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Review Article

Erythrocyte degradation, metabolism, secretion, and communication with immune cells in the blood during sepsis: A review

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Abstract

Sepsis is a health issue that affects millions of people worldwide. It was assumed that erythrocytes were affected by sepsis. However, in recent years, a number of studies have shown that erythrocytes affect sepsis as well. When a pathogen invades the human body, it infects the blood and organs, causing infection and sepsis-related symptoms. Pathogens change the internal environment, increasing the levels of reactive oxygen species, influencing erythrocyte morphology, and causing erythrocyte death, i.e., eryptosis. Characteristics of eryptosis include cell shrinkage, membrane blebbing, and surface exposure of phosphatidylserine (PS). Eryptotic erythrocytes increase immune cell proliferation, and through PS, attract macrophages that remove the infected erythrocytes. Erythrocyte-degraded hemoglobin derivatives and heme deteriorate infection; however, they could also be metabolized to a series of derivatives. The result that erythrocytes play an anti-infection role during sepsis provides new perspectives for treatment. This review focuses on erythrocytes during pathogenic infection and sepsis.

Keywords: *Eryptosis, Erythrocyte, Hemoglobin, Heme, Sepsis*

Introduction

Sepsis represents a response to pathogenic infection and is induced in many infectious diseases. According to a 2017 estimation, there were 48.9 million sepsis cases and 11 million sepsis-related deaths worldwide [1]. If sepsis is not diagnosed early and managed immediately, it may result in sepsis-related hemolysis, septic shock, and multiple organ failure, ultimately resulting in death [2]. Earlier, erythrocytes were thought to be affected by sepsis [3]; however, recent studies have shown that erythrocytes may have other functions in sepsis. During infection, pathogens require iron to maintain survival; thus, they may invade erythrocytes leading to membrane structure changes and eryptosis, which causes erythrocyte shrinkage, membrane blebbing, and phosphatidylserine (PS) exposure, resulting in degraded and reduced amounts of erythrocytes, thereby inducing anemia [4]. Erythrocytes in the eryptotic state secrete exosomes through blebbing. These exosomes act as signal transporters that enhance immune cell responses and increase T-cell proliferation in response to infection [5]. In contrast, when septic hemolysis occurs, erythrocytes undergo apoptosis and release cell-free hemoglobin that worsens sepsis [6]. However, cell-free hemoglobin may exhibit an antioxidant effect that results in decreased inflammation, and its immune cell degradation product, the derivative hemorphin, also exhibits the potential to inhibit inflammation [7]. For this review, we searched PubMed using the keywords "erythrocyte, infection, sepsis, and eryptosis" and reviewed the selected manuscripts in order to attain a comprehensive understanding of the effects of pathogen-induced sepsis on erythrocytes in the human body. In addition, potential anti-inflammatory drugs that affect erythrocytes during sepsis were explored.

Long-established red blood cell (erythrocyte) phenomena during infection

Pathogens alter red blood cell shape, induce anisocytosis, and decrease red blood cell deformability

When a pathogen invades the human body, it penetrates the blood and organs through the circulatory system, leading to systemic inflammation, and ultimately, sepsis [8]. In *in vitro* and *in vivo* studies, changes in the shape of human red blood cells (RBCs) during sepsis have been observed [9]; which has also been confirmed in humans [Figure 1] [10]. It was

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demonstrated in clinical studies that patients with sepsis may exhibit higher activity of neuraminidase, which cleaves the sialic acids moieties on the RBC membrane; thus, altering the shape of RBCs (rheology) [9]. Furthermore, changes in RBC morphology indicate multiple protruding spikes on the surface during septic shock [11] and RBC aggregation [12]. *In vivo* studies revealed similar results; in mouse models of cecal ligation and puncture (CLP)-induced sepsis, plasma-derived extracellular vesicles (EVs) increase RBC rigidity and influence RBC deformability [13]. In rat models of CLP-induced sepsis, oxidative stress alters the rheology of blood-influenced RBC deformability [14]. In addition, increased RBC distribution width (RDW) – also related to anisocytosis–is an independent risk factor for mortality in sepsis and septic shock [15,16]. However, some studies indicate that there is no relationship between RDW and sepsis [17]. In other studies, decreased ATP levels have been observed to influence RBC deformability and PS exposure [18]. Moreover, 3 days after admission to the intensive care unit (ICU), patients with sepsis exhibited increased creatinine and bilirubin levels [19] and lower hemoglobin and hematocrit values than patients without sepsis, and these phenomena are associated with RBC deformability [10].

PS flip-flops on the surface of RBCs and low ATP levels result in reduced RBCs in circulation; alterations in the phospholipid component of the cell membrane are induced by surface antigens or antibodies, and may promote the killing of RBCs in the spleen [18].

Normal RBCs elastic and can pass through capillaries through deformation. However, inhibition of RBC deformation may affect RBC function and hinder blood flow.

In summary, infection-induced RBC deformity and apoptosis are well known; however, the physiological underpinnings are still unknown.

Pathogens induce eryptosis and clearance of damaged red blood cells

RBCs are directly or indirectly influenced by pathogenic infection. Pathogens invade the RBC intracellular environment and produce endogenous substances, causing energy depletion, hyperosmotic and oxidative stress, and membrane structure changes [20]. These effects induce RBC cytosolic Ca^{2+} activity [21], leading to a state similar to apoptosis, termed eryptosis [Figure 1]. Eryptosis is characterized by RBC cell shrinkage, membrane blebbing, and membrane phospholipids that compete with PS on the cell surface; thus, membrane PS is a signal for macrophage clearance of eryptotic RBCs [Figure 1] [22]. In addition, an *in vitro* study showed that *Pseudomonas aeruginosa* might release the virulence factor pyocyanin, which increases cytosolic Ca^{2+} activity and PS externalization on erythrocytes, enhancing prothrombin activation and promoting RBC shrinkage (eryptosis), which leads to spleen and liver clearing eryptotic RBCs [23]. It is also reported that macrophages, epithelial cells, airway smooth muscle cells, and endothelial cells produce the cytokine interleukin (IL)-8, which also induces RBC eryptosis [Figure 1] [24].

Clinical phenomena of erythrocytes during sepsis

Anemia during sepsis and septic shock

We explored the clinical changes in erythrocytes during sepsis, and based on animal experiments, concluded that infection may result in the killing of RBCs. *In vivo* studies show that compared to hepcidin knockout mice, wild-type mice with inflammation–induced as a result of iron (Fe) and erythropoietin treatment–exhibit more anemia symptoms [25]. Additionally, in the CLP BALB/c mouse model, high-mobility group box 1, a chromatin protein, has been observed to mediate anemia through extramedullary erythropoiesis and increased reticulocyte counts [26].

