ANTIOXIDANTS & REDOX SIGNALING
Volume 8, Numbers 7 & 8, 2006

Forum Review

Mechanisms and Significance of Eryptosis

FLORIAN LANG, KARL S. LANG, PHILIPP A. LANG,
STEPHAN M. HUBER, and THOMAS WIEDER

ABSTRACT

Suicidal death of erythrocytes (eryptosis) is characterized by cell shrinkage, membrane blebbing, activation of proteases, and phosphatidylserine exposure at the outer membrane leaflet. Exposed phosphatidylserine is recognized by macrophages that engulf and degrade the affected cells. Eryptosis is triggered by erythrocyte injury after several stressors, including oxidative stress. Besides caspase activation after oxidative stress, two signaling pathways converge to trigger eryptosis: (a) formation of prostaglandin E_2 leads to activation of Ca^{2+}-permeable cation channels, and (b) the phospholipase A_2–mediated release of platelet-activating factor activates a sphingomyelinase, leading to formation of ceramide. Increased cytosolic Ca^{2+} activity and enhanced ceramide levels lead to membrane scrambling with subsequent phosphatidylserine exposure. Moreover, Ca^{2+} activates Ca^{2+}-sensitive K^+ channels, leading to cellular KCl loss and cell shrinkage. In addition, Ca^{2+} stimulates the protease calpain, resulting in degradation of the cytoskeleton. Eryptosis is inhibited by erythropoietin, which thus extends the life span of circulating erythrocytes. Eryptosis may be a mechanism of defective erythrocytes to escape hemolysis. Conversely, excessive eryptosis favors the development of anemia. Conditions with excessive eryptosis include iron deficiency, lead or mercury intoxication, sickle cell anemia, thalassemia, glucose 6- phosphate dehydrogenase deficiency, malaria, and infection with hemolysin-forming pathogens. *Antioxid. Redox Signal.* 8, 1183–1192.

INTRODUCTION

ABUNDANT, DEFECTIVE OR POTENTIALLY HARMFUL NUCLEATED CELLS are disposed by apoptosis (7, 19, 50, 51, 89, 113, 121), which is triggered either by stimulation of respective receptors such as CD95 (26, 74) or by cell exposure to stressors such as oxidants, cytostatic drugs, radiation, or osmotic shock (12, 50, 108, 122). Typical apoptosis is paralleled by cell shrinkage, nuclear condensation, DNA fragmentation, mitochondrial depolarization, cell membrane blebbing, and breakdown of phosphatidylserine asymmetry of the plasma membrane and is associated with a loss of intracellular potassium (13, 14, 48, 50–52, 71, 73, 90, 100, 102, 127–130). Cells exposing phosphatidylserine at the cell surface are recognized by macrophages equipped with receptors specific for phosphatidylserine (39) and rapidly engulf and degrade the affected cells (9, 37). Accordingly, apoptosis allows the elimination of those cells without release of intracellular proteins, which would otherwise cause inflammation (51).

Erythrocytes are devoid of nuclei and mitochondria and thus lack crucial elements in the machinery of apoptosis. Thus until recently, dying erythrocytes have been considered to be eliminated by mechanisms other than apoptosis. Exposure of erythrocytes to the Ca^{2+} ionophore ionomycin, however, triggers cell shrinkage, membrane blebbing, and phosphatidylserine exposure, all typical features of apoptotic, nucleated cells (6, 20, 28). The cell shrinkage results from activation of the Ca^{2+}-sensitive "Gardos" K^+ channels (83), and the phosphatidylserine exposure has previously been thought to result from the activation of a Ca^{2+}-sensitive scramblase (31, 125, 131) and/or inhibition of a Ca^{2+}-sensitive and ATP-dependent aminophospholipid translocase (112).

In view of the similarities to and differences from the apoptosis program of nucleated cells (for details, see also Table 1),

Department of Physiology, Eberhard-Karls-University of Tübingen, Tübingen, Germany.

Table 1. Hallmarks of Apoptosis and Eryptosis

Apoptosis	*Eryptosis*	*Literature*
Nuclear condensation, DNA fragmentation[1]	Nothing equivalent	1: (126)
Dissipation of the mitochondrial membrane potential[2,3]	Nothing equivalent	2: (94) 3: (49)
Cellular shrinkage[4]	Cellular shrinkage[4]	4: (72)
Apoptotic bodies[5]	Vesiculation[6]	5: (118) 6: (124)
Activation of caspases[7]	In most cases, caspase-independent[8,9,10] Activation of μ-calpain[8]	7: (122) 8: (6) 9: (20) 10: (77)
Phosphatidylserine exposure on the outer leaflet of the cell membrane[11]	Phosphatidylserine exposure on the outer leaflet of the erythrocyte membrane[9]	11: (40) 9: (20)
Expression of different death receptors[12]	Expression of CD95/FAS[13]	12: (26) 13: (92)
Accumulation of ceramide by sphingomyelinase-mediated sphingomyelin breakdown[14] or enhanced ceramide synthesis[15,16]	Sphingomyelinase-induced ceramide formation[17]	14: (53) 15: (123) 16: (15) 17: (81)
Increase of intracellular Ca^{2+} by release from the endoplasmic reticulum[18]	Activation of Ca^{2+} permeable cation channels in the erythrocyte membrane[19]	18: (111) 19: (75)

Apoptosis, programmed death of nucleated cells; eryptosis, programmed death of nucleus- and organelle-free, mature erythrocytes.

the term *eryptosis* has been coined to describe the suicidal death of erythrocytes (76). Eryptosis may be distinct from the mechanisms involved in erythrocyte aging (16, 66, 110) or neocytolysis, the death of newly formed erythrocytes (106). The present brief review describes the mechanisms leading to eryptosis and lists some diseases involving excessive eryptosis. Clearly, further research will disclose additional mechanisms operating in eryptosis and a variety of further clinical conditions displaying enhanced eryptosis.

CYCLOOXYGENASE ACTIVATION, PGE_2 FORMATION, AND ACTIVATION OF CATION CHANNELS

Hyperosmotic shock and Cl^- removal trigger the release of prostaglandin E_2 (PGE_2) (85). PGE_2, in turn, activates nonselective cation channels (62, 85), increases the cytosolic Ca^{2+} concentration (63, 85), stimulates phosphatidylserine exposure at the erythrocyte surface (85), and triggers cell membrane vesiculation (1). Accordingly, osmotic cell shrinkage activates the cation channels (58) and triggers erythrocyte Ca^{2+} uptake (75). The same or similar channels are activated by oxidative stress (36), which similarly stimulates Ca^{2+} entry (75) and triggers phosphatidylserine exposure at the cell surface (Fig. 1). The channels are inhibited by intracellular or extracellular Cl^- (36, 58), and the activation of the cation channels by Cl^- removal is abolished by the cyclooxygenase inhibitor diclophenac (85). Moreover, the phospholipase-A_2 inhibitors quinacrine and palmitoyl-trifluoromethyl-ketone and the cyclooxygenase inhibitors acetylsalicylic acid and diclophenac blunt the increase of phosphatidylserine exposure after Cl^- removal (85).

Besides its effect on phosphatidylserine scrambling (6, 20, 28), an increase of cytosolic Ca^{2+} stimulates the Ca^{2+}-sensitive "Gardos" K^+ channels in erythrocytes (10, 21, 41). The subsequent hyperpolarization of the cell membrane drives Cl^- in parallel to K^+ out of the cell. The cellular loss of KCl favors cell shrinkage. Moreover, the cellular loss of K^+ contributes to the triggering of eryptosis (83).

PGE_2 further activates the Ca^{2+}-dependent cysteine endopeptidase calpain, an effect, however, apparently not required for stimulation of phosphatidylserine exposure (85). Instead calpain degrades the cytoskeleton (103).

Energy depletion impairs the replenishment of GSH and thus weakens the antioxidative defense of the erythrocytes (8, 96). Accordingly, energy depletion similarly activates the cation channels and thus leads to eryptosis (80).

The cation channel inhibitors amiloride (75) and ethylisopropylamiloride (EIPA) (79) blunt the phosphatidylserine exposure after osmotic shock. Thus, activation of the cell volume–sensitive and oxidant-sensitive cation channel and subsequent Ca^{2+} entry contribute to the stimulation of erythrocyte scrambling after osmotic shock or oxidative stress.

