Human erythrocytes, nuclear factor kappaB (NFκB) and hydrogen sulfide (H₂S) – from non-genomic to genomic research

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ABSTRACT

Enculeated mature human erythrocytes possess NFκB and its upstream kinases. There is a negative correlation between the NFκB activity and the amount of NFκB subunits p50 and p65. This finding is based on the fact that young erythrocytes are the last NFκB and the highest extract level, while in old erythrocytes the opposite ratio prevails. Human erythrocytes (hRBCs) effectively control the homoeostasis of the cell membrane permeable anti-inflammatory signal molecule hydrogen sulfide (H₂S). They endogenously produce H₂S via both non-enzymatic (glutathione-dependent) and enzymes (mercaptopyruvate sulfur transferase-dependent). They uptake H₂S from diverse tissues and very effectively degrade H₂S via methemoglobin (HbFe³⁺)-catalyzed oxidation. Interestingly, a reciprocal correlation exists between the intensity of inflammatory diseases and endogenous levels of H₂S. H₂S deficiency has been observed in patients with diabetes, psoriasis, obesity, and chronic kidney disease (CKD). Furthermore, endogenous H₂S deficiency results in impaired renal erythropoietin (EPO) production and EPO-dependent erythropoiesis. In general we can say: dynamic reciprocal interaction between tumor suppressor and oncoproteins, orchestrated and sequential activation of pro-inflammatory NFκB heterodimers (RelA-p50) and the anti-inflammatory NFκB-p50 homodimers for optimal inflammation response, appropriate generation, subsequent degradation of H₂S etc., are prerequisites for a functioning cell and organism. Diseases arise when the fragile balance between different signaling pathways that keep each other in check is permanently disturbed. This work deals with the intact anti-inflammatory hRBCs and their role as guarantors to maintain the redox status in the physiological range, a basis for general health and well-being.

Human erythrocytes (hRBCs) and their methemoglobin (metHb/HbFe³⁺) as biological hydrogen sulfide (H₂S) carrier

Under physiological conditions, the auto-oxidation of about 1–3% of the total body hemoglobin (ferrous Hb/HbFe²⁺) results in the generation of methemoglobin (metHb/HbFe³⁺). Different organs such as liver, kidney, and brain produce the signal molecule hydrogen sulfide (H₂S). The cellular H₂S biogenesis, that is, desulfuration of cysteine or homocysteine, is primarily accomplished by three enzymes: Cystathionine b-synthase (CBS), γ-cystathionase (CSE), and mercaptopyruvate sulfur transferase (MST) [1–3]. hRBCs does possess MST, but not the other two H₂S-producing enzymes [4]. While H₂O flow through cell membrane is accelerated by aquaporins [5], the transmembrane diffusion of hydrophobic H₂S requires no facilitator and its permeability coefficient is still 10,000 times higher than that of water [6]. Based on this property, H₂S can exhibit broad toxicity effects or function as a signal molecule in a concentration-dependent manner. Besides the endogenously produced nitric oxide (NO) and carbon monoxide (CO), the cell membrane permeable H₂S [7,8] plays an important role as a gaseous signaling molecule in biological and physiological processes. H₂S regulates several biological and physiological processes, for instance: it shows anti-thrombotic effects [9], protects vascular tissues from atherogenic disease [10], enhances blood flow which protects against vascular ischemia [11] and inhibits glucose consumption and uptake. For reviews, see [12,13].

One hundred years ago, sophisticated experiment on animals provided the first evidence of fast H₂S detoxification via its metabolism. The practice
of a fast administered single dose of 10 ml of a 77 mmol/l Na₂S solution was always lethal, whereas dogs that received a five-fold dose over a period of 20 minutes survived and showed no obvious damage [14]. Mammalian tissues (e.g. heart, kidney, brain, and intestine) as well as human erythrocytes are able to produce H₂S. Under physiological conditions, ~30% of the short-lived H₂S exists in a non-dissociated form and ~70% in its hydrogen sulfide anion (H₂S ⇔ HS⁻). It is important to note that: a) the completion of net acid efflux by the H₅S/HS⁻ follows the same principle as that of CO₂/HCO₃⁻ in the Jacobs–Stewart cycle (see Figure 1b) due to the lack of extracellular hydration and intracellular dehydration, the net acid efflux in Cl⁻/HS⁻/H₂S cycle is faster than Cl⁻/HCO₃⁻/H₂CO₃ cycle, c) HS⁻ is a very good substrate for the anion exchanger 1 (AE1) and d) H₂S possesses a very high permeability coefficient in human erythrocytes [15]. The atomic structure of CO allows it to solely bind Fe²⁺ with its two unpaired electrons [16]. Therefore, all of the spectral work with CO and heme proteins employs the native (reduced) forms. NO is able to bind both Fe²⁺ and Fe³⁺ in heme proteins. H₂S binds rapidly to Fe³⁺ in heme proteins, for example, methemoglobin (HbFe³⁺) [4,17]. Vitvitsky et al. showed that hRBCs produce H₂S via MST and in addition to this effectively clear sulfide via MetHb-catalyzed oxidation of H₂S to thiosulfate and polysulfides [4], see also Fig. 1 and 2. Human erythrocytes are represented by ~5 billion per ml of blood, and each intact hRBC contains over 270 million hemoglobin molecules that are able to uptake H₂S from diverse tissues and very effectively control its clearance. This ensures the maintenance of the physiological plasma and tissues concentration of free H₂S in the range of 15 to 150 nM [18,19]. The very high lipid and water solubility of H₂S allows quick passage through the alveolar membrane, which is the best condition for achieving an almost perfect equilibrium between blood and alveolar air. Human alveolar air measurements showed negligible free H₂S, indicating very low blood concentration [18]. The fundamental findings of Furne et al. also revealed the conventional experimental errors involved in H₂S research. Considering that a) we have seen the work of Furne et al. 2008 as well as King et al. 2014, b) an adult healthy human has ~30 trillion (3 × 10¹³) circulating RBCs with a life span of 120 days [20], c) ~1% of the circulating hRBCs (~200–300 billion cells) are cleared per day and replaced by erythropoiesis, and d) that ~3.7 million (3.7 × 10⁶) cell-free, intact and respiratory competent mitochondria circulating per ml of blood plasma [21], those organelles apart from hRBCs contribute to the degradation of H₂S, maintain human plasma concentration of H₂S clearly below a µM range under physiological conditions.