Anemia always develops during sepsis and is presented in the emergency department, the ICU, or during ICU admission [27-29]. In one clinical study, the reduction i hemoglobin concentration was shown to correlate with initial creatinine levels, fluid resuscitation, and sepsis itself, compared to the control group [27]. The etiologies of anemia in sepsis have been studied, including diagnostic phlebotomy [30], stress ulcers [31], nutritional deficiency [32], inflammatory cytokines impairing iron homeostasis [33], IL-6-induced hepcidin-25 worsening anemia [34], vasopressors that inhibit hematopoietic precursors [35], and decrease in erythropoietin by impairment of renal function and cytokine inhibition [36]. In a clinical study, septic patients with higher hemoglobin levels exhibited increased levels of plasma IL-6 and IL-8 [25]. In another study, it was observed that compared to healthy individuals, aseptic patients exhibit significantly lower levels of hemoglobin and plasma iron and sTfR/log ferritin and increased plasma erythropoietin, soluble transferrin receptor, hepcidin, and IL-6 levels at the time of admission to ICU. In septic patients who exhibited similar parameters, nonsurvival plasma iron, erythropoietin sTfR/log ferritin decreased significantly; however, the levels of hepcidin, ferritin and IL-6 significantly increased [26]. During sepsis, the levels of inflammation-related cytokines, such as IL-6 and IL-8 increase significantly, and the expression of few iron-associated proteins, such as plasma iron, ferritin, erythropoietin, soluble transferrin receptor, and hepcidin is affected. In summary, inflammatory and iron associated-proteins are highly correlated with sepsis-induced anemia.

Sepsis-induced hemolysis and poor prognosis in patients with higher cell-free hemoglobin

An increase in cell-free hemoglobin, bilirubin, and ferritin levels has been observed during sepsis [37-39]. Hemolysis may occur during hemolytic anemia, malaria, hemorrhage, and ischemia-reperfusion. In a critical illness, erythrocyte deformability decreases and induces the release of intracellular contents such as cell-free hemoglobin into circulation [37]. Cell-free hemoglobin is released from RBCs during sepsis due to hemolysis [38]. The mechanisms include RBC transfusion reactions, complement activation, capillary stopped-flow, hemolytic pathogens, pore-forming toxins, RBC starvation without glucose, RBC apoptosis, disseminated intravascular coagulation, and lipopolysaccharide (LPS) invasion of RBC membranes [38]. Later, free heme damages tissues and worsens sepsis in LPS-induced systemic inflammation animal

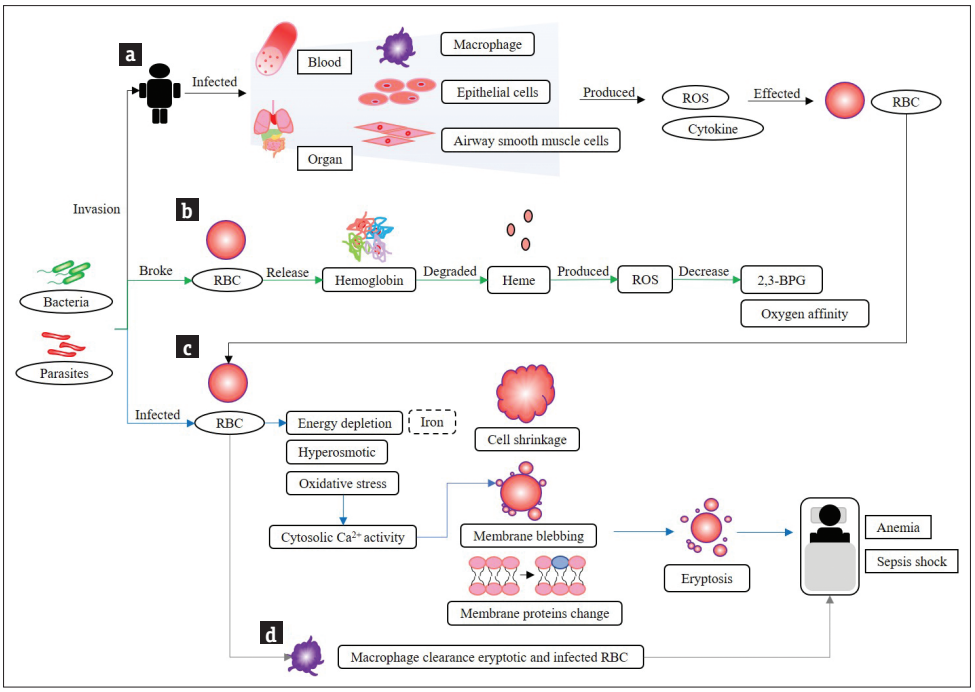

Figure 1: Pathogens influence red blood cells during sepsis. (a) Pathogens like bacterial and malarial parasite, which invade the blood and organ induce macrophage, epithelial cells and airway smooth muscle cells to secrete cytokine interleukin-8, and also cause phagocytes to produce reactive oxygen species against pathogens. However, reactive oxygen species can also effect red blood cell and lead to eryptosis. (b) Pathogen infected red blood cell release cell-free hemoglobin and heme, which could induce overdose of reactive oxygen species; subsequently causing organ damage. The infected red blood cells' 2,3-BPG expression level influences normal state hemoglobin oxygen affinity indirectly, causing organ dysfunction, leading to septic shock. Moreover, cell-free hemoglobin could release iron cause protein, lipid, and modified DNA that could induce inflammation. (c) Infection causes red blood cell energy depletion, and hyperosmotic and extracellular reactive oxygen species induced cell oxidative stress. Above mechanism effects red blood cell membrane structure and induces cytosolic Ca^{2+} activity leads to changes in red blood cell morphology, such as membrane blebbing, shrinkage, and membrane protein phospholipid changes to phosphatidylserine causing red blood cell eryptosis. (d) Macrophage through phosphatidylserine acts as a signal to clear eryptotic red blood cell and also engulfs infected red blood cell; thus, cell amount decreases resulting in anemia

models [Figure 1] [39,40]. Iron may be released from cell-free hemoglobin and modify lipids, proteins, and DNA, thereby inducing inflammation [41]. Furthermore, iron helps bacterial growth [42]. In clinical studies, compared to survivors, patients with severe sepsis exhibit a higher free hemoglobin level, and significant lower survival rate of 30-days [6,43]. 2,3-bisphosphoglycerate (2,3-BPG) is an important hemoglobin regulator, which is synthesized by RBCs under hypoxic conditions. It shifts the oxygen dissociation curve to the right and promotes release of oxygen; a decrease in 2,3-BPG is observed in *E. coli*-induced septic shock in baboons [44] and also in a critical human illness [Figure 1] [45]. During sepsis, the RBC oxygen dissociation curve shifts to the left, increasing hemoglobin affinity to oxygen; consequently, oxygen release into peripheral tissues becomes difficult [46].

Role of erythrocytes in immune function during sepsis

Erythrocytes are involved in innate immunity

It was demonstrated in a study that the bloodstream mean velocity in the aorta is 40 cm/s, in the vena cava superior and inferior, it is 15 cm/s, and in capillaries, 0.03 cm/s [47]. In the above conditions, leukocytes do not have enough time to detect pathogens and apprehend them. Thus, leukocyte phagocytosis of pathogens occurs in tissues, subepithelial, and in the lymph system [48]. In the bloodstream, erythrocytes are charged by the triboelectric effect, which attracts bacteria to their surface; subsequently, the engulfed bacteria are killed by hemoglobin-binding oxygen inside erythrocytes [49]. Inflammasomes are activated when the Toll-like receptor (TLR)-9 expressed on RBC surface binds to the mitochondrial DNA released by pathogens; this results in the induction of inflammation, activation of neutrophils, and expression of pro-inflammatory cytokines. Furthermore, if pro-inflammatory stimuli are inhibited, inflammation would be suppressed [50], as shown in Figure 2. Erythrocytes play a bactericidal role by entrapping and killing; hemoglobin has been suggested to participate in this process as RBCs lysed by bacteria would release hemoglobin as free radicals, which subsequently degrade the pathogen's cell wall and membrane [51].