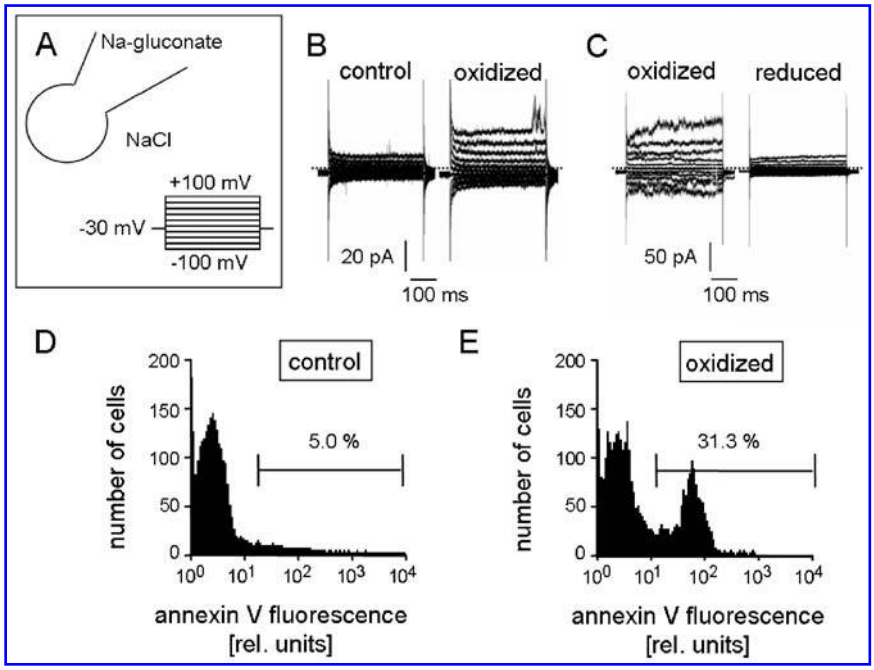

FIG. 1. Activation of cation channels and triggering of phosphatidylserine exposure by oxidative stress. A–C: Dependence of the nonselective cation channel of human erythrocytes on redox potential. (**A**) Whole-cell recording from human erythrocytes. Currents were recorded with Na-gluconate pipette and NaCl bath solution (upper panel). Lower panel, Applied pulse protocol. Currents were evoked by 11 successive square pulses to voltages between –100 and +100 mV delivered in 20-mV increments from a holding potential of –30 mV. The applied voltages refer to the cytoplasmic face of the membrane with respect to the extracellular space. (**B**) Current traces recorded by whole-cell patch-clamp recording before (control) and after addition of *tert*-butylhydroperoxide (*t*-BHP; 1 m*M*) to the bath solution (oxidation). (**C**) Current traces of oxidized erythrocytes (pretreatment with 1 m*M* *t*-BHP for 15 min) before (oxidized) and after bath application of dithiothreitol (1 m*M*; reduced). The currents of the individual square pulses are superimposed, and the zero current is indicated by a *gray line*. (**D, E**) Oxidation-stimulated phosphatidylserine (PS) exposure. Original histograms showing annexin V–dependent fluorescence as a measure of PS exposure of control (**D**) and oxidized (**E**) erythrocytes. PS exposure was analyzed after oxidizing erythrocytes with *t*-BHP (0 and 0.66 m*M* for 15 min) followed by further incubation in NaCl Ringer (24 h). The numbers depict the percentages of annexin V–positive cells.

PAF FORMATION AND STIMULATION OF SPHINGOMYELINASE

Beyond its effect on PGE_2 formation, erythrocyte shrinkage triggers the formation of platelet-activating factor (PAF) (81), which is involved in the regulation of inflammation, thrombosis, atherogenesis, and cardiovascular function (22, 45, 55, 97, 98, 114, 132). PAF then stimulates a sphingomyelinase, leading to the breakdown of sphingomyelin and release of ceramide from erythrocytes (81). Osmotic shock thus leads to the appearance of ceramide at the erythrocyte surface (77). At least partially because of ceramide formation, PAF triggers scrambling of the cell membrane with phosphatidylserine exposure at the erythrocyte surface. C_6-ceramide as well as treatment with purified, bacterial sphingomyelinase similarly triggers phosphatidylserine scrambling (77). Moreover, eryptosis after osmotic shock is blunted by the sphingomyelinase inhibitor 3,4-dichloroisocoumarin. The stimulation of phosphatidylserine exposure is blunted by genetic knockout of PAF receptors (PAF-receptor knockout mice) and by the PAF-receptor antagonist ABT491 (81). PAF further activates Ca^{2+}-sensitive K^+ channels (Gardos channels) in the erythrocyte cell membrane (43) by sensitizing them for the stimulating effects of cytosolic Ca^{2+} (107). Conversely, PAF is released from erythrocyte progenitor cells on increase of cytosolic Ca^{2+} activity (34). The signaling through PAF does, however, not necessarily require elevated cytosolic Ca^{2+} concentrations, and enhanced PAF levels at least partially account for Ca^{2+}-independent eryptosis (81).

RECEPTOR-MEDIATED ERYPTOSIS

In nucleated cells, ligation of death receptors at the cell surface leads to direct and fast activation of the death machinery (26, 88). Very recently, it was demonstrated that erythrocytes likewise undergo programmed cell death after ligation of specific surface antigens, such as glycophorin-C (56), the thrombospondin-1 receptor CD47 (57), and the death receptor CD95/Fas (92). Further efforts are required to elucidate the mechanisms and (patho-) physiologic role of receptor-mediated erythrocyte death.

REGULATION OF ERYPTOSIS BY PROTEIN KINASE C SIGNALING

Eythrocyte energy depletion enhances phosphorylation of membrane proteins by protein kinase C (PKC), leads to subsequent phosphatidylserine exposure at the cell surface and triggers cell shrinkage, effects mimicked by stimulation of PKC with phorbolesters or inhibition of protein phosphatases with okadaic acid (68). PKC activation has previously been

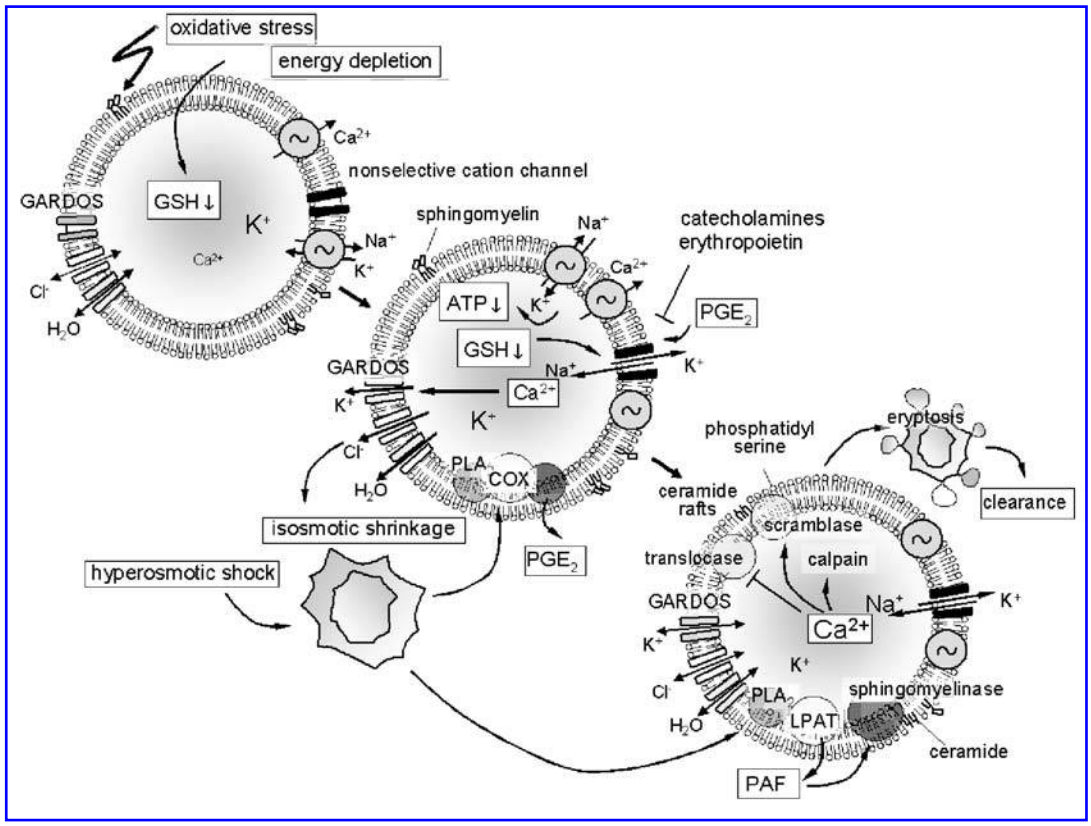

FIG. 2. Synopsis of the mechanisms and the signaling pathways involved in eryptosis. Signaling pathways leading to eryptosis (*i.e.*, cellular shrinkage, phosphatidylserine exposure and activation of calpains). COX, cyclooxygenase; GSH, reduced glutathione; Gardos, potassium channel; LPAT, lyso-PAF acetyl transferase; NSC, nonselective cation channel; PAF, platelet-activating factor; PGE_2, prostaglandin E_2; PLA_2, phospholipase A_2.

shown to stimulate erythrocyte Ca^{2+} entry (3) and phosphatidylserine exposure (30). PKC (EC 2.7.1.37) is a family of serine/threonine-specific protein kinases consisting of $\geq$10 members and requiring Ca^{2+}, diacylglycerol, and a phospholipid for activation. PKC isoenzymes play an essential role in the regulation of diverse cellular functions including proliferation, differentiation, and apoptosis (101). Human erythrocytes express PKC isoenzymes mediating the phosphorylation of cytoskeletal proteins, such as band 4.1, 4.9, and adducin (27), and the human Na^+/H^+ antiporter NHE 1 (17). To date PKCα, PKCi, PKCμ, and PKCζ have been reported to be expressed in erythrocytes (47). Additional experiments are required to define the PKC isoforms involved in eryptosis and the interaction of PKC with the PGE_2-dependent and PAF-dependent signaling pathways.

