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
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<sup>2+</sup> 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<sup>2+</sup> 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 in 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
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 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 engulfed infected red blood cell; thus, cell amount decreases resulting in anemia](http://example.com/figure1.png)
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 Ca2+ 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/CD40L 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 Heme to exert anti-inflammatory effects**

Free heme is derived from free hemoglobin or hemolysates, whose expression level is enhanced through oxidative substances, such as H2O2 [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].

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**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.
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-1β, and interferon (IFN)-γ [73]. In addition, human umbilical cord mesenchymal stem 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 phosphate 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                            | [39-41,77]           |
| HPX           | In vivo    | Inhibits pro-inflammatory factors                                          |                      |
| Hemorphin-7   | In vivo    | HPX targets heme to liver parenchymal cells for catabolism                | [39,78]              |
| Resveratrol   | In vitro   | Decreased plasma corticosterone and TNF-α levels in an LPS-induced rat sepsis model | [79]                 |
| Silymarin     | In vitro   | Blocked oxidants in the RBC lipid bilayer                                 | [80,81]              |
| Ascorbic acid | In vitro   | Prevented RBC lipid peroxidation by increasing GSH-Px, catalase, and GSH activity |                      |
| Flavonoid     | In vitro   | Increased GSH-Px and SOD antioxidant enzyme activity                       | [82]                 |
| Quercetin     | Randomized, double-blind, placebo-controlled pilot study                 | Inhibited NF-κB      | [83]                 |
|               |            | Restored β-spectrin levels                                                |                      |
|               |            | Increased membrane integrity                                              |                      |
|               |            | Inhibited H2O2-induced RBC hemolysis                                      | [84]                 |
|               |            | Increased RBC production by stimulating the secretion of erythropoietin    | [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.

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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 exosomes inhibit zymosan A- and LPS-induced macrophage inflammatory factors, such as TNF-\(\alpha\), 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-\(\gamma\), and CXCL-9 [97]. It was observed in an *in vivo* experiment that RBC-derived exosomes inhibit TNF-\(\alpha\) 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, exosomes, and exosomes, activate lymphocyte immune reactions mainly through cytokines to increase T-cell proliferation and inhibit

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**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-\(\gamma\), 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 CD40L to increase T-cell proliferation via cell-cell contact. In addition, exosomes 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-\(\alpha\), 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-\(\gamma\), 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 [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$_2$O$_2$-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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