Critical role of cell-free hemoglobin

Cell-free hemoglobin may be released from the intravascular to extravascular space [41]. It is hypothesized to oversupply oxygen to tissue and damage tissue via reactive oxygen formation and nitric oxide (NO) scavenging [Figure 1] [52,53]. *In vivo* studies have shown that cell-free hemoglobin worsens sepsis-induced organ damage in the lungs and kidneys [54,55]. Cell-free hemoglobin increases inflammatory cytokines and induces lung apoptosis and edema in polymicrobial septic mice, leading to higher sepsis mortality [54]. In a canine antibiotic-treated *Staphylococcus aureus* pneumonia model, cell-free hemoglobin combined with the hemoglobin complex

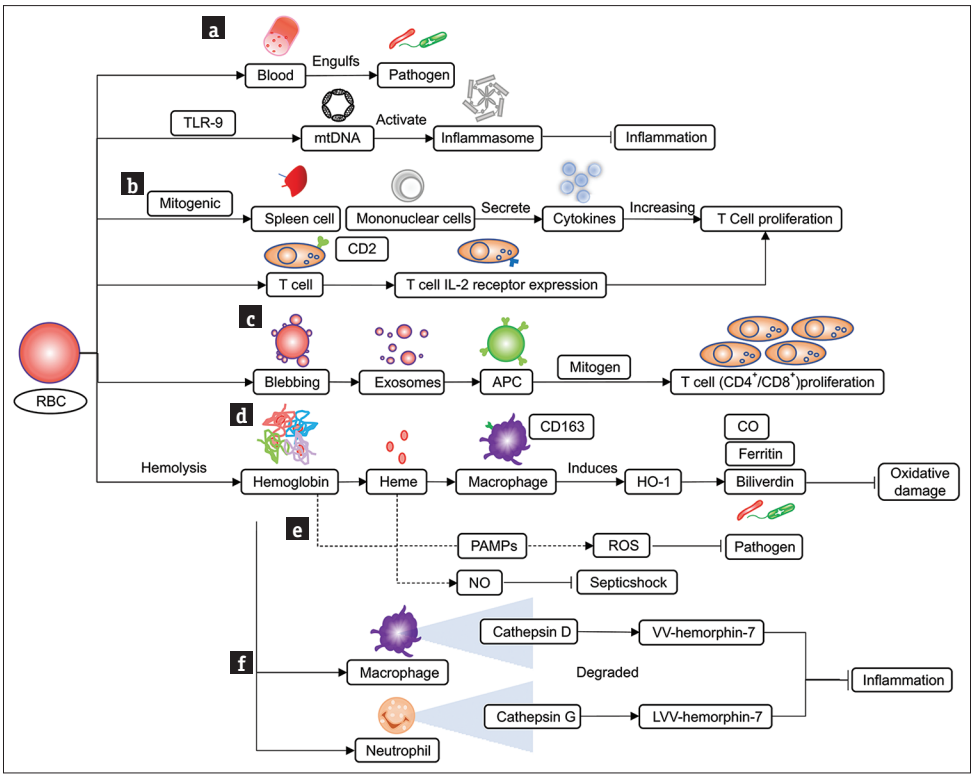

Figure 2: Red blood cells and their derivatives regulate immune responses in sepsis. (a) red blood cells in the bloodstream, through their negatively charged membrane, attract the pathogens and engulf them. Toll-like receptor-9 recognizes the pathogen's mitochondrial DNA (mtDNA), leading to activation of inflammasomes to inhibit pro-inflammatory stimuli and suppress inflammation. (b) Red blood cells, through mitogenic stimulation, enhance mouse spleen cells and mononuclear cells to secrete Colony-stimulating factors (CSFs) and cytokines, such as interleukin-2 to increase T-cell proliferation. Under conditions without mitogenic stimulation, red blood cells directly increase interleukin-2 receptor expression, which results in T-cell proliferation through nonspecific interactions with the CD2 antigen on T cells. (c) During eryptosis, red blood cells release exosomes through budding, which, through APCs, modulate mitogens leading to $CD4^+/CD8^+$ T-cell proliferation. (d) During hemolysis, when red blood cells lyse hemoglobin, hemoglobin is degraded to heme through binding of the CD163 antigen, leading to macrophage-induced heme oxygenase 1 expression, resulting in heme metabolism to biliverdin, ferritin, and carbon monoxide, which then inhibits oxidative damage. (e) Hemoglobin and heme, through pathogen-associated molecular patterns (PAMPs), produce reactive oxygen species against pathogen infection, while heme decreases the production of nitric oxide to inhibit septic shock. (f) Hemoglobin is degraded by macrophage cathepsin D or neutrophil cathepsin G to the opioid peptide hemorphin, such as VV-hemorphin-7 or LVV-hemorphin 7. Studies have shown their anti-inflammatory effects, but not the exact pathway

inhibits cell-free hemoglobin, and iron clearance decreases the benefit of hemoglobin in sepsis [56]. In a cecal slurry-induced mouse sepsis model, cell-free hemoglobin increases kidney injury indices, such as neutrophil gelatinase-associated lipocalin, kidney injury molecule-1, and renal tubular injury [55]. In addition, it causes kidney tubular obstruction and NO depletion, induces oxidative damage, and produces pro-inflammatory signals leading to acute kidney injury [57].

Leukocytes mediate erythrocyte immunity in sepsis

Erythrocytes and leukocytes are partners in antimicrobial missions in the bloodstream, although they have different functions [49]. Macrophage-engulfed erythrocytes during sepsis have been observed in some situations; for example, pathogens induce PS exposure on the erythrocyte surface, followed by macrophage engulfment [58]. Some pathogens produce sphingomyelinases that induce ceramide formation on erythrocytes, which are also engulfed by macrophages [Figure 1] [59].

Erythrocytes may enhance T lymphocyte survival [60] by secretion of cytokines, induction of IL^{-2} receptors [Figure 2b] [61], and modulation of $CD4^+/CD8^+$ ratios [62]. Recently, stored erythrocytes have been observed to release exosomes [63], and in another study, erythrocytes were stimulated by Ca^{2+} uptake such that exosomes were detected [64]. EVs, such as exosomes or microvesicles, from erythrocytes, interact with monocytes to induce pro-inflammatory cytokine secretion [5]. Moreover, through the antigen-presenting cell (APC) CD40/CD40 L pathway, active mitogen increases T-cell proliferation [Figure 2c] [5]. Hemoglobin fragment release from erythrocytes may also attract neutrophil aggregation [65], but its release is unknown. Therefore, erythrocytes may release messages via exosomes and cooperate with leukocytes.