ERYPTOSIS AFTER OXIDATIVE STRESS AND CASPASE ACTIVATION

Oxidative stress by exposure to *tert*-butylhydroperoxide or peroxynitrite, for instance, is a major cause of erythrocyte injury (5, 18). It has been shown to activate aspartyl and cysteinyl proteases (95). Caspases have been shown to be expressed in erythrocytes (20, 91), to cleave the anion exchanger band 3 *in vitro* (91), and to stimulate phosphatidylserine exposure of erythrocytes (93). Conversely, eryptosis after ionomycin or hyperosmotic shock does not require activation of caspases (6, 77, 119).

Besides its effect on caspases, oxidative stress or defects of antioxidative defense (8, 25, 96) enhance Ca^{2+} entry via activation of the cation channels and thus stimulate eryptosis at least partially through channel activation (36). As illustrated in Fig. 1A–C, oxidation of erythrocytes with *tert*-butylhydroperoxide leads to an increase of the cation permeability of the membrane, an effect reversed by the reducing agent dithiothreitol. Oxidation with *tert*-butylhydroperoxide also enhanced erythrocyte annexin-V binding as a measure of phosphatidylserine exposure by some sixfold (Fig. 1D and E). Interestingly, not all erythrocytes of one population showed the same sensitivity against oxidative stress, and only one third of the population was shown to be annexin-V positive (Fig. 1E). Erythrocyte concentrates from one test person did contain subpopulations of erythrocytes that differed in their susceptibility to programmed cell death (85).

The heterogeneity of erythrocytes may in part be due to different ages.

Oxidative stress further activates erythrocyte Cl^- channels (59, 115), which are required for erythrocyte shrinkage and thus also participate in the triggering of eryptosis (100).

Antioxidants, such as vitamin E (46, 60, 69), glutathione (33), or the semisynthetic flavonoid 7-monohydroxyethylrutoside (29) may protect erythrocytes from oxidative stress and thus presumably from eryptosis.

INHIBITION OF ERYPTOSIS BY ERYTHROPOIETIN AND CATECHOLAMINES

Erythropoietin not only inhibits apoptosis of erythrocytic progenitor cells (61, 104) but similarly blunts the suicidal death of mature erythrocytes. The hormone is effective through inhibition of the Ca^{2+}-permeable cation channels. Accordingly, the protective effect of erythropoietin is lost in the nominal absence of extracellular Ca^{2+} (99). The antieryptotic effect of erythropoietin presumably accounts for its ability to increase the life span of circulating cells (104).

Dopamine, isoproterenol, and epinephrine similarly inhibit the Ca^{2+}-permeable cation channels and thus interfere with eryptosis (84). The effect is presumably not relevant for physiologic regulation, as the concentrations of catecholamines required exceed those encountered *in vivo*. However, animal experiments revealed that the hematotoxicity (including red blood cell counts) of cyclophosphamide has been reduced by simultaneous dopamine treatment (70). Thus, administration of dopamine at pharmacologic doses may well be therapeutically applicable for the suppression of eryptosis.

CLINICAL CONDITIONS ASSOCIATED WITH ERYPTOSIS

A variety of clinical conditions decrease the life span of mature erythrocytes by facilitating eryptosis. Increased sensitivity of sickle cells and of glucose 6-phosphate dehydrogenase–deficient cells to osmotic shock and of sickle cells, thalassemic cells, and glucose 6-phosphate dehydrogenase–deficient cells to oxidative stress and to glucose depletion has been observed previously (80). The enhanced susceptibility to eryptosis is considered to decrease the erythrocyte life span in those disorders (80).

Similarly, iron-deficient erythrocytes are more sensitive to eryptosis (64). The enhanced eryptosis is at least partially the result of enhanced cation channel activity. Presumably, the decreased volume of iron-deficient erythrocytes decreases the threshold for the activation of the channel (36, 58). Interestingly, the enhanced exposure of phosphatidylserine on the surface of iron-deficient erythrocytes coincides with a substantial decrease of the life span of iron-deficient erythrocytes (64). Eryptosis is further triggered by exposure of erythrocytes to lead (65) or mercury (38), or by treatment with plasma from patients with recurrent hemolytic-uremic syndrome (86). Thus, the typical anemia after lead intoxication is at least partially due to enhanced eryptosis.

Eryptosis may be relevant for the intraerythrocyte survival of the malaria pathogen *Plasmodium falciparum*, which activates presumably endogenous host cell channels via oxidation of the cell membrane (35, 59, 115). *Plasmodium falciparum* depends on the activation of those channels, as they allow the uptake of nutrients and the disposal of waste products (67). Activation of the cation channel might be required for increased uptake of Na^+ and Ca^{2+} by the parasitized erythrocyte. Cellular accumulation of Na^+ and Ca^{2+} is needed by the parasite. Ca^{2+} uptake by the parasite buffers erythrocytic Ca^{2+} concentration and thus delays eryptosis of the parasitized erythrocyte. Nevertheless, *Plasmodium* infection induces eryptosis (19), and eryptosis might lead to the recognition of the parasitized erythrocytes by macrophages.

Oxidant injury of erythrocytes and/or erythrocyte precursors may further occur in HIV (4, 32, 44, 105, 116). Moreover, eryptosis is triggered after exposure of erythrocytes to hemolysin Kanagawa from *Vibrio parahaemolyticus* (82), a marine bacterium causing gastroenteritis after ingestion of contaminated seafood. Thus, infection with hemolysin-producing pathogens triggers hemolysis as well as eryptosis.

Eryptosis not only decreases the life span of circulating erythrocytes but may also increase their adhesivity (24, 42, 109, 117). Phosphatidylserine-exposing erythrocytes may bind to receptors in the vascular wall and thus impede microcirculation (2, 23). Accordingly, annexin-binding erythrocytes may be trapped in renal medulla after ischemia of the mouse kidney (78). Phosphatidylserine-exposing cells may further participate in hemostasis (2) and may thus play a role in atherothrombosis (11, 54, 120).

Even though it is a pathophysiologic mechanism, eryptosis serves an important physiologic function (*i.e.*, the prevention of hemolysis). Energy depletion, defective Na^+/K^+ATPase, or enhanced leakiness of the cell membrane eventually leads to cellular gain of Na^+ and Cl^- with osmotically obliged water, resulting in subsequent cell swelling (71). Initially, the entry of Na^+ is compensated by cellular loss of K^+; the decrease of the K^+ equilibrium potential leads, however, to gradual depolarization, which favors the entry of Cl^-. The cell swelling jeopardizes the integrity of the cell membrane. Rupture of the cell leads to release of hemoglobin, which may be filtered at the glomeruli of the kidney, precipitate in the acid lumen of the tubules, obliterate the tubules, and thus lead to renal failure. The phosphatidylserine exposure at the cell surface allows the macrophages to recognize defective erythrocytes and to clear them from circulating blood before hemolysis. Moreover, the activation of the Gardos K^+ channel delays swelling and disruption of defective erythrocytes, thus expanding the time allowed for macrophages to clear the injured erythrocytes from circulating blood.

CONCLUSIONS

As illustrated in Fig. 2, eryptosis is a complex machinery eventually leading to erythrocyte shrinkage and scrambling of the erythrocyte membrane, events preceding uptake of

affected erythrocytes by macrophages. The signaling of eryptosis includes PAF, ceramide, PGE_2, cation channels, protein kinase C, and in some instances, caspases. Eryptosis is presumably an important physiologic mechanism to forestall hemolysis of defective red blood cells. Accelerated eryptosis may lead to anemia due to excessive loss of circulating erythrocytes. We anticipate that future research will identify further signaling mechanisms participating in the triggering and execution of eryptosis and further disorders involving deranged eryptotic death of circulating erythrocytes.

ACKNOWLEDGMENTS

We acknowledge the meticulous preparation of the manuscript by Tanja Loch and Lejla Subasic. The author's research is supported by the Deutsche Forschungsgemeinschaft, La 315/4–3, La 315/6–1, and La 315/13–1, and the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (Center for Interdisciplinary Clinical Research) 01 KS 9602 and the Else-Übelmesser-Stiftung.

ABBREVIATIONS

ABT491, PAF receptor antagonist; CD47, cluster of differentiation 47; CD95/Fas, cluster of differentiation 95, a death receptor; COX, cyclooxygenase; EIPA, ethylisopropylamiloride; Gardos, potassium channel; GSH, reduced glutathione; HIV, human immunodeficiency virus; LPAT, lyso-PAF acetyl transferase; Na^+/K^+ATPase, sodium/potassium pump; NHE 1, Na^+/H^+ antiporter; NSC, nonselective cation channel; PAF, platelet-activating factor; PGE_2, prostaglandin E_2; PKC, protein kinase C; PLA_2, phospholipase A_2; PS, phosphatidylserine; *t*-BHP: *tert*-butylhydroperoxide.