**Role of PKC-α and other Ca²⁺-dependent pathways in IĸB-α phosphorylation/degradation and NFκB activation**

Ankyrin-containing proteins including IĸBs act as specific protein–protein interactors [22–24]. The

![Figure 1](image-url)  
*Figure 1.* Role of human red blood cells (hRBCs) in uptake and degradation of H₂S. For more details see the main text.
prevailing opinion is that cellular activation via numerous stimuli initiates IκB-α phosphorylation, its subsequent dissociation from and abolition of its inhibitory effect on NFκB; and these events precede the IκB-α proteolytically degradation. However, this does not reflect the sequence of events. In fact, the IκB-α phosphorylation and its subsequent degradation enables NFκB release, which rapidly translocates into the nucleus [25] to drive the expression of genes, for example, IL-8 expression [26]. The dual function of the chemokine IL-8 includes pathogen elimination by recruitment of neutrophils and being causative in several inflammatory diseases. Both IL-4 and human erythrocytes can curb IL-8 effects. IL-4 functions as an endogenous inhibitor of IL-8 expression [27] and hRBCs reduces the bioavailability of IL-8 substantially by acting as a sink for IL-8 [28–30]. Steffan et al. were the first to show a direct link between the synergistic effects of PKC- and Ca$^{2+}$-dependent phosphatase calci-neurin on the regulation of IκB-α phosphorylation and pointed to the necessity of the existence of an IκB-α kinase (IKK) [25,31]. In the meantime, the existence of IKKs [32,33], but also of an IKK kinase, have been proven [34].

Retrobulbarly collected whole blood, the subsequent isolation of erythrocytes from the homozygous NFκB-p50 deficient and congenic wild-type C57BL/6 and their subsequent incubation in Ringer solution enabled to demonstrate a direct correlation between NFκB-p50 deficiency and increased eryptosis [38]. Additional biological/physiological effects were: a) significant increase of white blood cell (WBCs) count and b) considerable weight loss in NFκB-p50 deficient mice. The former indicates systemic inflammation in NFκB-p50 deficient mice and the latter observation offers a possibility to treat obesity with NFκB inhibitors provided their bioavailability is sufficient [39,40]. It is known that NFκB-p50 homodimers are refractory to inflammation while NFκB heterodimers (e.g. RelA-p50 subunits) have an inflammatory function [41]. This is why impaired p50-p50 activation is associated with dysregulated inflammation and chronic inflammatory diseases.

The generation of a reduced form of glutathione (GSH), an intracellular antioxidant, is the result of two concerted ATP-consuming reactions conducted by 1) γ-glutamylcysteine synthetase (γ-GCS) and 2) GSH synthetase (GS) [42,43]. (Reaction 1): L-glutamate + L-cysteine + ATP → γ-L-glutamyl-L-cysteine + ADP + P$_i$ (Reaction 2): γ-glutamyl-L-cysteine + L-glycine + ATP → GSH + ADP + P$_i$

The first reaction is, however, feedback inhibited by GSH [44], see also Figure 2. In nucleated cells, GSH and NO, respectively, are able to inhibit IKK-b activity by reversible S-glutathionylation or S-nitrosylation, which ultimately impairs NFκB activation [45,46]. Protein kinase C-alpha (PKC-α) phosphorylates NFκB-p65 subunits [47] and this is associated with NFκB-dependent induction of γ-GCS and intracellular GSH de novo biosynthesis [48]. Interestingly, addition of exogenous NO donor DETA/NO results in NO-mediated release of “free” intracellular zinc, zinc-dependent increase of γ-GCS expression and GSH synthesis [49]. This could be a new IKK-NFκB-independent, NO-dependent pro-survival pathway connecting redox potential of a cell with intracellular “free” zinc concentration. Human erythrocytes (hRBCs) possess functional endothelial nitric oxide synthase (eNOS) (L-Arginine + O$_2$ + eNOS → L-Citulline + NO) [50,51] and much more important is the fact that pro-survival NO and pro-eryptotic Ca$^{2+}$ keep each other in check [52]. Under physiological

**Role of redox-sensitive canonical NFκB pathway in human erythrocytes (hRBCs). NFκBs in nucleated cells; the complexity of glutathione (GSH), NFκB, PKC, Ca$^{2+}$ and nitric oxide synthase (NOS) interactions**

Human erythrocytes (hRBCs) possess the main members of the canonical NFκB pathway [35–37]. Virtually all publications on erythrocytes’ NFκBs available to date originate from our laboratory, which demonstrate a reciprocal relationship between age and abundance of NFκBs in hRBCs; the NFκB protein abundance is highest in young and lowest in aged erythrocytes. There is a positive correlation between cell volume, and a negative correlation between eryptosis (cell death of erythrocytes) and the amount of NFκB subunits p50 and p65. This finding is based on the fact that young erythrocytes have the highest cell volume and the lowest eryptosis rate, while in old erythrocytes the opposite ratio prevails [36].
conditions one portion of the abundant serum albumin binds to NO, forming a relatively long-lived albumin-NO-adduct (~7 µM S-nitrosothiol) and thus functioning as a sink for NO, while free NO with a plasma concentration of ~3 nM serves predominantly to maintain the vascular tone [53], see also Figure 2. For more details about the biological roles of NO we refer to the following reviews [54,55].