Heme oxygenase 1 is the key factor in transforming hHeme to exert anti-inflammatory effects

Free heme is derived from free hemoglobin or hemolysates, whose expression level is enhanced through oxidative substances, such as H_2O_2 [66]. Heme is an activator of TLR-4 [47] and nuclear factor-κB [63] on endothelial cells that later induce inflammation. Cell-free hemoglobin in sepsis has pros and cons. Sometimes, free hemoglobin release induces the generation of reactive oxygen species (ROS) [64] that break down the bacterial cell wall and membrane, killing the bacteria with microbial proteases and enhance the release of pathogen-associated molecular patterns [Figure 2] [65].

During sepsis, cell-free hemoglobin induces NO degradation [66]. Cell-free hemoglobin may protect hemolytic microbes upon coming into contact with LPS and gives rise to microbicidal free radicals [67]. In addition, a study has shown that hemoglobin is oxidized to ferri- and ferryl-hemoglobin, and iron is released upon the reaction of free heme with lipid hydroperoxides in advanced atheromatous lesions [67]. Heme might induce macrophage and neutrophil activation of innate immune receptors. Heme is catabolized by heme oxygenase 1 (HO-1), which is a rate-limiting enzyme [Figure 2d]; HO-1 and cytochrome P450 reductase catalyze the production of biliverdin, ferrous iron, and carbon monoxide from heme, oxygen, and nicotinamide adenine dinucleotide phosphate [68]. Biliverdin is further converted to bilirubin by the cytosolic enzyme biliverdin reductase, and ferrous iron is bound by the iron storage protein, ferritin [69]. In other studies, it has been demonstrated that heme breakdown by HO (HMOX)–which has two isoforms (HMOX1 and HMOX2)—through the cleavage of the heme ring, produces the same amounts of ferrous iron, CO, and biliverdin [70].

In early sepsis animal models, early HO-1 mRNA expression in leukocytes may represent oxidative stress and may predict the severity of liver and renal dysfunction during sepsis [71]; thus, HO-1 may be a potential early biomarker to predict sepsis outcomes. Furthermore, in an early sepsis human study, arterial CO and monocyte HO-1 protein expression increased in critically ill patients, particularly those with severe sepsis or septic shock, suggesting that oxidative stress is closely related to HO-1 expression; thus, the HO-1/CO system may play an important role in early sepsis [72]. *In vivo* studies have shown that treating heme for 24 h before cecal slurry-induced sepsis in a mouse model increases liver and spleen HO-1 activity and decreases sepsis mortality and expression of pro-inflammatory cytokines such as chemokine ligand 5, C-X-C motif chemokine ligand (CXCL)-10, IL-1 β, and interferon (IFN)-γ [73]. In addition, human umbilical cord mesenchymal stromal cells induce an increase in peritoneal macrophage HO-1 through lipoxin A4 and prostaglandin E2 [74]. Transient receptor potential melastatin 2 also mediates the effect of peritoneal macrophage HO-1 expression on bacterial clearance [75].

Potential treatment mechanisms during sepsis

Animal model suggests that HO-1 attenuates septic injury by modulating TLR4-mediated mitochondrial quality control [Table 1] [76]. In other animal models with sepsis-related acute lung injury (ALI), endoplasmic reticulum (ER) stress is activated during CLP-induced ALI, and HO-1 activation inhibits CLP-induced lung ER stress and attenuates CLP-induced ALI [69]. In late sepsis animal models, administering appropriate doses of HO inhibitors is beneficial for the amelioration of sepsis-induced liver dysfunction [86]. In a clinical study, ELISA on samples from 70 septic patients admitted to the ICU, revealed the concentrations of hemoglobin, IL-10, and HO-1 to be positively correlated with the survival rate [87]. Thus, HO-1 may be a potential therapeutic target for survival in sepsis.

However, this also had negative effects on HO-1. In another late sepsis splenocyte animal model, HO-1 overexpression contributes to sepsis-induced immunosuppression during late-phase sepsis by promoting Th2 polarization and regulatory T-cell function [77].

In a normal situation, cell-free hemoglobin is bound and scavenged by haptoglobin and hemopexin (HPX) [52]. First, the haptoglobin-hemoglobin complex binds to the macrophage CD163 surface marker, and endocytosis of these complexes occurs, leading to degradation inside macrophages [Figure 2] [41,52,88]. Second, the heme sequestering protein HPX is reduced during severe infection [89]. Heme-catabolizing enzyme HPX and albumin may transport free heme into the liver so that it is degraded by hepatocytes [39,40,52,78]. The iron-sequestering ferritin H chain may attenuate oxidative inhibition of liver glucose 6 phosphatase and sustain liver gluconeogenesis, an important homeostasis mechanism during sepsis [90].

Table 1: Possible therapy targets or drugs to treat sepsis

Name	Experiment	Mechanism	Reference
HO-1	*In vivo*	Regulatory T-cell (Th and Treg) differentiation Inhibits pro-inflammatory factors	[39-41,77]
HPX	*In vivo*	HPX targets heme to liver parenchymal cells for catabolism	[39,78]
Hemorphin-7	*In vivo*	Decreased plasma corticosterone and TNF-α levels in an LPS-induced rat sepsis model	[79]
Resveratrol	*In vitro*	Blocked oxidants in the RBC lipid bilayer Prevented RBC lipid peroxidation by increasing GSH-Px, catalase, and GSH activity	[80,81]
Silymarin	*In vitro*	Increased GSH-PX and SOD antioxidant enzyme activity Inhibited NF-κB	[82]
Ascorbic acid	*In vitro*	Restored β-spectrin levels Reduced osmotic fragility Increased membrane integrity	[83]
Flavonoid	*In vitro*	Inhibited H2O2-induced RBC hemolysis	[84]
Quercetin	Randomized, double-blind, placebo-controlled pilot study	Increased RBC production by stimulating the secretion of erythropoietin Protects erythrocyte membranes against oxidative damage	[85]

HO-1: Heme oxygenase 1, HPX: Hemopexin, TNF: Tumor necrosis factor, LPS: Lipopolysaccharide, RBC: Red blood cell, GSH-Px: Erythrocyte glutathione peroxidase, SOD: Superoxide dismutase, NF-κB: Nuclear factor-kappa B

Furthermore, beta hemoglobin fragments that are antimicrobial hemoglobin-derived peptides have been purified from human erythrocytes and shown to inhibit bacterial growth [91]. Another hemoglobin-derived peptide such as hemorphin 7 has been observed to have a therapeutic effect on endotoxin-induced stress to lower corticosteroid and tumor necrosis factor alpha secretion in animal experiment [79]. Hemorphin 7 is defined as an evidence of hemoglobin proteolysis in an abdominal aortic aneurysm, which may attract increased neutrophil influx [65]. The authors observed that thrombus tissue contains hemorphin 7, while the digestion enzymes cathepsin D and cathepsin G originate from neutrophils [92,93]. However, cathepsin D comes from lysosomes in macrophages [94]. When bovine globin is incubated with mouse peritoneal macrophages, the lysosomal enzyme cathepsin D hydrolyzes globin and generates VV-hemorphin-7 [95]. When human hemoglobin is coincubated with cathepsin G, which is produced by neutrophils, cathepsin G generates LVV-hemorphin 7 [65]. This mechanism is important because neutrophil degranulation occurs in areas rich in free hemoglobin [65]. These results also provide evidence of erythrocyte and leukocyte cooperation during inflammation [Figure 2]. However, the anti-inflammatory effect of hemorphin and its production during sepsis are unknown.