REFERENCES

1. Allan D and Michell RH. Calcium ion-dependent diacylglycerol accumulation in erythrocytes is associated with microvesiculation but not with efflux of potassium ions. *Biochem J* 166: 495–499, 1977.
2. Andrews DA and Low PS. Role of red blood cells in thrombosis. *Curr Opin Hematol* 6: 76–82, 1999.
3. Andrews DA, Yang L, and Low PS. Phorbol ester stimulates a protein kinase C-mediated agatoxin-TK-sensitive calcium permeability pathway in human red blood cells. *Blood* 100: 3392–3399, 2002.
4. Barbaro G, Di Lorenzo G, Soldini M, Bellomo G, Belloni G, Grisorio B, and Barbarini G. Vagal system impairment in human immunodeficiency virus-positive patients with chronic hepatitis C: does hepatic glutathione deficiency have a pathogenetic role? *Scand J Gastroenterol* 32: 1261–1266, 1997.
5. Barvitenko NN, Adragna NC, and Weber RE. Erythrocyte signal transduction pathways, their oxygenation dependence and functional significance. *Cell Physiol Biochem* 15: 1–18, 2005.
6. Berg CP, Engels IH, Rothbart A, Lauber K, Renz A, Schlosser SF, Schulze-Osthoff K, and Wesselborg S. Human mature red blood cells express caspase-3 and caspase-8, but are devoid of mitochondrial regulators of apoptosis. *Cell Death Differ* 8: 1197–1206, 2001.
7. Bergamo P, Luongo D, and Rossi M. Conjugated linoleic acid–mediated apoptosis in Jurkat T cells involves the production of reactive oxygen species. *Cell Physiol Biochem* 14: 57–64, 2004.
8. Bilmen S, Aksu TA, Gumuslu S, Korgun DK, and Canatan D. Antioxidant capacity of G-6-PD-deficient erythrocytes. *Clin Chim Acta* 303: 83–86, 2001.
9. Boas FE, Forman L, and Beutler E. Phosphatidylserine exposure and red cell viability in red cell aging and in hemolytic anemia. *Proc Natl Acad Sci U S A* 95: 3077–3081, 1998.
10. Bookchin RM, Ortiz OE, and Lew VL. Activation of calcium-dependent potassium channels in deoxygenated sickled red cells. *Prog Clin Biol Res* 240: 193–200, 1987.
11. Borenstain-Ben Yashar V, Barenholz Y, Hy-Am E, Rachmilewitz EA, and Eldor A. Phosphatidylserine in the outer leaflet of red blood cells from beta-thalassemia patients may explain the chronic hypercoagulable state and thrombotic episodes. *Am J Hematol* 44: 63–65, 1993.
12. Bortner CD and Cidlowski JA. Caspase independent/dependent regulation of K(+), cell shrinkage, and mitochondrial membrane potential during lymphocyte apoptosis. *J Biol Chem* 274: 21953–21962, 1999.
13. Bortner CD and Cidlowski JA. Apoptotic volume decrease and the incredible shrinking cell. *Cell Death Differ* 9: 1307–1310, 2002.
14. Bortner CD and Cidlowski JA. The role of apoptotic volume decrease and ionic homeostasis in the activation and repression of apoptosis. *Pflugers Arch* 448: 313–318, 2004.
15. Bose R, Verheij M, Haimovitz-Friedman A, Scotto K, Fuks Z, and Kolesnick R. Ceramide synthase mediates daunorubicin-induced apoptosis: an alternative mechanism for generating death signals. *Cell* 82: 405–414, 1995.
16. Bosman GJ, Willekens FL, and Werre JM. Erythrocyte aging: a more than superficial resemblance to apoptosis? *Cell Physiol Biochem* 16: 1–8, 2005.
17. Bourikas D, Kaloyianni M, Bougoulia M, Zolota Z, and Koliakos G. Modulation of the Na(+)-H(+) antiport activity by adrenaline on erythrocytes from normal and obese individuals. *Mol Cell Endocrinol* 205: 141–150, 2003.
18. Bracci R, Perrone S, and Buonocore G. Oxidant injury in neonatal erythrocytes during the perinatal period. *Acta Paediatr Suppl* 91: 130–134, 2002.
19. Brand VB, Sandu CD, Duranton C, Tanneur V, Lang KS, Huber SM, and Lang F. Dependence of *Plasmodium falciparum* in vitro growth on the cation permeability of the human host erythrocyte. *Cell Physiol Biochem* 13: 347–356, 2003.
20. Bratosin D, Estaquier J, Petit F, Arnoult D, Quatannens B, Tissier JP, Slomianny C, Sartiaux C, Alonso C, Huart JJ, Montreuil J, and Ameisen JC. Programmed cell death in mature erythrocytes: a model for investigating death effector pathways operating in the absence of mitochondria. *Cell Death Differ* 8: 1143–1156, 2001.

21. Brugnara C, de Franceschi L, and Alper SL. Inhibition of Ca(2+)-dependent K+ transport and cell dehydration in sickle erythrocytes by clotrimazole and other imidazole derivatives. *J Clin Invest* 92: 520–526, 1993.
22. Bussolino F, Fischer E, Turrini F, Kazatchkine MD, and Arese P. Platelet-activating factor enhances complement-dependent phagocytosis of diamide-treated erythrocytes by human monocytes through activation of protein kinase C and phosphorylation of complement receptor type one (CR1). *J Biol Chem* 264: 21711–21719, 1989.
23. Closse C, Dachary-Prigent J, and Boisseau MR. Phosphatidylserine-related adhesion of human erythrocytes to vascular endothelium. *Br J Haematol* 107: 300–302, 1999.
24. Covas DT, de LA, I, Vianna Bonini PP, and Zago MA. Effects of hydroxyurea on the membrane of erythrocytes and platelets in sickle cell anemia. *Haematologica* 89: 273–280, 2004.
25. Damonte G, Guida L, Sdraffa A, Benatti U, Melloni E, Forteleoni G, Meloni T, Carafoli E, and De Flora A. Mechanisms of perturbation of erythrocyte calcium homeostasis in favism. *Cell Calcium* 13: 649–658, 1992.
26. Daniel PT, Wieder T, Sturm I, and Schulze-Osthoff K. The kiss of death: promises and failures of death receptors and ligands in cancer therapy. *Leukemia* 15: 1022–1032, 2001.
27. Danilov YN and Cohen CM. Wheat germ agglutinin but not concanavalin A modulates protein kinase C-mediated phosphorylation of red cell skeletal proteins. *FEBS Lett* 257: 431–434, 1989.
28. Daugas E, Cande C, and Kroemer G. Erythrocytes: death of a mummy. *Cell Death Differ* 8: 1131–1133, 2001.
29. de Franceschi L, Turrini F, Honczarenko M, Ayi K, Rivera A, Fleming MD, Law T, Mannu F, Kuypers FA, Bast A, van der Vijgh WJ, and Brugnara C. In vivo reduction of erythrocyte oxidant stress in a murine model of beta-thalassemia. *Haematologica* 89: 1287–1298, 2004.
30. de Jong K, Rettig MP, Low PS, and Kuypers FA. Protein kinase C activation induces phosphatidylserine exposure on red blood cells. *Biochemistry* 41: 12562–12567, 2002.
31. Dekkers DW, Comfurius P, Bevers EM, and Zwaal RF. Comparison between Ca2+-induced scrambling of various fluorescently labelled lipid analogues in red blood cells. *Biochem J* 362: 741–747, 2002.
32. Delmas-Beauvieux MC, Peuchant E, Couchouron A, Constans J, Sergeant C, Simonoff M, Pellegrin JL, Leng B, Conri C, and Clerc M. The enzymatic antioxidant system in blood and glutathione status in human immunodeficiency virus (HIV)-infected patients: effects of supplementation with selenium or beta-carotene. *Am J Clin Nutr* 64: 101–107, 1996.
33. Dumaswala UJ, Wilson MJ, Wu YL, Wykle J, Zhuo L, Douglass LM, and Daleke DL. Glutathione loading prevents free radical injury in red blood cells after storage. *Free Radic Res* 33: 517–529, 2000.
34. Dupuis F, Levasseur S, Jean-Louis F, Dulery C, Praloran V, Denizot Y, and Michel L. Production, metabolism and effect of platelet-activating factor on the growth of the human K562 erythroid cell line. *Biochim Biophys Acta* 1359: 241–249, 1997.
35. Duranton C, Huber S, Tanneur V, Lang K, Brand V, Sandu C, and Lang F. Electrophysiological properties of the *Plasmodium falciparum*-induced cation conductance of human erythrocytes. *Cell Physiol Biochem* 13: 189–198, 2003.
36. Duranton C, Huber SM, and Lang F. Oxidation induces a Cl(-)-dependent cation conductance in human red blood cells. *J Physiol* 539: 847–855, 2002.
37. Eda S and Sherman IW. Cytoadherence of malaria-infected red blood cells involves exposure of phosphatidylserine. *Cell Physiol Biochem* 12: 373–384, 2002.
38. Eisele K, Lang PA, Kempe DS, Klarl BA, Niemoller O, Wieder T, Huber SM, Duranton C, and Lang F. Stimulation of erythrocyte phosphatidylserine exposure by mercury ions. *Toxicol Appl Pharmacol* 210: 116–122, 2006.
39. Fadok VA, Bratton DL, Rose DM, Pearson A, Ezekewitz RA, and Henson PM. A receptor for phosphatidylserine-specific clearance of apoptotic cells. *Nature* 405: 85–90, 2000.
40. Fadok VA, Voelker DR, Campbell PA, Cohen JJ, Bratton DL, and Henson PM. Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. *J Immunol* 148: 2207–2216, 1992.
41. Franco RS, Palascak M, Thompson H, Rucknagel DL, and Joiner CH. Dehydration of transferrin receptor-positive sickle reticulocytes during continuous or cyclic deoxygenation: role of KCl cotransport and extracellular calcium. *Blood* 88:4359–4365, 1996.
42. Gallagher PG, Chang SH, Rettig MP, Neely JE, Hillery CA, Smith BD, and Low PS. Altered erythrocyte endothelial adherence and membrane phospholipid asymmetry in hereditary hydrocytosis. *Blood* 101: 4625–4627, 2003.
43. Garay R and Braquet P. Involvement of K+ movements in the membrane signal induced by PAF-acether. *Biochem Pharmacol* 35: 2811–2815, 1986.
44. Gil L, Martinez G, Gonzalez I, Tarinas A, Alvarez A, Giuliani A, Molina R, Tapanes R, Perez J, and Leon OS. Contribution to characterization of oxidative stress in HIV/AIDS patients. *Pharmacol Res* 47: 217–224, 2003.
45. Goggel R, Winoto-Morbach S, Vielhaber G, Imai Y, Lindner K, Brade L, Brade H, Ehlers S, Slutsky AS, Schutze S, Gulbins E, and Uhlig S. PAF-mediated pulmonary edema: a new role for acid sphingomyelinase and ceramide. *Nat Med* 10: 155–160, 2004.
46. Gokkusu C and Mostafazadeh T. Changes of oxidative stress in various tissues by long-term administration of vitamin E in hypercholesterolemic rats. *Clin Chim Acta* 328: 155–161, 2003.
47. Govekar RB and Zingde SM. Protein kinase C isoforms in human erythrocytes. *Ann Hematol* 80: 531–534, 2001.
48. Grassme H, Kirschnek S, Riethmueller J, Riehle A, von Kurthy G, Lang F, Weller M, and Gulbins E. CD95/CD95 ligand interactions on epithelial cells in host defense to *Pseudomonas aeruginosa*. *Science* 290: 527–530, 2000.
49. Green DR and Kroemer G. The pathophysiology of mitochondrial cell death. *Science* 305: 626–629, 2004.
50. Green DR and Reed JC. Mitochondria and apoptosis. *Science* 281: 1309–1312, 1998.
51. Gulbins E, Jekle A, Ferlinz K, Grassme H, and Lang F. Physiology of apoptosis. *Am J Physiol Renal Physiol* 279: F605–F615, 2000.