**Human erythrocytes (hRBCs), free and unbound Calcium (Ca^{2+}), Ca^{2+} dependent Protein kinase C-alpha (PKC-α) and NFκB activation. The Bermuda Triangle Ca^{2+}-PKC-α-NFκB and its association with respiratory diseases**

Intact hRBCs among mammalian cells have the lowest free, unbound intracellular concentration of calcium ions [Ca^{2+}]_{i} under physiological conditions ranging between 20 and 30 nM [56]. In contrast to organelles-free mature hRBCs, other mammalian cells possess multiple organelles with a wide variety of free, unbound calcium distributions, for example, [Ca^{2+}]_{c} ~50–100 nM, [Ca^{2+}]_{mt} ~100 nM, [Ca^{2+}]_{nucleus} ~100 nM, [Ca^{2+}]_{ER} ~100–700 nM, for review see [57]. [Ca^{2+}]_{i} directly impairs the transmembrane equilibrium distribution of the phospholipids, that is, their inward translocation from the outer to the inner leaflet of erythrocytes’ plasma membrane. For instance, [Ca^{2+}]_{i} of ~50 and ≥200 nM affect the inward translocation of phosphatidylethanolamine and acidic phosphatidylinerine (PS), respectively [58], a process directly related to Ca^{2+}-dependent inhibition of aminophospholipid translocase (or flipase) activity. A sustained cytosolic calcium elevation [Ca^{2+}]_{i} concomitantly promotes the activity of the phospholipid scramblase which then unspecifically initiates bidirectional PS translocation on both sides of the plasma membrane. In contrast to internalized PS, PS externalization or depletion is associated with a cell-type independent weakening of the plasma membrane Ca^{2+}-ATPase (PMCA)-mediated Ca^{2+}-efflux [59,60].

The following reviews illustrate Ca^{2+} transporting systems, for example, PMCA and the Ca^{2+}-activated K+ channel, known as Gardos channel [61–63]: In view of the antagonistic roles of NO and Ca^{2+} [52] and association of early eryptosis with the removal/translocation of PS from the inner to the outer leaflet of the bilayer plasma membrane, it is not surprising that intact hRBCs maintain their [Ca^{2+}]_{i} as low as possible and as much as necessary (see Figure 3).

It is undisputed that changes in [Ca^{2+}]_{i} are associated with changes in cell functions [64]. [Ca^{2+}]_{i} mediates both cell survival and apoptosis depending on its oscillation and excessive elevation, respectively [57,65,66]. The most prominent member of the protein kinase C (PKC) family is the Ca^{2+}-, acidic phospholipids (e.g. PS-) and diacylglycerol (DAG)-dependent conventional PKC-alpha (cPKC-α) [67–71]. According to structure and cofactor regulation, the PKC family is divided...
Figure 3. Qualitative illustration of four major plasma membrane phospholipids as well as intraerythrocytic distribution of potassium (K⁺) and calcium (Ca²⁺) ions in intact and eryptotic human erythrocyte.

into three classes: cPKCs (α, βI, βII, and γ), novel, that is, nPKCs (δ, ε, η, and θ), and atypical, that is, aPKCs (ζ and iota (ι)). The first class being Ca²⁺-, PS, and DAG-dependent; the second being Ca²⁺-independent but PS- and DAG-dependent, and the third class being Ca²⁺- and DAG-independent but acidic phospholipids and ceramides dependent. hRBCs possess four cytosolic isoforms of PKCs: alpha, zeta, mu, and iota, of which only PKC-α with membrane translocation capability [72,73], that is, induction of eryptosis [74,75]. Using chelerythrine as a specific PKC-α inhibitor, we were able to show a direct correlation between the costunolide-induced GSH-depletion and PKC-α activation in hRBCs [76], a phenomenon also observed in nucleated mammalian cells. Thus, it is not astonishing that the capacity of hRBCs to synthesize ~2 mM of the pro-survival [GSH]i [77,78] exceeds the rate GSH turnover by 150-fold [79] to avoid a PKC-α mediated induction of erythrocytes death (eryptosis). Furthermore, H₂S can be endogenously produced in the presence of GSH [80,81]. GSH is a linchpin of cellular defense protecting both prokaryotic [82] and eukaryotic cells [83,84] including hRBCs from biotic and abiotic stresses. In nucleated mammalian cells, PKC-α activation drives the pro-survival machinery [85–87], and its inhibition commonly triggers apoptosis in these cells [88,89]. In addition to this, respiratory diseases of viral [90] and bacterial [91] origin are associated with PKC-α activation. It is to note that intact hRBCs are actively involved in bacterial [92] and viral clearance from circulation [93–98], for reviews see [99,100]. The message is clear: specific PKC-α inhibitors, for example, the bioactive molecule chelerythrine [101–104], as a natural product of plant origin can dose-dependently cause a pro-apoptotic effect in nucleated cells, thus creating a hostile environment for intracellular parasites including viruses and simultaneously can create a pro-survival effect in enucleated hRBCs [76]. Therefore, hRBCs in combination with PKC-α inhibitors (e.g., chelerythrine) should be a promising approach to treat COVID-19 [105]. PKC-α as the upstream kinase of the NFkB signaling pathway as well as NFkB itself, represent a link between nucleated and enucleated mammalian cells, which can be designated as: “NFkB, from non-genomic to genomic research”.

Inflammation vs. anti-inflammation.
NFkB-p65, glycolysis, inflammatory diseases vs. hRBCs, H₂S, insulin

Obesity- and psoriasis-associated chronic low-grade inflammation and NFκB activation are two sides of the same coin that perpetuate each other. It is known that NFκB is a positive physiological regulator of glycolysis [106], for review see [107]. The following review clearly illustrates the relationship between the anti-inflammatory effects of insulin and the pro-inflammatory effects of glucose with NFκB as a common target [108]. Interestingly, glucose uptake is negatively
correlated with in adipose tissue up-regulation of H₂S system. As already mentioned, a negative correlation exists between the intensity of inflammatory diseases and endogenous H₂S levels. Psoriasis is directly associated with low serum H₂S levels [109], for review, see [110]. Diminished adipose tissue H₂S has been observed in obesity. H₂S inhibits the expression of highly pro-inflammatory IL-8 in human keratinocytes and shows potential for psoriasis treatment [111]. In addition, hRBCs function as a sink for IL-8, thus minimizing the deleterious effects of NFκB-mediated IL-8 expression. Recently, Mezouari et al. demonstrated that H₂S enhances the secretion of the glucoregulatory hormone glucagon-like peptide 1 and improves glucose clearance in mice [112], for review see [113]. In addition to these, endogenous H₂S deficiency in patients with chronic kidney disease (CKD) is associated with impaired renal erythropoietin (EPO) production and EPO-dependent erythropoiesis [114]. Taken together, the role of anti-inflammatory hRBCs to regulate H₂S homeostasis and to maintain its physiological concentration in the blood as well as to function as a sink for a many inflammatory cytokines and chemokines, is essential for maintaining cellular health as the basis for general health and well-being.