Erythrocyte extracellular vesicles activate the lymphocyte immune reaction

When intracellular Ca^{2+} levels increase, or protein kinase C active erythrocytes produce microvesicles (MVs) via blebbing [Figure 3] [64], exosomes released from stored erythrocytes bind to monocytes and induce the secretion of the pro-inflammatory cytokine TNF-alpha [5]. An *in vitro* experiment has shown that erythrocyte-derived ectosomes inhibit zymosan A- and LPS-induced macrophage inflammatory factors, such as TNF-α, IL-8, and IL-10, through TLR-2 and TLR-4 [96]. In addition, *in vivo* experiments have also demonstrated that in septic mice, exosomes derived from erythrocytes induce Th1/Th2 cell differentiation through IL-4 and IL-12 and enhance T-cell proliferation through IL-4, IFN-γ, and CXCL-9 [97]. It was observed in an *in vivo* experiment | that RBC-derived exosomes inhibit TNF-α and IL-10 in serum [97], as observed *in vitro*. *Plasmodium falciparum*-infected RBCs release extracellular vesicles that contain RNAs, which could be transferred to endothelial cells where they regulate vascular function [98]. In addition, an *in vitro* experiment showed that the extracellular vesicles released by *P. falciparum*-infected RBCs could activate the innate immune response [99]. Thus, we can conclude that erythrocyte EVs, including microvesicles, ectosomes, and exosomes, activate lymphocyte immune reactions mainly through cytokines to increase T-cell proliferation and inhibit

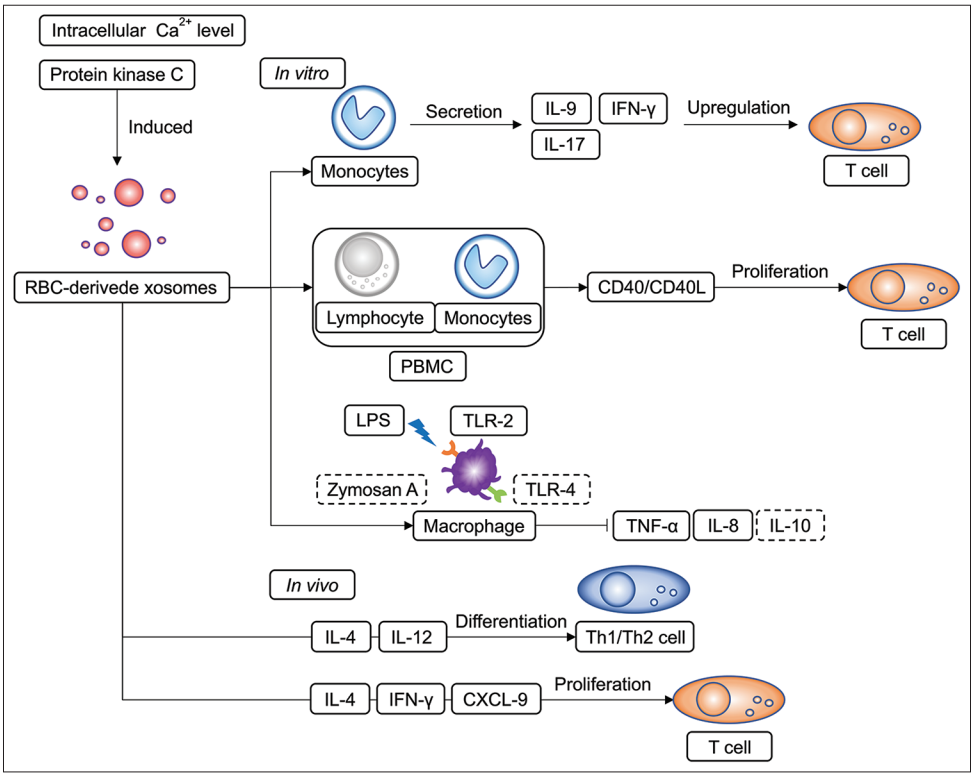

Figure 3: Red blood cell-derived exosome effects on immune cells. Intracellular Ca^{2+} levels and protein kinase C activate erythrocyte blebbing, producing exosomes. *In vitro* studies have shown that red blood cell-derived exosomes interact with monocytes, causing the secretion of cytokines, such as IFN-γ, interleukin-9, and interleukin-17. These cytokines upregulate T helper cells, such as Th1, Th17, and Th9. Exosomes interact with peripheral blood mononuclear cells (PBMCs), including lymphocytes and monocytes, through CD40 or CD40 L to increase T-cell proliferation via cell-cell contact. In addition, ectosomes from red blood cells, via Toll-like receptor-2 and Toll-like receptor-4 binding to macrophages, decrease lipopolysaccharide-or zymosan-A-induced pro-inflammatory cytokine release, including tumor necrosis factor-α, interleukin-8, and interleukin-10. *In vivo* studies have shown similar results as *in vitro* experiments. Red blood cell-derived exosomes from septic mice enhance T-cell proliferation via interleukin-4- and interleukin-12-induced Th1/Th2 cell differentiation or through interleukin-4, IFN-γ, and C-X-C motif chemokine ligand-9

inflammatory cytokine secretion. However, complete details of the EV contents released from RBCs are still unknown and should be studied in detail.

Possible drugs for sepsis treatment

ROS plays an important role in RBC membranes, causing RBC oxidative stress and membrane peroxidation, leading to changes in RBC structure and dysfunction [100]. A previous study revealed that some drugs have an antioxidant ability that could protect RBCs from ROS injury [Table 1] [101]. *In vitro* research has shown that resveratrol and silymarin increase RBC antioxidant enzyme activity to protect RBC membranes [80-82]. Ascorbic acid reduces osmotic fragility to maintain RBC membrane integrity [83]. Flavonoids inhibit ROS H_2O_2-induced RBC hemolysis through antioxidant activity [84]. In addition, a clinical study has shown that quercetin also protects RBCs from oxidatively-induced membrane damage and increases erythropoietin production to stimulate RBC proliferation [85].

Conclusion

Erythrocytes not only sacrifice themselves but also join the antimicrobial battle during sepsis. Although blood transfusion is necessary if severe anemia develops after systemic infection and multiple organ dysfunction, adequate erythrocyte transfusion is not beneficial for survival in sepsis [102]. Erythrocytes have innate immunity, and their metabolites have potential antimicrobial effects [50]. They also secrete exosomes that may transmit important anti-infection messages [5]. Further studies are needed to identify potential therapies to enhance the antimicrobial function of erythrocytes and their metabolites for the treatment of sepsis.