52. Han H, Wang J, Zhang Y, Long H, Wang H, Xu D, and Wang Z. HERG K channel conductance promotes H2O2-induced apoptosis in HEK293 cells: cellular mechanisms. *Cell Physiol Biochem* 14: 121–134, 2004.
53. Hannun YA. Functions of ceramide in coordinating cellular responses to stress. *Science* 274: 1855–1859, 1996.
54. Hassoun H, Wang Y, Vassiliadis J, Lutchman M, Palek J, Aish L, Aish IS, Liu SC, and Chishti AH. Targeted inactivation of murine band 3 (AE1) gene produces a hypercoagulable state causing widespread thrombosis in vivo. *Blood* 92: 1785–1792, 1998.
55. Haynes J Jr and Obiako B. Activated polymorphonuclear cells increase sickle red blood cell retention in lung: role of phospholipids. *Am J Physiol Heart Circ Physiol* 282: H122–H130, 2002.
56. Head DJ, Lee ZE, Poole J, and Avent ND. Expression of phosphatidylserine (PS) on wild-type and Gerbich variant erythrocytes following glycophorin-C (GPC) ligation. *Br J Haematol* 129: 130–137, 2005.
57. Head DJ, Lee ZE, Swallah MM, and Avent ND. Ligation of CD47 mediates phosphatidylserine expression on erythrocytes and a concomitant loss of viability in vitro. *Br J Haematol* 130: 788–790, 2005.
58. Huber SM, Gamper N, and Lang F. Chloride conductance and volume-regulatory nonselective cation conductance in human red blood cell ghosts. *Pflugers Arch* 441: 551–558, 2001.
59. Huber SM, Uhlemann AC, Gamper NL, Duranton C, Kremsner PG, and Lang F. *Plasmodium falciparum* activates endogenous Cl(-) channels of human erythrocytes by membrane oxidation. *EMBO J* 21: 22–30, 2002.
60. Jain SK. Vitamin E and stabilization of membrane lipid organization in red blood cells with peroxidative damage. *Biomed Biochim Acta* 42: S43–S47, 1983.
61. Jelkmann W. Erythropoietin: Structure, control of production, and function. *Physiol Rev* 72: 449–489, 1992.
62. Kaestner L, Bernhardt I. Ion channels in the human red blood cell membrane: their further investigation and physiological relevance. *Bioelectrochemistry* 55: 71–74, 2002.
63. Kaestner L, Tabellion W, Lipp P, and Bernhardt I. Prostaglandin E2 activates channel-mediated calcium entry in human erythrocytes: an indication for a blood clot formation supporting process. *Thromb Haemost* 92: 1269–1272, 2004.
64. Kempe DS, Lang PA, Duranton C, Akel A, Lang KS, Huber SM, Wieder T, and Lang F. Enhanced programmed cell death of iron deficient erythrocytes. *FASEB J* 20: 368–370, 2006.
65. Kempe DS, Lang PA, Eisele K, Klarl BA, Wieder T, Huber SM, Duranton C, and Lang F. Stimulation of erythrocyte phosphatidylserine exposure by lead ions. *Am J Physiol Cell Physiol* 288: C396–C402, 2005.
66. Kiefer CR and Snyder LM. Oxidation and erythrocyte senescence. *Curr Opin Hematol* 7: 113–116, 2000.
67. Kirk K. Membrane transport in the malaria-infected erythrocyte. *Physiol Rev* 81: 495–537, 2001.
68. Klarl B, Lang PA, Kempe DS, Niemoeller OM, Akel A, Sibiesiak M, Eisele K, Podolski M, Huber SM, Wieder T, and Lang F. Protein kinase C mediates erythrocyte "programmed cell death" following glucose depletion. *Am J Physiol Cell Physiol* 290: C244–253, 2006.
69. Kobayashi S, Moriya H, Aso K, and Ohtake T. Vitamin E-bonded hemodialyzer improves atherosclerosis associated with a rheological improvement of circulating red blood cells. *Kidney Int* 63: 1881–1887, 2003.
70. Lakshmi C, Deb C, Ray C, and Ray MR. Reduction of hematotoxicity and augmentation of antitumor efficacy of cyclophosphamide by dopamine. *Neoplasma* 52: 68–73, 2005.
71. Lang F, Busch GL, Ritter M, Völkl H, Waldegger S, Gulbins E, and Häussinger D. Functional significance of cell volume regulatory mechanisms. *Physiol Rev* 78: 247–306, 1998.
72. Lang F, Gulbins E, Szabo I, Lepple-Wienhues A, Huber SM, Duranton C, Lang KS, Lang PA, and Wieder T. Cell volume and the regulation of apoptotic cell death. *J Mol Recogn* 17: 473–480, 2004.
73. Lang F, Ritter M, Gamper N, Huber S, Fillon S, Tanneur V, Lepple-Wienhues A, Szabo I, and Gulbins E. Cell volume in the regulation of cell proliferation and apoptotic cell death. *Cell Physiol Biochem* 10: 417–428, 2000.
74. Lang F, Szabo I, Lepple-Wienhues A, Siemen D, and Gulbins E. Physiology of receptor-mediated lymphocyte apoptosis. *News Physiol Sci* 14: 194–200, 1999.
75. Lang KS, Duranton C, Poehlmann H, Myssina S, Bauer C, Lang F, Wieder T, and Huber SM. Cation channels trigger apoptotic death of erythrocytes. *Cell Death Differ* 10: 249–256, 2003.
76. Lang KS, Lang PA, Bauer C, Duranton C, Wieder T, Huber SM, and Lang F. Mechanisms of suicidal erythrocyte death. *Cell Physiol Biochem* 15: 195–202, 2005.
77. Lang KS, Myssina S, Brand V, Sandu C, Lang PA, Berchtold S, Huber SM, Lang F, and Wieder T. Involvement of ceramide in hyperosmotic shock-induced death of erythrocytes. *Cell Death Differ* 11: 231–243, 2004.
78. Lang KS, Myssina S, Lang PA, Tanneur V, Kempe DS, Mack AF, Huber SM, Wieder T, Lang F, and Duranton C. Inhibition of erythrocyte phosphatidylserine exposure by urea and Cl-. *Am J Physiol Renal Physiol* 286: F1046–F1053, 2004.
79. Lang KS, Myssina S, Tanneur V, Wieder T, Huber SM, Lang F, and Duranton C. Inhibition of erythrocyte cation channels and apoptosis by ethylisopropylamiloride. *Naunyn Schmiedebergs Arch Pharmacol* 367: 391–396, 2003.
80. Lang KS, Roll B, Myssina S, Schittenhelm M, Scheel-Walter HG, Kanz L, Fritz J, Lang F, Huber SM, and Wieder T. Enhanced erythrocyte apoptosis in sickle cell anemia, thalassemia and glucose-6-phosphate dehydrogenase deficiency. *Cell Physiol Biochem* 12: 365–372, 2002.
81. Lang PA, Kempe DS, Tanneur V, Eisele K, Klarl BA, Myssina S, Jendrossek V, Ishii S, Shimizu T, Weidmann M, Huber SM, Lang F, and Wieder T. Stimulation of erythrocyte ceramide formation by platelet activating factor. *J Cell Sci* 118: 1233–1243, 2005.
82. Lang PA, Kaiser S, Myssina S, Birka C, Weinstock C, Northoff H, Wieder T, Lang F, and Huber SM. Effect of *Vibrio parahaemolyticus* haemolysin on human erythrocytes. *Cell Microbiol* 6: 391–400, 2004.
83. Lang PA, Kaiser S, Myssina S, Wieder T, Lang F, and Huber SM. Role of Ca2+-activated K+ channels in human

