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Phosphatidylserine as Red Cell Eryptosis Marker Consolidating Phagocytotic Clearance

Hisham Ali Waggiallah

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Abstract

Erythrocyte damage such as osmotic shock, oxidative stress, or energy depletion promotes the generation of prostaglandin E2 by activating cyclooxygenase, which triggers a permeable Ca+2 cation channel. Ca^{+2} further promotes the transfer of Phosphatidylserine from the cell membrane inside to the outer by a scramblase. Macrophages recognize phosphatidylserine at the surface of the erythrocyte which engulfs and degrade the affected cells. Also, the revealing phosphatidylserine red cell may bind to the vascular wall, interfering with microvessels. Erythrocyte shrinkage and exposure to phosphatidylserine ("eryptosis") mimic apoptosis characteristics in nucleated cells which, however, involve many processes lacking in red cells. Several conditions cause premature eryptosis, thereby favoring anemia progress. Moreover, eryptosis may be a faulty mechanism for erythrocytes to avoid hemolysis. Phosphatidylserine has been considered the key marker of eryptosis and serves as the primary booster in the reticuloendothelial system to eliminate red blood cells by the macrophage.

Key words: Phagocytotic, Red Cell Eryptosis, Phosphatidylserine

Introduction

Today, the concern of many people in the world is access to health care with the best quality (Poornowrooz et al., 2017). Nature has provided a complete store-house of remedies to cure all aliments of mankind (Krishnaa et al., 2018). Radiation triggers DNA repair pathways and cell cycle checkpoints in typical cells and results in recuperation or cell passing With advances in the science of technology, human exploitation is increasing the new energy sources and endless such as nuclear energy (Gheisari et al., 2017). Red cells are usually passed via stress regions. These regions comprise the lungs where the red cell is exposed to oxidative stress or is exposed to osmotic shock through the kidneys where the erythrocyte is. The erythrocyte membrane will then be harmfully influenced. This can result in the release of hemoglobin in extracellular plasma, which is then filtered into the kidneys and collects in the acidic lumen of the renal tubules, contributing eventually to renal failure (Lang et al., 2005; Lang et al., 2006).

Hisham Ali Waggiallah

Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Prince Sattam Bin Abdulaziz University.

***Email:** hishamwagg30 @ hotmail.com

Red cells live about 120 days in the body before being eliminated from the bloodstream by the senescence process. Under certain circumstances, the erythrocytes undergo a mechanism of cell death, called eryptosis, before reaching their maximum lifespan (Ghashghaeinia et al., 2012). This form of cell death can be caused by erythrocyte injury and can be caused by a wide range of causes, ranging from hyperosmolarity, oxidative stress, loss of energy, exposure to heavy metals, xenobiotics and antibiotics prescribed for various health problems (Lang et al., 2005).

Human RBC phospholipids are distributed asymmetrically within the red cell membrane bilayer (Mario et al., 1999). Maintaining asymmetry of the plasma membrane, also at the cost of energy consumption (Devaux et al., 1994), is important for cells. Indeed, loss of normal phospholipid asymmetry, particularly the appearance of PS (an aminophospholipid normally confined to the inner leaflet of the membrane) inside the outer leaflet of the erythrocyte membrane. Especially, PS exposure encourages the recognition of macrophage and the splenic clearance of aged or abnormal erythrocytes (McEvoy et al., 1986). The characteristics of eryptosis are identical to apoptosis because this form of cell death also shows similar characteristics, including cell shrinkage, membrane blebbing, and cell membrane phosphatidylserine exposure (Berg et al., 2001).

This review briefly explains processes lead to eryptosis, phosphatidylserine exposure, and removal of eryptotic red cell by scavengers

Eryptosis

Eryptosis serves as a protective system by reducing the survival of red cells when damage or other clinical conditions affect them, and by eliminating these erythrocytes from the bloodstream (Lang et al., 2006). Eryptosis is described by cell shrinkage and cell membrane scrambling and is triggered by calcium entry via Ca2+ permeable, PGE2-activated cation channels, ceramide caspases, calpain, complement, hyperosmotic shock, energy depletion, oxidative stress and the deranged activity of several kinases (e.g. AMPK, GK, PAK2, JAK3) (Pretorius et al., 2016). This mechanism happens as follows: The non-selective Ca2 + permeable cation channels in the membrane are triggered by hyperosmotic shock, energy depletion, or removal of extracellular Cl-. This allows Ca2 + to join and leak into the cell followed by PS-flip to display PS on the surface of the erythrocyte (Lang et al., 2005).