**H₂S-mediated regulation of biochemical pathways in human erythrocytes (hRBCs)**

For adequate supply of the organism with molecular oxygen, hRBCs divert 20% of the uptaken glucose to Rapoport and Luebering glycolytic shunt [115], for review see [107]. In this process erythrocyte 2,3-bisphosphoglycerate (2,3-BPG) plays a central role. It negatively regulates hemoglobin oxygen (O₂) binding affinity, facilitates O₂ release from oxyhemoglobin [116] improving tissue oxygenation. H₂S regulates 2,3-BPG production and it exists a reciprocal correlation between H₂S concentration and 2,3-BPG production. H₂S level increases during normoxic and decreases during hypoxic conditions [117]. This ensures maximum O₂ uptake in the lungs and maximum O₂ release in the peripheral tissues. It is to note that the reduced form of glutathione (GSH), glycolytic, and pentose phosphate pathways positively regulate H₂S production in hRBCs [80]. hRBCs possess an active and functional endothelial nitric oxide synthase (eNOS) and are a major source of NO (hRBC-eNOS → NO production), contributing to the circulating NO pool [50,118]. The ability of hRBCs to take up endothelium-derived NO, thereby limiting NO available for vasodilation: Fe²⁺-HbO₂ (oxy-Hb) + NO → Fe³⁺-Hb (metHb) + NO₃⁻, does not invalidate our statement just described. The localization of homodimeric hRBC-eNOS at the cytoplasm leaflet preferentially increase local metHb concentration which in turn acts like a shield to protect NO molecules – produced by hRBC-eNOS – from scavenging by oxyhemoglobin (oxy-Hb). This allows NO molecules not only to leave the erythrocytes but also to interact with their targets located in the immediate vicinity of hRBC-eNOS. Another important aspect is that metHb molecules generated in this process can now be used to clear sulfide via MetHb-catalyzed oxidation of H₂S to thiosulfate and polysulfides. It is to note that high concentration of NO impairs dimer stability of eNOS as well as its activity and this loss of dimer (eNOS monomerization) can be reversed by thioredoxin/thioreductase system [119]. These sophisticated and coordinated processes curtail exuberant NO production in vivo. The following work illustrates in a very compact form the physical and chemical properties of NO and its physiological roles [120]. NO inhibits erythrocyte cell death (erytosis) [52] and reduction of NO bioavailability has been observed in several diseases, for example, in sickle cell anemia [121]. Recently, we observed systemic inflammation and enhanced rate of erytosis in NFκB-p50 (p50) deficient mice [38]. It is known that NFκB-p50 homodimers are refractory to inflammation while NFκB heterodimers (e.g. NFκB-p65-p50 subunits) have an inflammatory function [41]. NFκB-p65 (p65) activity is regulated by several reversible post-translational modification mechanisms. p65 is activated by phosphorylation [122] or acetylation [123] and inhibited by deacetylation [123,124]. To date, there is no single publication that has investigated the influence of H₂S on NFkB in hRBCs. We tend to believe that H₂S with its anti-inflammatory properties exerts an inhibitory effect on p65 and positively regulates p50. We will clarify this experimentally in the near
future. In nucleated cells, publications on the influence of H₂S on NFκBαs are contradictory. According to several publications, H₂S-mediated p65 sulphydrination can lead to its activation and inhibition. These inconsistencies are rather due to a lack of standardized methods for determining H₂S concentration.

**Conclusion and perspectives**

Human erythrocytes (hRBCs) are a mobile organ that traverse our entire organism. They are involved in innumerable biological and physiological processes, are directly involved in virus and bacterial elimination from circulation, maintain the concentrations of many signaling molecules and antioxidants in physiological range, possess transcription factors such as NFκBαs and their upstream kinases and act as a sink for many inflammatory cytokines and chemokines, thus minimizing their deleterious effects. Therefore, treatment of many pathological diseases without considering hRBCs, is myopic and not an adequate remedy.

**Acknowledgments**

Not applicable

**Funding**

This work was financed by Mehrdad Ghashghaeinia

**Authors contributions**

MG designed the project and mainly wrote the manuscript. All figures were made by MG. All authors read, discussed, improved, and approved the final version of the manuscript.

**Disclosure statement**

The authors declare that no competing financial interests or otherwise exist.

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**References**

[1] Stipanuk MH, Beck PW. Characterization of the enzymic capacity for cysteine desulfhydration in liver and kidney of the rat. Biochem J. 1982;206(2):267–277.

[2] Shibuya N, Mikami Y, Kimura Y, et al. Vascular endothelium expresses 3-mercaptopuruvate sulfuryltransferase and produces hydrogen sulfide. J Biochem. 2009;146(5):623–626.

[3] Tanizawa K. Production of H₂S by 3-mercaptopuruvate sulphurtransferase. J Biochem. 2011;149(4):357–359.

[4] Vitvitsky V, Yadav PK, Kurthen A, et al. Sulfide oxidation by a noncanonical pathway in red blood cells generates thiosulfate and polysulfides. J Biol Chem. 2015;290(13):8310–8320.

[5] Carbrey JM, Agre P. Discovery of the aquaporins and development of the field. Handb Exp Pharmacol. 2009;190:3–8.

[6] Mathai JC, Mer A, Kugler P, et al. No facilitator required for membrane transport of hydrogen sulfide. Proc Natl Acad Sci U S A. 2009;106(39):16633–16638.

[7] Cuevasanta E, Denicola A, Alvarez B, et al. Solubility and permeation of hydrogen sulfide in lipid membranes. PLoS One. 2012;7(4):e34562.

[8] Riahi S, Rowley CN. Why can hydrogen sulfide permeate cell membranes? J Am Chem Soc. 2014;136(43):15111–15113.