erythrocyte apoptosis. *Am J Physiol Cell Physiol* 285: C1553–C1560, 2003.
84. Lang PA, Kempe DS, Akel A, Klarl BA, Eisele K, Podolski M, Hermle T, Niemoeller OM, Attanasio P, Huber SM, Wieder T, Lang F, and Duranton C. Inhibition of erythrocyte "apoptosis" by catecholamines. *Naunyn Schmiedebergs Arch Pharmacol* 372: 228–235, 2005.
85. Lang PA, Kempe DS, Myssina S, Tanneur V, Birka C, Laufer S, Lang F, Wieder T, and Huber SM. PGE(2) in the regulation of programmed erythrocyte death. *Cell Death Differ* 12: 415–428, 2005.
86. Lang PA, Beringer O, Nicolay J, Amon O, Kempe DS, Hermle T, Attanasio P, Akel A, Schäfer R, Friedrich B, Risler T, Baur M, Olbricht C, Zimmerhackl L, Zipfel P, Wieder T, and Lang F. Suicidal death of erythrocytes in recurrent hemolytic-uremic syndrome. *J Mol Med* 84: 378– 388, 2006.
87. Lang PA, Warskulat U, Heller-Stilb B, Huang DY, Grenz A, Myssina S, Duszenko M, Lang F, Haussinger D, Vallon V, and Wieder T. Blunted apoptosis of erythrocytes from taurine transporter deficient mice. *Cell Physiol Biochem* 13: 337–346, 2003.
88. Lavrik I, Golks A, and Krammer PH. Death receptor signaling. *J Cell Sci* 118: 265–267, 2005.
89. Long H, Han H, Yang B, and Wang Z. Opposite cell density-dependence between spontaneous and oxidative stress-induced apoptosis in mouse fibroblast L-cells. *Cell Physiol Biochem* 13: 401–414, 2003.
90. Maeno E, Ishizaki Y, Kanaseki T, Hazama A, and Okada Y. Normotonic cell shrinkage because of disordered volume regulation is an early prerequisite to apoptosis. *Proc Natl Acad Sci U S A* 97: 9487–9492, 2000.
91. Mandal D, Baudin-Creuza V, Bhattacharyya A, Pathak S, Delaunay J, Kundu M, and Basu J. Caspase 3-mediated proteolysis of the N-terminal cytoplasmic domain of the human erythroid anion exchanger 1 (band 3). *J Biol Chem* 278: 52551–52558, 2003.
92. Mandal D, Mazumder A, Das P, Kundu M, and Basu J. FAS-,caspase 8-and caspase 3-dependent signaling regulate the activity of the aminophospholipid translocase and phosphatidylserine externalization in human erythrocytes. *J Biol Chem* 280: 39460–39467, 2005.
93. Mandal D, Moitra PK, Saha S, and Basu J. Caspase 3 regulates phosphatidylserine externalization and phagocytosis of oxidatively stressed erythrocytes. *FEBS Lett* 513: 184–188, 2002.
94. Martinou JC, Green DR. Breaking the mitochondrial barrier. *Nat Rev Mol Cell Biol* 2: 63–67, 2001.
95. Matarrese P, Straface E, Pietraforte D, Gambardella L, Vona R, Maccaglia A, Minetti M, and Malorni W. Peroxynitrite induces senescence and apoptosis of red blood cells through the activation of aspartyl and cysteinyl proteases. *FASEB J* 19: 416–418, 2005.
96. Mavelli I, Ciriolo MR, Rossi L, Meloni T, Forteleoni G, De Flora A, Benatti U, Morelli A, and Rotilio G. Favism: a hemolytic disease associated with increased superoxide dismutase and decreased glutathione peroxidase activities in red blood cells. *Eur J Biochem* 139: 13–8, 1984.
97. Montrucchio G, Alloatti G, and Camussi G. Role of platelet-activating factor in cardiovascular pathophysiology. *Physiol Rev* 80: 1669–1699, 2000.
98. Montrucchio G, Alloatti G, Mariano F, Lupia E, Lucchina PG, Musso E, Emanuelli G, and Camussi G. Role of platelet-activating factor in hypotension and platelet activation induced by infusion of thrombolytic agents in rabbits. *Circ Res* 72: 658–670, 1993.
99. Myssina S, Huber SM, Birka C, Lang PA, Lang KS, Friedrich B, Risler T, Wieder T, and Lang F. Inhibition of erythrocyte cation channels by erythropoietin. *J Am Soc Nephrol* 14: 2750–2757, 2003.
100. Myssina S, Lang P, Kempe D, Kaiser S, Huber S, Wieder T, and Lang F. Cl channel blockers NPPB and niflumic acid blunt Ca(2+)-induced erythrocyte 'apoptosis'. *Cell Physiol Biochem* 14: 241–248, 2004.
101. Nishizuka Y. Discovery and prospect of protein kinase C research: epilogue. *J Biochem (Tokyo)* 133: 155–158, 2003.
102. Okada Y, Maeno E, Shimizu T, Dezaki K, Wang J, and Morishima S. Receptor-mediated control of regulatory volume decrease (RVD) and apoptotic volume decrease (AVD). *J Physiol* 532: 3–16, 2001.
103. Pant HC, Virmani M, and Gallant PE. Calcium-induced proteolysis of spectrin and band 3 protein in rat erythrocyte membranes. *Biochem Biophys Res Commun* 117: 372–377, 1983.
104. Polenakovic M and Sikole A. Is erythropoietin a survival factor for red blood cells? *J Am Soc Nephrol* 7: 1178–1182, 1996.
105. Repetto M, Reides C, Gomez Carretero ML, Costa M, Griemberg G, and Llesuy S. Oxidative stress in blood of HIV infected patients. *Clin Chim Acta* 255: 107–117, 1996.
106. Rice L and Alfrey CP. The negative regulation of red cell mass by neocytolysis: physiologic and pathophysiologic manifestations. *Cell Physiol Biochem* 15: 245–250, 2005.
107. Rivera A, Jarolim P, and Brugnara C. Modulation of Gardos channel activity by cytokines in sickle erythrocytes. *Blood* 99: 357–603, 2002.
108. Rosette C and Karin M. Ultraviolet light and osmotic stress: activation of the JNK cascade through multiple growth factor and cytokine receptors. *Science* 274: 1194–1197, 1996.
109. Samocha-Bonet D, Lichtenberg D, Tomer A, Deutsch V, Mardi T, Goldin Y, Abu-Abeid S, Shenkerman G, Patshornik H, Shapira I, and Berliner S. Enhanced erythrocyte adhesiveness/aggregation in obesity corresponds to low-grade inflammation. *Obes Res* 11: 403–407, 2003.
110. Schwarzer E, Kühn H, Valente E, and Arese P. Band 3/COMPLEMENT-mediated recognition and removal of normally senescent and pathological human erythrocytes. *Cell Physiol Biochem* 16: 133–146, 2005.
111. Scorrano L, Oakes SA, Opferman JT, Cheng EH, Sorcinelli MD, Pozzan T, and Korsmeyer SJ. BAX and BAK regulation of endoplasmic reticulum Ca2+: a control point for apoptosis. *Science* 300: 135–139, 2003.
112. Seigneuret M, Devaux PF. ATP-dependent asymmetric distribution of spin-labeled phospholipids in the erythrocyte membrane: relation to shape changes. *Proc Natl Acad Sci U S A* 81: 3751–3755, 1984.
113. Sturm J, Zhang H, Magdeburg R, Hasenberg T, Bonninghoff R, Oulmi J, Keese M, and McCuskey R. Altered