Causative factors of eryptosis:

A. Hyperosmolarity and Oxidative Stress. Stimulate channels and Cl− channels of Ca2 + -penetrable cation and activate aspartyl and cysteinyl proteases (Lang et al., 2006). The loss of Cl− ions triggers the formation of prostaglandin E2, which in turn is the driving factor behind the rise in Ca2 + ion levels causing eryptosis. Oxidative stress also stimulates caspases displayed by erythrocytes which facilitate exposure to phosphatidylserine resulting in the recognition and engulfment of the erythrocyte by circulating macrophages. Hyperosmolarity doesn't involve caspase activation (Lang et al., 2006).
B. Reduction of energy; Glutathione replenishment (GSH) is affected during the energy deficiency and thus reduces erythrocyte antioxidant activity. Energy depletion also results in the activation of Ca2 + -permeable cation channels in red cell membranes that cause eryptosis via the formation of PGE2 (Lang et al., 2005). Energy depletion may also affect the function of protein kinase C (PKC) and membrane protein phosphorylation leading to cell shrinking and exposure of phosphatidylserine. Additionally, PKC activation induces an increase in intracellular Ca2 + ion concentration and direct eryptosis activation (Lang et al., 2006).
C. α -lipoic acid. α-Lipoic acid can induce eryptosis and trigger caspase 3 activation (Bhavsar et al., 2010). In comparison, α-lipoic acid has antioxidant properties in the red cell; in the existence of α-lipoic acid, in eryptotic erythrocytes, phosphatidylserine exposure is inactivated, leading to the assumption that only high doses of α-lipoic acid will cause eryptosis (Bhavsar et al., 2010).
D. Cadmium. Cadmium poisoning leads to eryptosis by rising Ca2 + ion levels of the erythrocyte, diminishing K+ ion standards, and erythrocyte shrinkage. This latter describes the appearance of cadmium-poisoned anemia in patients (Sopjani et al., 2008).
E. Anti-A IgG. Anti-A IgG antibodies are also known to induce the influx of Ca2 + ions into red cells, resulting in the removal of damaged red cells. This coincides with the response of the immune system to antigen A for autoimmune diseases, or an ABO blood transplant (Attanasio et al., 2007). Any foreign substance presents in the blood has the potential to injure erythrocytes and trigger eryptosis (Akel et al., 2006).

Chronic renal disease:

or End-stage renal disease (ESRD) is characterized as a reduction in kidney function in the filtration of waste materials and excess body fluid (Lang et al., 2017). ESRD also results in decreased production and release of erythropoietin which dwindles red cell production by erythropoiesis leading to anemia (Lang et al., 2017; Lang and Lang, 2015; Lang et al., 2012). Researches have shown that the number of erythrocytes exposed to phosphatidylserine in ESRD patients is considerably higher compared with healthy subjects (Lang et al., 2017). In this patient population, the substantial rise in eryptosis is specifically associated with the increased number of uremic toxins, such as methylglyoxal in patients with ESRD (Lang et al., 2017).

Drugs:

Some drugs can also cause eryptosis in clinical conditions. Which includes an antipsychotic drug called chlorpromazine. According to research released by (Akel et al., 2006), the concentrations of the drug administered to patients are sufficient to induce eryptosis by phosphatidylserine cell membrane exposure. Chlorpromazine acts by decreasing erythrocyte ATP standards, and also by raising the Ca+2 ion levels that together contribute to hallmarks of eryptosis to include hyperosmolarity, glucose depletion, and erythrocyte shrinkage (Akel et al., 2006)

Malaria:

Plasmodium falciparum: The microorganism is capable to use non-selective canals of cation to get nutrients (Foller et al., 2009). Plasmodium falciparum uses Ca+2 ions for the activation of the cation channel, thereby obtaining the required nutrients present in the cell undergoing eryptosis, thus enabling the erythrocyte to survive longer in eryptosis as it decreases cytosolic Ca+2 ion concentrations (Foller et al., 2009). This results in damage to the membrane phospholipid asymmetry and phosphatidylserine exposure at the outer membrane leaflet during the late stages of parasite growth. It may also promote the phagocytotic clearance of macrophages to infected cells (Lang et al., 2004).