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Conflicts of interest

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References

1. Rudd KE, Johnson SC, Agesa KM, Shackelford KA, Tsoi D, Kievlan DR, et al. Global, regional, and national sepsis incidence and mortality, 1990-2017: Analysis for the Global Burden of Disease Study. Lancet 2020;395:200-11.
2. Hotchkiss RS, Moldawer LL, Opal SM, Reinhart K, Turnbull IR, Vincent JL. Sepsis and septic shock. Nat Rev Dis Primers 2016;2:16045.
3. Serroukh Y, Djebara S, Lelubre C, Zouaoui Boudjeltia K, Biston P, Piagnerelli M. Alterations of the erythrocyte membrane during sepsis. Crit Care Res Pract 2012;2012:702956.
4. Bissinger R, Bhuyan AAM, Qadri SM, Lang F. Oxidative stress, eryptosis and anemia: A pivotal mechanistic nexus in systemic diseases. FEBS J 2019;286:826-54.
5. Danesh A, Inglis HC, Jackman RP, Wu S, Deng X, Muench MO, et al. Exosomes from red blood cell units bind to monocytes and induce proinflammatory cytokines, boosting T-cell responses *in vitro*. Blood 2014;123:687-96.
6. Adamzik M, Hamburger T, Petrat F, Peters J, de Groot H, Hartmann M. Free hemoglobin concentration in severe sepsis: Methods of measurement and prediction of outcome. Crit Care 2012;16:R125.
7. Gomes I, Dale CS, Casten K, Geigner MA, Gozzo FC, Ferro ES, et al. Hemoglobin-derived peptides as novel type of bioactive signaling molecules. AAPS J 2010;12:658-69.
8. Bosmann M, Ward PA. The inflammatory response in sepsis. Trends Immunol 2013;34:129-36.
9. Piagnerelli M, Boudjeltia KZ, Rapotec A, Richard T, Brohée D, Babar S, et al. Neuraminidase alters red blood cells in sepsis. Crit Care Med 2009;37:1244-50.
10. Reggiori G, Occhipinti G, De Gasperi A, Vincent JL, Piagnerelli M. Early alterations of red blood cell rheology in critically ill patients. Crit Care Med 2009;37:3041-6.
11. Oliveira YP, Pontes-de-Carvalho LC, Couto RD, Noronha-Dutra AA. Oxidative stress in sepsis. Possible production of free radicals through an erythrocyte-mediated positive feedback mechanism. Braz J Infect Dis 2017;21:19-26.
12. Bateman RM, Sharpe MD, Singer M, Ellis CG. The effect of sepsis on the erythrocyte. Int J Mol Sci 2017;18:1932.
13. Subramani K, Raju SP, Chu X, Warren M, Pandya CD, Hoda N, et al. Effect of plasma-derived extracellular vesicles on erythrocyte deformability in polymicrobial sepsis. Int Immunopharmacol 2018;65:244-7.
14. Bostanci H, Dikmen K, Comu FM, Arslan M, Kucuk A. Investigation of the effects of thymoquinone on erythrocyte deformability in sepsis treatment which created by cecal perforation in rat. Bratisl Lek Listy 2018;119:152-5.
15. Sadaka F, O'Brien J, Prakash S. Red cell distribution width and outcome in patients with septic shock. J Intensive Care Med 2013;28:307-13.
16. Kim CH, Park JT, Kim EJ, Han JH, Han JS, Choi JY, et al. An increase in red blood cell distribution width from baseline predicts mortality in patients with severe sepsis or septic shock. Crit Care 2013;17:R282.
17. Fontana V, Spadaro S, Bond O, Cavicchi FZ, Annoni F, Donadello K, et al. No relationship between red blood cell distribution width and microcirculatory alterations in septic patients. Clin Hemorheol Microcirc 2017;66:131-41.
18. McMahon TJ. Red blood cell deformability, vasoactive mediators, and adhesion. Front Physiol 2019;10:1417.
19. Donadello K, Piagnerelli M, Reggiori G, Gottin L, Scolletta S, Occhipinti G, et al. Reduced red blood cell deformability over time is associated with a poor outcome in septic patients. Microvasc Res 2015;101:8-14.
20. McCullough J. RBCs as targets of infection. Hematology Am Soc Hematol Educ Program 2014;2014:404-9.
21. Jemaà M, Fezai M, Bissinger R, Lang F. Methods employed in cytofluorometric assessment of eryptosis, the suicidal erythrocyte death. Cell Physiol Biochem 2017;43:431-44.
22. Lang F, Lang E, Föller M. Physiology and pathophysiology of eryptosis. Transfus Med Hemother 2012;39:308-14.
23. Qadri SM, Donkor DA, Bhakta V, Eltringham-Smith LJ, Dwivedi DJ, Moore JC, et al. Phosphatidylserine externalization and procoagulant activation of erythrocytes induced by *Pseudomonas aeruginosa* virulence factor pyocyanin. J Cell Mol Med 2016;20:710-20.
24. Bester J, Matshailwe C, Pretorius E. Simultaneous presence of hypercoagulation and increased clot lysis time due to IL-1β, IL-6 and IL-8. Cytokine 2018;110:237-42.
25. Loftus TJ, Mira JC, Stortz JA, Ozrazgat-Baslanti T, Ghita GL, Wang Z, et al. Persistent inflammation and anemia among critically ill septic