apoptotic response and different liver structure during liver regeneration in FGF-2-deficient mice. *Cell Physiol Biochem* 14: 249–260, 2004.
114. Subbanagounder G, Leitinger N, Shih PT, Faull KF, and Berliner JA. Evidence that phospholipid oxidation products and/or platelet-activating factor play an important role in early atherogenesis: in vitro and in vivo inhibition by WEB 2086. *Circ Res* 85: 311–318, 1999.
115. Tanneur V, Duranton C, Brand VB, Sandu CD, Akkaya C, Kasinathan RS, Gachet C, Sluyter R, Barden JA, Wiley JS, Lang F, and Huber SM. Purinoceptors are involved in the induction of an osmolyte permeability in malaria-infected and oxidized human erythrocytes. *FASEB J* 20: 133–135, 2006.
116. Trotti R, Rondanelli M, Anesi A, Gabanti E, Brustia R, and Minoli L. Increased erythrocyte glutathione peroxidase activity and serum tumor necrosis factor-alpha in HIV-infected patients: relationship to on-going prothrombotic state. *J Hematother Stem Cell Res* 11: 369–375, 2002.
117. Wandersee NJ, Punzalan RC, Rettig MP, Kennedy MD, Pajewski NM, Sabina RL, Paul SJ, Low PS, and Hillery CA. Erythrocyte adhesion is modified by alterations in cellular tonicity and volume. *Br J Haematol* 131: 366–377, 2005.
118. Weedon D, Searle J, and Kerr JF. Apoptosis: Its nature and implications for dermatopathology. *Am J Dermatopathol* 1: 133–144, 1979.
119. Weil M, Jacobson MD, and Raff MC. Are caspases involved in the death of cells with a transcriptionally inactive nucleus? Sperm and chicken erythrocytes. *J Cell Sci* 111: 2707–2715, 1998.
120. Weinbrenner T, Cladellas M, Isabel CM, Fito M, Tomas M, Senti M, Bruguera J, and Marrugat J. High oxidative stress in patients with stable coronary heart disease. *Atherosclerosis* 168: 99–106, 2003.
121. Wenzel U and Daniel H. Early and late apoptosis events in human transformed and non-transformed colonocytes are independent on intracellular acidification. *Cell Physiol Biochem* 14: 65–76, 2004.
122. Wieder T, Essmann F, Prokop A, Schmelz K, Schulze-Osthoff K, Beyaert R, Dorken B, and Daniel PT. Activation of caspase-8 in drug-induced apoptosis of B-lymphoid cells is independent of CD95/Fas receptor-ligand interaction and occurs downstream of caspase-3. *Blood* 97: 1378–1387, 2001.
123. Wieder T, Orfanos CE, and Geilen CC. Induction of ceramide-mediated apoptosis by the anticancer phospholipid analog, hexadecylphosphocholine. *J Biol Chem* 273: 11025–11031, 1998.
124. Willekens FL, Werre JM, Kruijt JK, Roerdinkholder-Stoelwinder B, Groenen-Dopp YA, van den Bos AG, Bosman GJ, and van Berkel TJ. Liver Kupffer cells rapidly remove red blood cell-derived vesicles from the circulation by scavenger receptors. *Blood* 105: 2141–2145, 2005.
125. Woon LA, Holland JW, Kable EP, and Roufogalis BD. Ca2+ sensitivity of phospholipid scrambling in human red cell ghosts. *Cell Calcium* 25: 313–320, 1999.
126. Wyllie AH. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature* 284: 555–556, 1980.
127. Yu SP. Regulation and critical role of potassium homeostasis in apoptosis. *Prog Neurobiol* 70: 363–386, 2003.
128. Yu SP, Canzoniero LM, and Choi DW. Ion homeostasis and apoptosis. *Curr Opin Cell Biol* 13: 405–411, 2001.
129. Yurinskaya VE, Goryachaya TS, Moshkov AV, Rozanov YuM, Sakuta GA, Shirokova AV, Shumilina EV, Vassilieva IO, Lang F, and Vereninov AA. Potassium and sodium balance in U937 cells during apoptosis with and without cell shrinkage. *Cell Physiol Biochem* 16: 155–162, 2005.
130. Yurinskaya VE, Moshkov AV, Rozanov YM, Shirokova AV, Vassilieva IO, Shumilina EV, Lang F, Volgareva EV, and Vereninov AA. Thymocyte K+, Na+ and water balance during dexamethasone- and etoposide-induced apoptosis. *Cell Physiol Biochem* 16: 15–22, 2005.
131. Zhou Q, Zhao J, Wiedmer T, and Sims PJ. Normal hemostasis but defective hematopoietic response to growth factors in mice deficient in phospholipid scramblase 1. *Blood* 99: 4030–4038, 2002.
132. Zimmerman GA, McIntyre TM, Prescott SM, and Stafforini DM. The platelet-activating factor signaling system and its regulators in syndromes of inflammation and thrombosis. *Crit Care Med* 30: S294–S301, 2002.

Address reprint requests to:
Prof. Dr. Florian Lang
Physiologisches Institut der Universität Tübingen
Gmelinstr. 5
D-72076 Tübingen, Germany

E-mail: florian.lang@uni-tuebingen.de

Date of first submission to ARS Central, January 19, 2006; February 6, 2006.

This article has been cited by:

1. Elisabetta Straface , Alessandra Marchesi , Lucrezia Gambardella , Alessio Metere , Isabella Tarissi de Jacobis , Marina Viora , Luciana Giordani , Alberto Villani , Domenico Del Principe , Walter Malorni , Donatella Pietraforte . 2012. Does Oxidative Stress Play a Critical Role in Cardiovascular Complications of Kawasaki Disease?. *Antioxidants & Redox Signaling* **17**:10, 1441-1446. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
2. Nabil Foudi, Ingrid Gomez, Chabha Benyahia, Dan Longrois, Xavier Norel. 2012. Prostaglandin E2 receptor subtypes in human blood and vascular cells. *European Journal of Pharmacology* . [CrossRef]
3. Sandra Belij, Djordje Miljkovi#, Aleksandra Popov, Vesna Subota, Gordana Timotijevi#, Marija Slavi#, Ivana Mirkov, Dragan Kataranovski, Milena Kataranovski. 2012. Effects of subacute oral warfarin administration on peripheral blood granulocytes in rats. *Food and Chemical Toxicology* **50**:5, 1499-1507. [CrossRef]
4. Erin Wei Qian, Daniel Tianfang Ge, Siu-Kai Kong. 2012. Salidroside Protects Human Erythrocytes against Hydrogen Peroxide-Induced Apoptosis. *Journal of Natural Products* 120406090817002. [CrossRef]
5. Mehrdad Ghashghaeinia, Judith C. A. Cluitmans, Ahmed Akel, Peter Dreischer, Mahmoud Toulany, Martin Köberle, Yuliya Skabytska, Mohammad Saki, Tilo Biedermann, Michael Duszenko, Florian Lang, Thomas Wieder, Giel J. C. G. M. Bosman. 2012. The impact of erythrocyte age on eryptosis. *British Journal of Haematology* n/a-n/a. [CrossRef]
6. Shobana Ganesan, Narayan D. Chaurasiya, Rajnish Sahu, Larry A. Walker, Babu L. Tekwani. 2012. Understanding the mechanisms for metabolism-linked hemolytic toxicity of primaquine against glucose 6-phosphate dehydrogenase deficient human erythrocytes: Evaluation of eryptotic pathway. *Toxicology* . [CrossRef]
7. Mehrdad Ghashghaeinia, Mahmoud Toulany, Mohammad Saki, H. Peter Rodemann, Ulrich Mrowietz, Florian Lang, Thomas Wieder. 2012. Roles of the NF#B and glutathione pathways in mature human erythrocytes. *Cellular & Molecular Biology Letters* **17**:1, 11. [CrossRef]
8. John W. HarveyEvaluation of Erythrocytes 49-121. [CrossRef]
9. Alexandre P. Rogerio, Fernanda F. Anibal. 2012. Role of Leukotrienes on Protozoan and Helminth Infections. *Mediators of Inflammation* **2012**, 1-13. [CrossRef]
10. Yasmina Serroukh, Sarah Djebara, Christophe Lelubre, Karim Zouaoui Boudjeltia, Patrick Biston, Michael Piagnerelli. 2012. Alterations of the Erythrocyte Membrane during Sepsis. *Critical Care Research and Practice* **2012**, 1-7. [CrossRef]
11. Yuliya V. Kucherenko, Florian Lang. 2012. Inhibitory Effect of Furosemide on Non-Selective Voltage-Independent Cation Channels in Human Erythrocytes. *Cellular Physiology and Biochemistry* **30**:4, 863-875. [CrossRef]
12. Per-Arne Oldenborg. 2012. Role of CD47 and Signal Regulatory Protein Alpha (SIRPa) in Regulating the Clearance of Viable or Aged Blood Cells. *Transfusion Medicine and Hemotherapy* **39**:5, 315-320. [CrossRef]
13. Antonio Macciò, Clelia Madeddu. 2012. Management of Anemia of Inflammation in the Elderly. *Anemia* **2012**, 1-20. [CrossRef]
14. Inna Freikman, Eitan Fibach. 2011. Distribution and shedding of the membrane phosphatidylserine during maturation and aging of erythroid cells. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1808**:12, 2773-2780. [CrossRef]
15. D. A. Korzhenevskii, V. N. Kuptsov, V. A. Mityanina, A. A. Selishcheva, S. V. Saveliev, T. Yu. Kalashnikova. 2011. Identification of the individual molecular species of ceramides derived from human erythrocytes using HPLC/MS and HPLC/MS/MS. *Journal of Analytical Chemistry* **66**:13, 1270-1275. [CrossRef]
16. Erwin Weiss, Urszula M. Cytlak, David C. Rees, Anna Osei, John S. Gibson. 2011. Deoxygenation-induced and Ca2+ dependent phosphatidylserine externalisation in red blood cells from normal individuals and sickle cell patients. *Cell Calcium* . [CrossRef]
17. Krishnakumar Balasubramanian, Alan J. SchroitMembrane Lipid Asymmetry in Aging and Apoptosis 289-313. [CrossRef]
18. Shagun Sabarwal, S. V. Subramanian, Marie C. McCormick, Jay G. Silverman. 2011. Husband's preference for a son and women's nutrition: examining the role of actual and desired family composition on women's anaemia and body mass index in India. *Paediatric and Perinatal Epidemiology* no-no. [CrossRef]
19. Silvana Balzan, Angelo Carpi, Monica Evangelista, Giuseppina Nicolini, Alberto Pollastri, Antonio Bottoni, Giorgio Iervasi. 2011. Acute effect of TSH on oxygenation state and volume of erythrocytes from subjects thyroidectomized for differentiated thyroid carcinoma. *Biomedicine & Pharmacotherapy* . [CrossRef]
20. Si Jin , Fan Zhou , Foad Katirai , Pin-Lan Li . Lipid Raft Redox Signaling: Molecular Mechanisms in Health and Disease. *Antioxidants & Redox Signaling*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]