Sickle Cell Anemia:

In SCD, Erythrocytes more vulnerable to eryptotic stimulators such as oxidative stress and energy depletion (Lang et al., 2006). Phosphatidylserine exposure occurs in patients with sickle cell disease (SCD) in red blood cells (RBCs), which is increased by deoxygenation. RBCs from SCD patients often have increased cation permeability and, particularly, a deoxygenation-induced cation conductance that facilitates the entry of Ca+2, providing an obvious link to phosphatidylserine exposure. High phosphatidylserine exposure in SCD patients' RBCs increased upon deoxygenation. As the extracellular Ca+2 increased, phosphatidylserine exposure was further elevated when deoxygenated. This impact was blocked by dipyridamole, intracellular Ca^{+2} chelation, and Gardos channel inhibition. Phosphatidylserine exposure decreased in high K+ saline. The amount of Ca+2 needed to elicit exposure to phosphatidylserine was in the low micromolar range. Ca^{+2} entry via the deoxygenation-induced pathway (Psickle), triggering the Gardos channel. [Ca^{+2}] demanded for phosphatidylserine scrambling is in the range can be done in vivo (Foller. 2008).

Iron Deficiency Anemia:

Patient erythrocytes have iron deficiency anemia commonly show with reduced size of cells. The other causes raised cation channel stimulation and subsequently increasing in cytosolic Ca+2 and resulted in eryptosis and a shortened lifespan of erythrocytes (Foller. 2008). The limited lifespan of iron-deficient red cells often represents increased phosphatidylserine exposure on the

erythrocyte surface and correlates with eryptosis (Lang et al., 2004).

Thalassemia:

a disorder that results in decreased and defective development of the erythrocyte hemoglobin. This results in erythrocytes becoming more vulnerable to oxidative stress, and increased anemia occurrence as erythrocytes are prematurely eliminated from circulation by eryptosis due to exposure of phosphatidylserine (Lang et al., 2004). Previous studies have shown insulin can shield thalassemic erythrocytes from increased oxidative effects. They undergo the latter, as insulin reduces the incidence of phosphatidylserine exposure and raises glycolysis in red cells, which elevates the amount of ATP in the red cells leading to protein C kinase (PKC) inhibition (Jermnim et al., 2011). Although it is known that PKC significantly affects eryptosis caused by energy depletion, the treatment of these patients with insulin will lead to inhibition of eryptosis (Jermnim et al., 2011).

Thrombosis:

Changes in the red cell membrane, like externalization of phosphatidylserine (PS) and generation of PS-bearing microvesicles, may allow red cells involved in blood coagulation, cell adhesion, and premature erythrocyte clearance. PS exposed on red cell generates a site for prothrombinase and tenase association, leading to thrombin production and coagulation (Chung et al., 2007). Additionally, PS-exposing red cells become stickier to endothelial cells, participating in vas occlusion. It is also shown that PS-bearing microvesicles shed from red cell membrane as well exhibit procoagulant activity. These findings strongly indicate that in certain conditions where cellular membrane perturbation occurs; erythrocytes may play an important role in normal or abnormal hemostasis (Chung et al., 2007).

Phosphatidylserine recognition by macrophage:

The lifespan of the erythrocyte approaches 100–120 days, in the absence of injury (Jelkmann et al., 2012). It is eventually restricted by senescence (Bosman et al., 2005) resulting from attaching altered hemoglobin to band 3, followed by band 3 alteration, disruption of band 3-dependent cytoskeleton anchorage to the lipid bilayer, and formation of vesicles that exposing of phosphatidylserine to their surface (Lang et al., 2010). The vesicles are thereafter bound, engulfed, and therefore eliminated by scavenger receptors (Bosman et al., 2005). Erythrocytes can suffer survival-threatening injury before senescence, as described above. They can then undergo either programmed cell death or eryptosis (Lang et al., 2010). Comparable to programmed nucleated cell death or apoptosis, eryptosis is a coordinated suicidal death that finally leads to the elimination of defective cells without cell membrane rupture and the release of intracellular material (Lang et al., 2010). However, contrary to nucleated cells, erythrocytes lack nuclei and mitochondria (Jermnim et al., 2011; Bosman et al., 2005) which participates actively in the machinery that underlies apoptosis (Bosman et al., 2005; Lang et al., 2010). Thus, eryptosis lacks many apoptosis hallmarks, such as mitochondrial depolarization and nucleic condensation. The signaling that eventually contributes to eryptosis (Lang et al., 2010) is not similar to that underlying apoptosis (Lanza et al., 2010; Wlodkowic et al., 2011). Even so, eryptosis shares many apoptosis characteristics such as cell shrinkage, blebbing of the cell membrane, and scrambling of the cell membrane with phosphatidylserine exposure on the cell surface (Lang et al., 2010). Similar to apoptotic cells and particles, macrophages ingest, and degrade the eryptotic cells and particles (Lang et al., 2010; Lang et al., 2012). Equally similar to apoptosis, eryptosis induces the elimination of cells that are damaged, infected, or otherwise potentially harmful.