patients. J Trauma Acute Care Surg 2019;86:260-7.
26. Jiang Y, Jiang FQ, Kong F, An MM, Jin BB, Cao D, et al. Inflammatory anemia-associated parameters are related to 28-day mortality in patients with sepsis admitted to the ICU: A preliminary observational study. Ann Intensive Care 2019;9:67.
27. Jansma G, de Lange F, Kingma WP, Vellinga NA, Koopmans M, Kuiper MA, et al. 'Sepsis-related anemia' is absent at hospital presentation; a retrospective cohort analysis. BMC Anesthesiol 2015;15:55.
28. Nissenson AR, Dylan ML, Griffiths RI, Yu HT, Dubois RW. Septicemia in patients with ESRD is associated with decreased hematocrit and increased use of erythropoietin. Clin J Am Soc Nephrol 2006;1:505-10.
29. van Beest PA, Hofstra JJ, Schultz MJ, Boerma EC, Spronk PE, Kuiper MA. The incidence of low venous oxygen saturation on admission to the intensive care unit: A multi-center observational study in The Netherlands. Crit Care 2008;12:R33.
30. Vincent JL, Baron JF, Reinhart K, Gattinoni L, Thijs L, Webb A, et al. Anemia and blood transfusion in critically ill patients. JAMA 2002;288:1499-507.
31. Cook D, Heyland D, Griffith L, Cook R, Marshall J, Pagliarello J. Risk factors for clinically important upper gastrointestinal bleeding in patients requiring mechanical ventilation. Canadian Critical Care Trials Group. Crit Care Med 1999;27:2812-7.
32. Rodriguez RM, Corwin HL, Gettinger A, Corwin MJ, Gubler D, Pearl RG. Nutritional deficiencies and blunted erythropoietin response as causes of the anemia of critical illness. J Crit Care 2001;16:36-41.
33. Fleming RE, Bacon BR. Orchestration of iron homeostasis. N Engl J Med 2005;352:1741-4.
34. van Eijk LT, Kroot JJ, Tromp M, van der Hoeven JG, Swinkels DW, Pickkers P. Inflammation-induced hepcidin-25 is associated with the development of anemia in septic patients: An observational study. Crit Care 2011;15:R9.
35. Fonseca RB, Mohr AM, Wang L, Sifri ZC, Rameshwar P, Livingston DH. The impact of a hypercatecholamine state on erythropoiesis following severe injury and the role of IL-6. J Trauma 2005;59:884-9.
36. von Ahsen N, Müller C, Serke S, Frei U, Eckardt KU. Important role of nondiagnostic blood loss and blunted erythropoietic response in the anemia of medical intensive care patients. Crit Care Med 1999;27:2630-9.
37. Janz DR, Ware LB. The role of red blood cells and cell-free hemoglobin in the pathogenesis of ARDS. J Intensive Care 2015;3:20.
38. Effenberger-Neidnicht K, Hartmann M. Mechanisms of hemolysis during sepsis. Inflammation 2018;41:1569-81.
39. Larsen R, Gozzelino R, Jeney V, Tokaji L, Bozza FA, Japiassú AM, et al. A central role for free heme in the pathogenesis of severe sepsis. Sci Transl Med 2010;2:51ra71.
40. Dutra FF, Bozza MT. Heme on innate immunity and inflammation. Front Pharmacol 2014;5:115.
41. Schaer DJ, Buehler PW, Alayash AI, Belcher JD, Vercellotti GM. Hemolysis and free hemoglobin revisited: Exploring hemoglobin and hemin scavengers as a novel class of therapeutic proteins. Blood 2013;121:1276-84.
42. Pishchany G, McCoy AL, Torres VJ, Krause JC, Crowe JE Jr., Fabry ME, et al. Specificity for human hemoglobin enhances *Staphylococcus aureus* infection. Cell Host Microbe 2010;8:544-50.
43. Janz DR, Bastarache JA, Peterson JF, Sills G, Wickersham N, May AK, et al. Association between cell-free hemoglobin, acetaminophen, and mortality in patients with sepsis: An observational study. Crit Care Med 2013;41:784-90.
44. Johnson G Jr., McDevitt NB, Proctor HJ. Erythrocyte 2,3-diphosphoglycerate in endotoxic shock in the subhuman primate: Response to fluid and-or methylprednisolone succinate. Ann Surg 1974;180:783-6.
45. Ibrahim Eel D, McLellan SA, Walsh TS. Red blood cell 2,3-diphosphoglycerate concentration and *in vivo* P50 during early critical illness. Crit Care Med 2005;33:2247-52.
46. Myburgh JA, Webb RK, Worthley LI. The P50 is reduced in critically ill patients. Intensive Care Med 1991;17:355-8.
47. Minasyan H. Mechanisms and pathways for the clearance of bacteria from blood circulation in health and disease. Pathophysiology 2016;23:61-6.
48. Bicker H, Höflich C, Wolk K, Vogt K, Volk HD, Sabat R. A simple assay to measure phagocytosis of live bacteria. Clin Chem 2008;54:911-5.
49. Minasyan HA. Erythrocyte and leukocyte: Two partners in bacteria killing. Int Rev Immunol 2014;33:490-7.
50. Anderson HL, Brodsky IE, Mangalmurti NS. The evolving erythrocyte: Red blood cells as modulators of innate immunity. J Immunol 2018;201:1343-51.
51. Minasyan H. Erythrocyte and blood antibacterial defense. Eur J Microbiol Immunol (Bp) 2014;4:138-43.
52. Rother RP, Bell L, Hillmen P, Gladwin MT. The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: A novel mechanism of human disease. JAMA 2005;293:1653-62.
53. Winslow RM. Oxygen: The poison is in the dose. Transfusion 2013;53:424-37.
54. Meegan JE, Shaver CM, Putz ND, Jesse JJ, Landstreet SR, Lee HNR, et al. Cell-free hemoglobin increases inflammation, lung apoptosis, and microvascular permeability in murine polymicrobial sepsis. PLoS One 2020;15:e0228727.
55. Shaver CM, Paul MG, Putz ND, Landstreet SR, Kuck JL, Scarfe L, et al. Cell-free hemoglobin augments acute kidney injury during experimental sepsis. Am J Physiol Renal Physiol 2019;317:F922-9.
56. Remy KE, Cortés-Puch I, Sun J, Feng J, Lertora JJ, Risoleo T, et al. Haptoglobin therapy has differential effects depending on severity of canine septic shock and cell-free hemoglobin level. Transfusion 2019;59:3628-38.
57. Kerchberger VE, Ware LB. The role of circulating cell-free hemoglobin in sepsis-associated acute kidney injury. Semin Nephrol 2020;40:148-59.
58. Kempe DS, Akel A, Lang PA, Hermle T, Biswas R, Muresanu J, et al. Suicidal erythrocyte death in sepsis. J Mol Med (Berl) 2007;85:273-81.
59. Lang KS, Myssina S, Brand V, Sandu C, Lang PA, Berchtold S, et al. Involvement of ceramide in hyperosmotic shock-induced death of erythrocytes. Cell Death Differ 2004;11:231-43.
60. Fonseca AM, Pereira CF, Porto G, Arosa FA. Red blood cells promote survival and cell cycle progression of human peripheral blood T cells independently of CD58/LFA-3 and heme compounds. Cell Immunol 2003;224:17-28.
61. Kalechman Y, Herman S, Gafter U, Sredni B. Enhancing effects of autologous erythrocytes on human or mouse cytokine secretion and IL-2R expression. Cell Immunol 1993;148:114-29.
62. Porto B, Fonseca AM, Godinho I, Arosa FA, Porto G. Human red blood cells have an enhancing effect on the relative expansion of CD8+ T lymphocytes *in vitro*. Cell Prolif 2001;34:359-67.
63. Almizraq RJ, Seghatchian J, Holovati JL, Acker JP. Extracellular vesicle characteristics in stored red blood cell concentrates are influenced by the method of detection. Transfus Apher Sci 2017;56:254-60.
64. Nguyen DB, Ly TB, Wesseling MC, Hittinger M, Torge A, Devitt A, et al. Characterization of microvesicles released from human red blood cells. Cell Physiol Biochem 2016;38:1085-99.
65. Dejouvencel T, Féron D, Rossignol P, Sapoval M, Kauffmann C, Piot JM, et al. Hemorphin 7 reflects hemoglobin proteolysis in abdominal aortic aneurysm. Arterioscler Thromb Vasc Biol 2010;30:269-75.
66. Oh JY, Williams A, Patel RP. The role of redox-dependent mechanisms in heme release from hemoglobin and erythrocyte hemolysates. Arch Biochem Biophys 2019;662:111-20.
67. Nagy E, Eaton JW, Jeney V, Soares MP, Varga Z, Galajda Z, et al. Red cells, hemoglobin, heme, iron, and atherogenesis. Arterioscler Thromb Vasc Biol 2010;30:1347-53.