21. Giel J.C.G.M. Bosman, Judith C.A. Cluitmans, Yvonne A.M. Groenen, Jan M. Werre, Frans L.A. Willekens, V#ra M.J. Novotný. 2011. Susceptibility to hyperosmotic stress-induced phosphatidylserine exposure increases during red blood cell storage. *Transfusion* **51**:5, 1072-1078. [CrossRef]
22. I.T. Ivanov, A. Zheleva, I. Zlatanov. 2011. Anion exchanger and the resistance against thermal haemolysis. *International Journal of Hyperthermia* **27**:3, 286-296. [CrossRef]
23. Emilia Maellaro, Silvia Leoncini, Daniele Moretti, Barbara Del Bello, Italo Tanganelli, Claudio De Felice, Lucia Ciccoli. 2011. Erythrocyte caspase-3 activation and oxidative imbalance in erythrocytes and in plasma of type 2 diabetic patients. *Acta Diabetologica* . [CrossRef]
24. Inna Freikman, Israel Ringel, Eitan Fibach. 2011. Oxidative Stress-Induced Membrane Shedding from RBCs is Ca Flux-Mediated and Affects Membrane Lipid Composition. *The Journal of Membrane Biology* **240**:2, 73-82. [CrossRef]
25. Kathrin M. Felder, Katharina Hoelzle, Mathias Ritzmann, Tim Kilchling, Daniela Schiele, Karl Heinritzi, Katrin Groebel, Ludwig E. Hoelzle. 2011. Hemotrophic Mycoplasmas Induce Programmed Cell Death in Red Blood Cells. *Cellular Physiology and Biochemistry* **27**:5, 557-564. [CrossRef]
26. Erwin Weiss, David Charles Rees, John Stanley Gibson. 2011. Role of Calcium in Phosphatidylserine Externalisation in Red Blood Cells from Sickle Cell Patients. *Anemia* **2011**, 1-8. [CrossRef]
27. Duc Bach Nguyen, Lisa Wagner-Britz, Sara Maia, Patrick Steffen, Christian Wagner, Lars Kaestner, Ingolf Bernhardt. 2011. Regulation of Phosphatidylserine Exposure in Red Blood Cells. *Cellular Physiology and Biochemistry* **28**:5, 847-856. [CrossRef]
28. D. SAGAN, N. JERMNIM, O. TANGVARASITTICHAI. 2010. CD95 is not functional in human erythrocytes. *International Journal of Laboratory Hematology* **32**:6p1, e244-e247. [CrossRef]
29. Marcel H.A.M. Fens, Gert Storm, Ralf C.M. Pelgrim, Anton Ultee, Annette T. Byrne, Carlo A. Gaillard, Wouter W. van Solinge, Raymond M. Schiffelers. 2010. Erythrophagocytosis by angiogenic endothelial cells is enhanced by loss of erythrocyte deformability. *Experimental Hematology* **38**:4, 282-291. [CrossRef]
30. I. V. Mindukshev, V. V. Krivoshlyk, I. A. Dobrylko, N. V. Goncharov, E. V. Vivulanets, S. V. Kuznetsov, A. I. Krivchenko. 2010. Abnormalities of elastic and transporting properties of red blood cells under development of apoptosis. *Biochemistry (Moscow) Supplement Series A: Membrane and Cell Biology* **4**:1, 22-31. [CrossRef]
31. Johnny Amer, Mutaz Dana, Eitan Fibach. 2010. The Antioxidant Effect of Erythropoietin on Thalassemic Blood Cells. *Anemia* **2010**, 1-11. [CrossRef]
32. Inna Freikman, Johnny Amer, Israel Ringel, Eitan Fibach. 2009. A flow cytometry approach for quantitative analysis of cellular phosphatidylserine distribution and shedding. *Analytical Biochemistry* **393**:1, 111-116. [CrossRef]
33. Philippe Chadebech, Anoosha Habibi, Ruben Nzouakou, Dora Bachir, Natacha Meunier-Costes, Philippe Bonin, Martine Rodet, Btissam Chami, Frederic Galacteros, Philippe Bierling, France Noizat-Pirenne. 2009. Delayed hemolytic transfusion reaction in sickle cell disease patients: evidence of an emerging syndrome with suicidal red blood cell death. *Transfusion* **49**:9, 1785-1792. [CrossRef]
34. Latorya A. Barber, Mary B. Palascak, Clinton H. Joiner, Robert S. Franco. 2009. Aminophospholipid translocase and phospholipid scramblase activities in sickle erythrocyte subpopulations. *British Journal of Haematology* **146**:4, 447-455. [CrossRef]
35. Hasan Mahmud, Michael Föller, Florian Lang. 2009. Arsenic-induced suicidal erythrocyte death. *Archives of Toxicology* **83**:2, 107-113. [CrossRef]
36. Donatella Zappulla. 2008. Environmental Stress, Erythrocyte Dysfunctions, Inflammation, and the Metabolic Syndrome: Adaptations to CO 2 Increases?. *Journal of the CardioMetabolic Syndrome* **3**:1, 30-34. [CrossRef]
37. G. J. C. G. M. Bosman, J. M. Werre, F. L. A. Willekens, V. M. J. Novotný. 2008. Erythrocyte ageing in vivo and in vitro : structural aspects and implications for transfusion. *Transfusion Medicine* **18**:6, 335-347. [CrossRef]
38. Inna Freikman, Johnny Amer, Jack S. Cohen, Israel Ringel, Eitan Fibach. 2008. Oxidative stress causes membrane phospholipid rearrangement and shedding from RBC membranes—An NMR study. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1778**:10, 2388-2394. [CrossRef]
39. F LAPAIX, G BOUYER, S THOMAS, S EGEE. 2008. Further characterization of cation channels present in the chicken red blood cell membrane. *Bioelectrochemistry* **73**:2, 129-136. [CrossRef]
40. Giel J.C.G.M. Bosman, Edwin Lasonder, Marleen Luten, Bregt Roerdinkholder-Stoelwinder, V#ra M.J. Novotný, Harry Bos, Willem J. De Grip. 2008. The proteome of red cell membranes and vesicles during storage in blood bank conditions. *Transfusion* **48**:5, 827-835. [CrossRef]

41. John W. HarveyThe Erythrocyte 173-240. [CrossRef]
42. Julian C. K. Lui, Judy W. Y. Wong, Y. K. Suen, T. T. Kwok, K. P. Fung, S. K. Kong. 2007. Cordycepin induced eryptosis in mouse erythrocytes through a Ca2+-dependent pathway without caspase-3 activation. *Archives of Toxicology* **81**:12, 859-865. [CrossRef]
43. Ivan Tanev Ivanov. 2007. Allometric dependence of the life span of mammal erythrocytes on thermal stability and sphingomyelin content of plasma membranes. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **147**:4, 876-884. [CrossRef]
44. Luigi Ferrucci, Jack M. Guralnik, Stefania Bandinelli, Richard D. Semba, Fulvio Lauretani, Annamaria Corsi, Carmelinda Ruggiero, William B. Ershler, Dan L. Longo. 2007. Unexplained anaemia in older persons is characterised by low erythropoietin and low levels of pro-inflammatory markers. *British Journal of Haematology* **136**:6, 849-855. [CrossRef]
45. Donatella Pietraforte, Paola Matarrese, Elisabetta Straface, Lucrezia Gambardella, Alessio Metere, Giuseppe Scorza, Thomas L. Leto, Walter Malorni, Maurizio Minetti. 2007. Two different pathways are involved in peroxynitrite-induced senescence and apoptosis of human erythrocytes. *Free Radical Biology and Medicine* **42**:2, 202-214. [CrossRef]