[9] Kram L, Grambow E, Mueller-Gräf F, et al. The anti-thrombotic effect of hydrogen sulfide is partly mediated by an upregulation of nitric oxide synthases. Thromb Res. 2013;132(2):e112–7.

[10] Mani S, Li H, Untereiner A, et al. Decreased endogenous production of hydrogen sulfide accelerates atherosclerosis. Circulation. 2013;127(25):2523–2534.

[11] Pardue S, Kolluru GK, Shen X, et al. Hydrogen sulfide stimulates xanthine oxidoreductase conversion to nitrite reductase and formation of NO. Redox Biol. 2020;34:101447.

[12] Mani S, Cao W, Wu L, et al. Hydrogen sulfide and the liver. Nitric Oxide. 2014;41:62–71.

[13] Altaany Z, Moccia F, Munaron L, et al. Hydrogen sulfide and endothelial dysfunction: relationship with nitric oxide. Curr Med Chem. 2014;21(32):3646–3661.

[14] Haggard HW, Thomas JC. THE FATE OF SULFIDES IN THE BLOOD. J Biol Chem. 1921;49(2):519–529.

[15] Jennings ML. Transport of H₂S and HS(-) across the human red blood cell membrane: rapid H₂S diffusion and AE1-mediated Cl(-)/HS(-) exchange. Am J Physiol Cell Physiol. 2013;305(9):C941–50.

[16] Omura T, Sato R. The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. J Biol Chem. 1964;239(7):2370–2378.
[17] Jensen B, Fago A. Reactions of ferric hemoglobin and myoglobin with hydrogen sulfide under physiological conditions. J Inorg Biochem. 2018;182:133–140.

[18] Furne J, Saeed A, Levitt MD. Whole tissue hydrogen sulfide concentrations are orders of magnitude lower than presently accepted values. Am J Physiol Regul Integr Comp Physiol. 2008;295(5):R1479–85.

[19] King AL, Polhemus DJ, Bhushan S, et al. Hydrogen sulfide cytoprotective signaling is endothelial nitric oxide synthase-nitric oxide dependent. Proc Natl Acad Sci U S A. 2014;111(8):3182–3187.

[20] Callender EOP ST, Witts LJ. The life-span of the red cell in men. J Pathol Bacteriol. 1945;57(1):129–139.

[21] Al Amir Dache Z, Otandault A, Tanos R, et al. Blood contains circulating cell-free respiratory competent mitochondria. FASEB J. 2020;34(3):3616–3630.

[22] Voronin DA, Kiseleva EV. Functional role of proteins containing ankyrin repeats. Cell Tissue Biol. 2008;2(1):1–12.

[23] Jernigan KK, Bordenstein SR. Ankyrin domains across the tree of life. PeerJ. 2014;2:e264.

[24] Galpern EA, Freiberger MI, Ferreiro DU. Large Ankyrin repeat proteins are formed with similar and energetically favorable units. PLoS One. 2020;15(6):e0233865.

[25] Steffan NM, Bren GD, Frantz B, et al. Regulation of IkB alpha phosphorylation by PKC- and Ca(2+)-dependent signal transduction pathways. J Immunol. 1995;155:4685–4691.

[26] Yoshida A, Yoshida S, Khalil AK, et al. Role of NF-kappaB-mediated interleukin-8 expression in intraocular neovascularization. Invest Ophthalmol Vis Sci. 1998;39:1097–1106.

[27] Standiford TJ, Strieter RM, Chensue SW, et al. IL-4 inhibits the expression of IL-8 from stimulated human monocytes. J Immunol. 1990;145:1435–1439.

[28] Darbonne WC, Rice GC, Mohler MA, et al. Red blood cells are a sink for interleukin 8, a leukocyte chemotaxin. J Clin Invest. 1991;88(4):1362–1369.

[29] De Winter RJ, Manten A, de Jong YP, et al. Interleukin 8 released after acute myocardial infarction is mainly bound to erythrocytes. Heart. 1997;78(6):598–602.

[30] Horuk R, Colby TJ, Darbonne WC, et al. The human erythrocyte inflammatory peptide (chemokine) receptor. Biochemical characterization, solubilization, and development of a binding assay for the soluble receptor. Biochemistry. 1993;32(22):5733–5738.

[31] Lanucara F, Lam C, Mann J, et al. Dynamic phosphorylation of RelA on Ser42 and Ser45 in response to TNFalpha stimulation regulates DNA binding and transcription. Open Biol. 2016;6(7).

[32] Trushin SA, Pennington KN, Algeciras-Schimnich A, et al. Protein kinase C and calcineurin synergize to activate IkappaB kinase and NF-kappaB in T lymphocytes. J Biol Chem. 1999;274(33):22923–22931.

[33] Trushin SA, Pennington KN, Carmona EM, et al. Protein kinase Calpha (PKCalpha) acts upstream of PKCtheta to activate IkappaB kinase and NF-kappaB in T lymphocytes. Mol Cell Biol. 2003;23:7068–7081.

[34] Tojima Y, Fujimoto A, Delhase M, et al. NAK is an IkappaB kinase-activating kinase. Nature. 2000;404(6779):778–782.

[35] Ghashghaeinia M, Toulany M, Saki M, et al. The NFkB pathway inhibitors Bay 11–7082 and parthenolide induce programmed cell death in anucleated Erythrocytes. Cell Physiol Biochem. 2011;27(1):45–54.

[36] Ghashghaeinia M, Cluitmans JC, Toulany M, et al. Age sensitivity of NFkappaB abundance and programmed cell death in erythrocytes induced by NFkappaB inhibitors. Cell Physiol Biochem. 2013;32(4):801–813.

[37] Ghashghaeinia M, Toulany M, Saki M, et al. Potential roles of the NFkappaB and glutathione pathways in mature human erythrocytes. Cell Mol Biol Lett. 2012;17(1):11–20.

[38] Ghashghaeinia M, Mrowietz U, Dreischer P, et al. Association between nuclear factor of kappa B (NFkappaB) deficiency and induction of eryptosis in mouse erythrocytes. Apoptosis. 2021;26(1–2):4–6.

