPK (see above), and ETB receptor (see above). Gene-targeted mice lacking the AE1 similarly suffer from excessive eryptosis leading to anemia despite enhanced formation of new erythrocytes [3]. Erythropoietin-overexpressing mice generate erythrocytes with enhanced sensitivity to eryptotic stimuli [3]. Eryptosis is further enhanced in Klotho-deficient mice [3]. In those mice, the excessive eryptosis could be reversed with a vitamin D-deficient diet, and thus directly or indirectly results from excessive $1,25(\text{OH})_2\text{D}_3$ formation [3]. In adenomatous polyposis coli (APC)-deficient mice, eryptosis appears in parallel to the development of intestinal tumors [40].

Decreased eryptosis has been observed in gene-targeted mice lacking phosphoinositide-dependent kinase (PDK1) [3], PAF receptor [3], TRPC6 [3], JAK3 [10], and taurine transporter (TAUT) [3].

Conclusion

Much has been learned in the past few years about cellular mechanisms triggering and counteracting suicidal erythrocyte death or eryptosis. It is obvious that suicidal erythrocyte death is triggered by a myriad of xenobiotics and endogenous challenges and that it participates in the pathophysiology of several clinical conditions. Nevertheless, our knowledge about mechanisms underlying eryptosis is still incomplete. Moreover, a wide variety of further xenobiotics and endogenous small molecules presumably stimulate or inhibit eryptosis. Excessive eryptosis most likely also plays a role in other, yet unrecognized, clinical conditions. Clearly, extensive additional experimental effort is required to fully understand this physiologically and pathophysiologically important fundamental cellular mechanism.

Disclosure Statement

The authors have nothing to disclose.

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