The attachment and digestion of phosphatidylserine (PS)-expressing cells tend to involve multiple receptor-mediated systems that directly or indirectly recognize the lipid through intermediary proteins that form a molecular connection between the cells. It has been demonstrated that β_2-glycoprotein I (β_2GPI), a 50-kDa serum glycoprotein, attaches PS-containing vesicles and acts as moderate for the interaction of these vesicles with macrophages (Balasubramanian and Schroit, 1998)

β2GPI is a well-defined glycoprotein plasma, which binds negatively charged phospholipids (Balasubramanian and Schroit, 1998). Similar PS binding behaviors with synthetic, negatively charged phospholipid vesicles have also been demonstrated (Nimpf et al 1986; Chonn et al., 1995). Forming those PS. β2GPI complexes are associated with the rapid removal of PS-expressive particles from the peripheral circulation and PS-expressing cell phagocytosis (Chonn et al., 1995). Interestingly, the binding of β2GPI to PS has resulted in the expression of a new epitope, which induces an autoimmune reaction in certain individuals (Balasubramanian and Schroit, 1998).

Numerous researches have proposed that the reticuloendothelial system redistributes PS from the cells inside to the outer leaflet act as signals to remove these cells (Balasubramanian and Schroit, 1998). Despite many processes may be responsible for the phagocyte recognition of PS expressing apoptotic cells, (Bhalla et al., 1984) Suggested that the interaction of β2GPI circulating with redistributed anionic phospholipid could in itself produce a specific ligand by which apoptotic cells are recognized.

Also, other studies indicate that β 2GPI may play a major role in this mechanism of recognition (Balasubramanian and Schroit, 1998).

Schroit et al., 1985 observed that the outer surface of erythrocytes enriched with phosphatidylserine analogs (injection into mouse erythrocytes of various quantities of fluorescent phosphatidylserine analogs) was removed five times quicker than the corresponding normal cells. Such cells accumulated in Kupffer and splenic macrophages. Cell clearance depended on the amount of exogenously inserted phosphatidylserine and occurred when only about 1 mol-percent of phosphatidylserine was in the cells.

The clearance of senescent RBCs is a basic process and the existence of several redundant pathways in lactadherin deficient cells is not surprising. It is important to note that several proteins have been identified which bind to apoptotic cells that express phosphatidylserine including gas-6, (Ishimoto et al., 2000) del-1, (Hanayama et al., 2004) and complement components (Wang et al., 1993)

Gas6, a ligand of receptor tyrosine kinases Axl, Sky, and Mer stimulates cell proliferation and inhibits cell death. This also contains γ-carboxy glutamic acid residues which mediate the interaction of certain blood coagulation factors with negatively charged phospholipids. Gas6 interacts with PS liposome via its domain N-terminal Gla, and with macrophages via its domain C-terminal. Like that of PS liposomes, in the presence of Gas6, the uptake of apoptotic cells by macrophages was also increased, roughly double. These results indicate that Gas6 may assist phagocytic cells to identify PS-exposed cells on their surfaces, which is considered one of the processes to clear dying cells away. By promoting the clearance of PS-expressing cells, Gas6 may thus play a critical role in homeostasis (Ishimoto et al., 2000).

Conclusion

We conclude that most of the pathological effects of red cells lead to exposure to phosphatidylserine, which serves as the key marker of eryptosis and plays an important role in the identification of erythrocytes by macrophage and enhances the removal of eryptotic cells from blood circulation.

Compliance with Ethical Standards

Disclosure of potential conflicts of interest

The author declares that they do not have a conflict of interest

Research involving human participants and/or animals

No need for ethical approval since the manuscript is a review article

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Conflict of interest

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