[39] Ghashghaeinia M (2019) Pharmaceutical composition containing Bay 11–7082, parthenolide or a combination thereof for the treatment of obesity or cardiovascular diseases. US Patent 10,420,746, B2.

[40] Ghashghaeinia M, Giustarini D, Koralkova P, et al. Pharmacological targeting of glucose-6-phosphate dehydrogenase in human erythrocytes by Bay 11–7082, parthenolide and dimethyl fumarate. Sci Rep. 2016;6(1):28754.

[41] Singh AK, Jiang Y. Differential activation of NF kappa B/RelA-p50 and NF kappa B/p50-p50 in control and alcohol-drinking rats subjected to carrageenin-induced pleurisy. Mediators Inflamm. 2004;13(4):255–262.

[42] Anderson ME, Meister A. Transport and direct utilization of gamma-glutamylcyst(e)ine for glutathione synthesis. Proc Natl Acad Sci U S A. 1983;80(3):707–711.

[43] Raftos JE, Willhite S, Kuchel PW. Glutathione synthesis and turnover in the human erythrocyte: alignment of a model based on detailed enzyme kinetics with experimental data. J Biol Chem. 2010;285(31):23557–23567.

[44] Richman PG, Meister A. Regulation of gamma-glutamyl-cysteine synthetase by nonallosteric feedback inhibition by glutathione. J Biol Chem. 1975;250(4):1422–1426.

[45] Reynaert NL, van der Vliet A, Guala AS, et al. Dynamic redox control of NF-kappaB through glutaredoxin-regulated S-glutathionylation of inhibitory kappaB kinase beta. Proc Natl Acad Sci U S A. 2006;103(35):13086–13091.
[46] Reynaert NL, Ckless K, Korn SH, et al. Nitric oxide represses inhibitory kappaB kinase through S-nitrosylation. Proc Natl Acad Sci U S A. 2004;101(24):8945–8950.

[47] Mut M, Amos S, Hussaini IM. PKC alpha phosphorylates cytosolic NF-kappaB/p65 and PKC delta delays nuclear translocation of NF-kappaB/p65 in U1242 glioblastoma cells. Turk Neurosurg. 2010;20:277–285.

[48] Iwanaga M, Mori K, Iida T, et al. Nuclear factor kappa B dependent induction of gamma glutamylcysteine synthetase by ionizing radiation in T98G human glioblastoma cells. Free Radic Biol Med. 1998;24(7–8):1256–1268.

[49] Cortese-Krott MM, Suschek CV, Wetzal W, et al. Nitric oxide-mediated protection of endothelial cells from hydrogen peroxide is mediated by intracellular zinc and glutathione. Am J Physiol Cell Physiol. 2009;296(4):C811–20.

[50] Kleinbongard P, Schulz R, Rassaf T, et al. Red blood cells express a functional endothelial nitric oxide synthase. Blood. 2006;107(7):2943–2951.

[51] Chen LY, Mehta JL. Evidence for the presence of L-arginine-nitric oxide pathway in human red blood cells: relevance in the effects of red blood cells on platelet function. J Cardiovasc Pharmacol. 1998;32(1):57–61.

[52] Ghashghaeinia M, Wesseling MC, Ramos E, et al. Trifluoperazine-Induced suicidal erythroid death and S-Nitrosylation inhibition, reversed by the nitric oxide donor sodium nitroprusside. Cell Physiol Biochem. 2017;42:1985–1998.

[53] Stamler JS, Jaraki O, Osborne J, et al. Nitric oxide circulates in mammalian plasma primarily as an S-nitroso adduct of serum albumin. Proc Natl Acad Sci U S A. 1992;89(16):7674–7677.

[54] Traylor TG, Sharma VS. Why NO? Biochemistry. 1992;31(11):2847–2849.

[55] Snyder SH, Bredt DS. Biological roles of nitric oxide. Sci Am. 1992;266(5):68–77.

[56] Lew VL, Tsien RY, Miner C, et al. Physiological [Ca2+]i level and pump-leak turnover in intact red cells measured using an incorporated Ca chelator. Nature. 1982;298(5873):478–481.

[57] Suzuki Y, Inoue T, Ra C. L-type Ca2+ channels: a new player in the regulation of Ca2+ signaling, cell activation and cell survival in immune cells. Mol Immunol. 2010;47(4):640–648.

[58] Bitbol M, Fellmann P, Zachowski A, et al. Ion regulation of phosphatidylinositol and phosphatidylethanolamine outside-inside translocation in human erythrocytes. Biochim Biophys Acta. 1987;904(2):268–282.

[59] Zhang J, Xiao P, Zhang X. Phosphatidylinositol externalization in caveolae inhibits Ca2+ efflux through plasma membrane Ca2+-ATPase in ECV304. Cell Calcium. 2009;45(2):177–184.

[60] Niggl V, Penniston JT, Carafoli E. Purification of the (Ca2+-Mg2+)-ATPase from human erythrocyte membranes using a calmodulin affinity column. J Biol Chem. 1979;254(20):9955–9958.

[61] Brini M, Carafoli E. The plasma membrane Ca(2+)-ATPase and the plasma membrane sodium calcium exchanger cooperate in the regulation of cell calcium. Cold Spring Harb Perspect Biol. 2011;3(2):a004168–a004168.

[62] Lopreti R, Giacomello M, Carafoli E. The plasma membrane calcium pump: new ways to look at an old enzyme. J Biol Chem. 2014;289(15):10261–10268.

[63] Maher AD, Kuchel PW. The Gardos channel: a review of the Ca2+-activated K+ channel in human erythrocytes. Int J Biochem Cell Biol. 2003;35(8):1182–1197.

[64] De Konincx P, Schulman H. Sensitivity of CaM Kinase II to the frequency of Ca2+ Oscillations. Science. 1998;279(5348):227–230.

[65] La Rovere RM, Roest G, Bultynck G, et al. Intracellular Ca(2+) signaling and Ca(2+) microdomains in the control of cell survival, apoptosis and autophagy. Cell Calcium. 2016;60:74–87.