68. Araujo JA, Zhang M, Yin F. Heme oxygenase-1, oxidation, inflammation, and atherosclerosis. Front Pharmacol 2012;3:119.
69. Chiang SK, Chen SE, Chang LC. A dual role of heme oxygenase-1 in cancer cells. Int J Mol Sci 2018;20:39.
70. Bonkovsky HL, Guo JT, Hou W, Li T, Narang T, Thapar M. Porphyrin and heme metabolism and the porphyrias. Compr Physiol 2013;3:365-401.
71. Jao HC, Lin YT, Tsai LY, Wang CC, Liu HW, Hsu C. Early expression of heme oxygenase-1 in leukocytes correlates negatively with oxidative stress and predicts hepatic and renal dysfunction at late stage of sepsis. Shock 2005;23:464-9.
72. Takaki S, Takeyama N, Kajita Y, Yabuki T, Noguchi H, Miki Y, et al. Beneficial effects of the heme oxygenase-1/carbon monoxide system in patients with severe sepsis/septic shock. Intensive Care Med 2010;36:42-8.
73. Fujioka K, Kalish F, Zhao H, Lu S, Wong S, Wong RJ, et al. Induction of heme oxygenase-1 attenuates the severity of sepsis in a non-surgical preterm mouse model. Shock 2017;47:242-50.
74. Jerkic M, Gagnon S, Rabani R, Ward-Able T, Masterson C, Otulakowski G, et al. Human umbilical cord mesenchymal stromal cells attenuate systemic sepsis in part by enhancing peritoneal macrophage bacterial killing via heme oxygenase-1 induction in rats. Anesthesiology 2020;132:140-54.
75. Qian X, Cheng H, Chen X. Transient receptor potential melastatin 2-mediated heme oxygenase-1 has a role for bacterial clearance by regulating autophagy in peritoneal macrophages during polymicrobial sepsis. Innate Immun 2019;25:530-8.
76. Park JS, Choi HS, Yim SY, Lee SM. Heme oxygenase-1 protects the liver from septic injury by modulating TLR4-mediated mitochondrial quality control in mice. Shock 2018;50:209-18.
77. Yoon SJ, Kim SJ, Lee SM. Overexpression of HO-1 contributes to sepsis-induced immunosuppression by modulating the Th1/Th2 balance and regulatory T-cell function. J Infect Dis 2017;215:1608-18.
78. Smith A, McCulloh RJ. Hemopexin and haptoglobin: Allies against heme toxicity from hemoglobin not contenders. Front Physiol 2015;6:187.
79. Barkhudaryan N, Zakaryan H, Sarukhanyan F, Gabrielyan A, Dosch D, Kellermann J, et al. Hemorphins act as homeostatic agents in response to endotoxin-induced stress. Neurochem Res 2010;35:925-33.
80. Suwalsky M, F Villena, Gallardo MJ. *In vitro* protective effects of resveratrol against oxidative damage in human erythrocytes. Biochim Biophys Acta 2015;1848:76-82.
81. Huyut Z, Şekeroğlu MR, Balahoroğlu R, Karakoyun T, Çokluk E. The relationship of oxidation sensitivity of red blood cells and carbonic anhydrase activity in stored human blood: Effect of certain phenolic compounds. Biomed Res Int 2016;2016:3057384.
82. Darvishi Khezri H, Salehifar E, Kosaryan M, Aliasgharian A, Jalali H, Hadian Amree A. Potential effects of silymarin and its flavonolignan components in patients with β-thalassemia major: A comprehensive review in 2015. Adv Pharmacol Sci 2016;2016:3046373.
83. Sanford K, Fisher BJ, Fowler E, Fowler AA, Natarajan R. Attenuation of red blood cell storage lesions with Vitamin C. Antioxidants (Basel) 2017;6:55.
84. Raman ST, Ganeshan AK, Chen C, Jin C, Li SH, Chen HJ, et al. *In vitro* and *in vivo* antioxidant activity of flavonoid extracted from mulberry fruit (*Morus alba* L.). Pharmacogn Mag 2016;12:128-33.
85. Pasdar Y, Oubari F, Zarif MN, Abbasi M, Pourmahmoudi A, Hosseinikia M. Effects of quercetin supplementation on hematological parameters in non-alcoholic fatty liver disease: A randomized, double-blind, placebo-controlled pilot study. Clin Nutr Res 2020;9:11-9.
86. Iwasashi H, Suzuki M, Unno M, Utiyama T, Oikawa M, Kondo N, et al. Inhibition of heme oxygenase ameliorates sepsis-induced liver dysfunction in rats. Surg Today 2003;33:30-8.
87. Ekregbesi P, Shankar-Hari M, Bottomley C, Riley EM, Mooney JP. Relationship between anaemia, haemolysis, inflammation and haem oxygenase-1 at admission with sepsis: A pilot study. Sci Rep 2018;8:11198.
88. Schaer CA, Schoedon G, Imhof A, Kurrer MO, Schaer DJ. Constitutive endocytosis of CD163 mediates hemoglobin-heme uptake and determines the noninflammatory and protective transcriptional response of macrophages to hemoglobin. Circ Res 2006;99:943-50.
89. Lin T, Maita D, Thundivalappil SR, Riley FE, Hambsch J, Van Marter LJ, et al. Hemopexin in severe inflammation and infection: Mouse models and human diseases. Crit Care 2015;19:166.
90. Weis S, Carlos AR, Moita MR, Singh S, Blankenhaus B, Cardoso S, et al. Metabolic adaptation establishes disease tolerance to sepsis. Cell 2017;169:1263-75.e14.
91. Liepke C, Baxmann S, Heine C, Breithaupt N, Ständker L, Forssmann WG. Human hemoglobin-derived peptides exhibit antimicrobial activity: A class of host defense peptides. J Chromatogr B Analyt Technol Biomed Life Sci 2003;791:345-56.
92. Starkey PM, Barrett AJ. Human cathepsin G. Catalytic and immunological properties. Biochem J 1976;155:273-8.
93. Korkmaz B, Horwitz MS, Jenne DE, Gauthier F. Neutrophil elastase, proteinase 3, and cathepsin G as therapeutic targets in human diseases. Pharmacol Rev 2010;62:726-59.
94. Diment S, Leech MS, Stahl PD. Cathepsin D is membrane-associated in macrophage endosomes. J Biol Chem 1988;263:6901-7.
95. Dagouassat N, Garreau I, Sannier F, Zhao Q, Piot JM. Generation of VV-hemorphin-7 from globin by peritoneal macrophages. FEBS Lett 1996;382:37-42.
96. Sadallah S, Eken C, Schifferli JA. Erythrocyte-derived ectosomes have immunosuppressive properties. J Leukoc Biol 2008;84:1316-25.
97. Gao K, Jin J, Huang C, Li J, Luo H, Li L, et al. Exosomes derived from septic mouse serum modulate immune responses via exosome-associated cytokines. Front Immunol 2019;10:1560.
98. Babatunde KA, Mbagwu S, Hernández-Castañeda MA, Adapa SR, Walch M, Filgueira L, et al. Malaria infected red blood cells release small regulatory RNAs through extracellular vesicles. Sci Rep 2018;8:884.
99. Mantel PY, Hoang AN, Goldowitz I, Potashnikova D, Hamza B, Vorobjev I, et al. Malaria-infected erythrocyte-derived microvesicles mediate cellular communication within the parasite population and with the host immune system. Cell Host Microbe 2013;13:521-34.
100. Maurya PK, Kumar P, Chandra P. Biomarkers of oxidative stress in erythrocytes as a function of human age. World J Methodol 2015;5:216-22.
101. Mohanty JG, Nagababu E, Rifkind JM. Red blood cell oxidative stress impairs oxygen delivery and induces red blood cell aging. Front Physiol 2014;5:84.
102. Raghunathan K, Singh M, Nathanson BH, Bennett-Guerrero E, Lindenauer PK. Early blood transfusions in sepsis: Unchanged survival and increased costs. Am J Crit Care 2018;27:205-11.