[66] Orrenius S, Zhivotovsky B, Nicotera P. Regulation of cell death: the calcium-apoptosis link. Nat Rev Mol Cell Biol. 2003;4(7):552–565.

[67] Stahelin RV, Cho W. Roles of calcium ions in the membrane binding of C2 domains. Biochem J. 2001;359(3):679–685.

[68] Bittova L, Stahelin RV, Cho W. Roles of ionic residues of the C1 domain in protein kinase C-alpha activation and the origin of phosphatidylserine specificity. J Biol Chem. 2001;276(6):4218–4226.

[69] Swanson CJ, Sommese RF, Petersen KJ, et al. Calcium stimulates self-assembly of protein kinase C alpha in vitro. PLoS One. 2016;11(10):e0162331.

[70] Ryves WJ, Evans AT, Olivier AR, et al. Activation of the PKC-isotypes α, β1, γ, δ, and ε by phosphor esters of different biological activities. FEBS Lett. 1991;288(1–2):5–9.

[71] Orr JW, Keranen LM, Newton AC. Reversible exposure of the pseudosubstrate domain of protein kinase C by phosphatidyserine and diacylglycerol. J Biol Chem. 1992;267(22):15263–15266.

[72] Govetak RB, Zingde SM. Protein kinase C isoforms in human erythrocytes. Ann Hematol. 2001;80(9):531–534.

[73] Palfrey CE, Waseem A. Protein kinase C in the human erythrocyte. Translocation to the plasma membrane and phosphorylation of bands 4.1 and 4.9 and other membrane proteins. J Biol Chem. 1985;260(29):16021–16029.

[74] Klair BA, Lang PA, Kempe DS, et al. Protein kinase C mediates erythrocyte “programmed cell death” following glucose depletion. Am J Physiol Cell Physiol. 2006;290(1):C244–53.

[75] de Jong K, Rettig MP, Low PS, et al. Protein kinase C activation induces phosphatidyserine exposure on
red blood cells. Biochemistry. 2002;41 (41):12562–12567.

[76] Ghashghaieinia M, Koralkova P, Giustarini D, et al. The specific PKC-alpha inhibitor chelerythrine blunts costunolide-induced eryptosis. Apoptosis. 2020;25(9–10):674–685.

[77] May JM, Qu Z, Morrow JD. Mechanisms of ascorbic acid recycling in human erythrocytes. Biochim Biophys Acta. 2001;1528(2–3):159–166.

[78] van ’t Erve TJ, Wagner BA, Ryckman KK, et al. The concentration of glutathione in human erythrocytes is a heritable trait. Free Radic Biol Med. 2013;65:742–749.

[79] Minnich V, Smith MB, Brauner MJ, et al. Glutathione biosynthesis in human erythrocytes. I. Identification of the enzymes of glutathione synthesis in hemolysates. J Clin Invest. 1971;50(3):507–513.

[80] Searcy DG, Lee SH. Sulfur reduction by human erythrocytes. J Exp Zool. 1998;282(3):310–322.

[81] Ishigami M, Hiraki K, Umemura K, et al. A source of hydrogen sulfide and a mechanism of its release in the brain. Antioxid Redox Signal. 2009;11(2):205–214.

[82] Smirnova GV, Oktyabrsky ON. Glutathione in bacteria. (Mosc. Biochemistry (1982);70 (11):1199–1211.

[83] Franco R, Schoneveld OJ, Pappa A, et al. The central role of glutathione in the pathophysiology of human diseases. Arch Physiol Biochem. 2007;113(4–5):234–258.

[84] Rana SV, Allen T, Singh R. Inevelative glutathione, then and now. Indian J Exp Biol. 2002;40:706–716.

[85] Takami M, Katayama K, Noguchi K, et al. Protein kinase C alpha-mediated phosphorylation of PIM-1L promotes the survival and proliferation of acute myeloid leukemia cells. Biochem Biophys Res Commun. 2018;503(3):1364–1371.

[86] Valdes-Rives SA, de la Fuente-Granada M, Velasco-Velazquez MA, et al. LPAl1 receptor activation induces PKCalpha nuclear translocation in glioblastoma cells. Int J Biochem Cell Biol. 2019;110:91–102.

[87] Kim CW, Asai D, Kang JH, et al. Reversal of efflux of an anticancer drug in human drug-resistant breast cancer cells by inhibition of protein kinase Calpha (PKCalpha) activity. Tumour Biol. 2016;37 (2):1901–1908.

[88] Yun BR, Lee MJ, Kim JH, et al. Enhancement of parthenolide-induced apoptosis by a PKC-alpha inhibition through heme oxygenase-1 blockage in cholangiocarcinoma cells. Exp Mol Med. 2010;42 (11):787–797.

[89] Jasinski P, Zwolak P, Terai K, et al. PKC-alpha inhibitor MT477 slows tumor growth with minimal toxicity in in vivo model of non-Ras-mutated cancer via induction of apoptosis. Invest New Drugs. 2011;29 (1):33–40.

[90] Maharaj NP, Wies E, Stoll A, et al. Conventional protein kinase C-alpha (PKC-alpha) and PKC-beta negatively regulate RIG-I antiviral signal transduction. J Virol. 2012;86(3):1358–1371.

[91] Chen F, Kumar S, Yu Y, et al. PKC-dependent phosphorylation of eNOS at T495 regulates eNOS coupling and endothelial barrier function in response to G+ -toxins. PLoS One. 2014;9(7):e99823.

[92] Thielen AJF, Zeerleder S, Wouters D. Consequences of dysregulated complement regulators on red blood cells. Blood Rev. 2018;32(4):280–288.

[93] Beck Z, Brown BK, Wieczorek L, et al. Human erythrocytes selectively bind and enrich infectious HIV-1 virions. PLoS One. 2009;4(12):e8297.

[94] Paul RW, Lee PW. Glycophorin is the reovirus receptor on human erythrocytes. Virology. 1987;159 (1):94–101.

[95] Engen Bjorn, Burness AT. Chemical structure of attachment sites for viruses on human erythrocytes. Nature. 1977;268(5620):536–537.

[96] Eaton BT, Cramer GS. The site of bluetongue virus attachment to glycoporphins from a number of animal erythrocytes. J Gen Virol. 1989;70(Pt 12):3347–3353.

[97] Nishimura H, Sugawara K, Kitame F, et al. Attachment of influenza C virus to human erythrocytes. J Gen Virol. 1988;69(Pt 10):2545–2553.

[98] Ruvonen-Clouet N, Blanchard D, Andre-Fontaine G, et al. Partial characterization of the human erythrocyte receptor for rabbit haemorrhagic disease virus. Res Virol. 1995;146(1):33–41.

[99] Carlisle RC, Di Y, Cerny AM, et al. Human erythrocytes bind and inactivate type 5 adenovirus by presenting Coxsackie virus-adenovirus receptor and complement receptor 1. Blood. 2009;113 (9):1909–1918.

[100] Moyaite J, Iwasaki Y, Takahashi A, et al. Regulation of circulating immune complexes by complement receptor type 1 on erythrocytes in chronic viral liver diseases. Gut. 2002;51(4):591–596.

[101] Andrews DA, Yang L, Low PS. Phorbol ester stimulates a protein kinase C-mediated agatoxin-TK-sensitive calcium permeability pathway in human red blood cells. Blood. 2002;100(9):3392–3399.

[102] Chmura SJ, Dolan ME, Cha A, et al. In vitro and in vivo activity of protein kinase C inhibitor chelerythrine chloride induces tumor cell toxicity and growth delay in vivo. Clin Cancer Res. 2000;6:737–742.

[103] Shi B, Li S, Ju H, et al. Protein kinase C inhibitor chelerythrine attenuates partial unilateral ureteral obstruction induced kidney injury in neonatal rats. Life Sci. 2019;216:85–91.

[104] Herbert JM, Augereau JM, Gleye J, et al. Chelerythrine is a potent and specific inhibitor of protein kinase C. Biochem Biophys Res Commun. 1990;172(3):993–999.

[105] Ghashghaieinia M, Dreischer P, Wieder T, et al. Coronavirus disease 2019 (COVID-19), human erythrocytes and the PKC-alpha/-beta inhibitor
chelerythrine - possible therapeutic implication. Cell Cycle. 2020;19(24):3399–3405.

[106] Moretti M, Bennett J, Tornatore L, et al. Cancer: NF-kappaB regulates energy metabolism. Int J Biochem Cell Biol. 2012;44(12):2238–2243.

[107] Ghashghaeinia M, Koberle M, Mrowietz U, et al. Proliferating tumor cells mimic glucose metabolism of mature human erythrocytes. Cell Cycle. 2019;18 (12):1316–1334.

[108] Dandona P, Chaudhuri A, Ghanim H, et al. Anti-inflammatory effects of insulin and the pro-inflammatory effects of glucose. Semin Thorac Cardiovasc Surg. 2006;18(4):293–301.

[109] Alshorafa AK, Guo Q, Zeng F, et al. Psoriasis is associated with low serum levels of hydrogen sulfide, a potential anti-inflammatory molecule. Tohoku J Exp Med. 2012;228(4):325–332.

[110] Coavoy-Sanchez SA, Costa SKP, Muscara MN. Hydrogen sulfide and dermatological diseases. Br J Pharmacol. 2020;177(4):857–865.

[111] Mirandola P, Gobbi G, Micheloni C, et al. Hydrogen sulfide inhibits IL-8 expression in human keratinocytes via MAP kinase signaling. Lab Invest. 2011;91 (8):1188–1194.

[112] Mezouari A, Nangia R, Gagnon J. The protective role of hydrogen sulfide against obesity-associated cellular stress in blood glucose regulation. Antioxidants (Basel). 2020;9(11):1038.

[113] Gemici B, Elsheikh W, Feitosa KB, et al. H2S-releasing drugs: anti-inflammatory, cytoprotective and chemopreventative potential. Nitric Oxide. 2015;46:25–31.

[114] Leigh J, Juriasingani S, Akbari M, et al. Endogenous H2S production deficiencies lead to impaired renal erythropoietin production. Can Urol Assoc J. 2018;13 (7):E210–E9.

[115] Duhm J, Deuticke B, Gerlach E. Metabolism of 2,3-diphosphoglycerate and glycolysis in human red blood cells under the influence of dipyridamole and inorganic sulfur compounds. Biochim Biophys Acta. 1968;170(2):452–454.

[116] Benesch R, Benesch RE, Yu CI. Reciprocal binding of oxygen and diphosphoglycerate by human hemoglobin. Proc Natl Acad Sci U S A. 1968;59 (2):526–532.

[117] Wang G, Huang Y, Zhang N, et al. Hydrogen Sulfide Is a Regulator of Hemoglobin Oxygen-Carrying Capacity via Controlling 2,3-BPG Production in Erythrocytes. Oxid Med Cell Longev. 2021;2021:8877691.

[118] Cortese-Krott MM, Kelm M. Endothelial nitric oxide synthase in red blood cells: key to a new erythrocrine function? Redox Biol. 2014;2:251–258.

[119] Broadley SP, Plaumann A, Coletti R, et al. Dual-Track clearance of circulating bacteria balances rapid restoration of blood sterility with induction of adaptive immunity. Cell Host Microbe. 2016;20(1):36–48.

[120] Butler AR, Williams DLH. The physiological role of nitric oxide. Chem Soc Rev. 1993;22(4):233–241.

[121] Wood KC, Hsu LL, Gladwin MT. Sickle cell disease vasculopathy: a state of nitric oxide resistance. Free Radic Biol Med. 2008;44:1506–1528.

[122] Naumann M, Scheidereit C. Activation of NF-kappa B in vivo is regulated by multiple phosphorylations. EMBO J. 1994;13(19):4597–4607.

[123] Chen L, Fischle W, Verdin E, et al. Duration of nuclear NF-kappaB action regulated by reversible acetylation. Science. 2001;293(5535):1653–1657.

[124] Yang H, Zhang W, Pan H, et al. SIRT1 activators suppress inflammatory responses through promotion of p65 deacetylation and inhibition of NF-kappaB activity. PLoS One. 2012;7(9):e46364.