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REVIEW PAPER

Oxycytosis and the role of triboelectricity and oxidation in bacteria clearing from the bloodstream

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ABSTRACT

Until recently, little was known about the mechanism for killing and clearing bacteria from the bloodstream. Leukocyte phagocytosis could not be a mechanism for catching, killing and removing bacteria from the bloodstream because of many reasons. Recently accumulated data have led to the conclusion that in bacteremia, bacteria are quickly removed from the blood and erythrocytes are the main cells that capture, kill and remove bacteria. Data were also obtained that erythrocytes catch bacteria by triboelectric charge attraction and kill them by oxygen released from oxyhemoglobin. This phenomenon has been named oxycytosis by analogy with the term phagocytosis. Oxycytosis has been discussed in a number of published articles, but the specific mechanism of triboelectric charging and the mechanism of killing bacteria by oxidation, have not yet been detailed. The purpose of this review is to provide a more detailed explanation of the process of triboelectric charging and capture of bacteria by erythrocytes and destruction of bacteria by oxidation. For the first time, the review presents various variants of oxycytosis (two-stage, three-stage, multi-stage), depending on the resistance of the pathogen to oxidation. The review also discusses the biological significance of oxycytosis and its impact on the understanding of bacteremia and sepsis.

KEYWORDS

innate immunity, phagocytosis, oxycytosis, bacteremia, sepsis

INTRODUCTION

Human innate immunity has humoral and cellular components [1, 2]. Innate antibacterial humoral factors (complement, the naturally occurring antibodies (NAb), pentraxins, contact cascades, host defense antibacterial peptids and others) are available in both tissues and the bloodstream [3]. Cellular innate immunity includes two main antibacterial mechanisms: phagocytosis and oxycytosis [4]. Phagocytosis occurs in the tissues whereas oxycytosis takes place in the bloodstream. Tissue resident macrophages, myeloid-derived suppressor cells, innate lymphoid cells and leukocytes transmigrated from the bloodstream clear bacteria from the tissues by phagocytosis [5] while erythrocytes clear bacteria from the bloodstream by oxycytosis [4]. Phagocytosis is bacteria-killing by engulfing and digesting [6, 7] whereas oxycytosis is bacteria catching by electric charge attraction and killing by oxidation [4]. Phagocytosis is a complicated time-consuming immune reaction with recognition, chemotaxis, engulfment and digestion of pathogens [8, 9]; oxycytosis is a simpler and faster electrochemical phenomenon [10]. If infectious agents enter the bloodstream after overcoming humoral innate immunity and phagocytosis in the tissues, it becomes resistant to humoral immunity in the bloodstream as well [11, 12]. As a result, oxycytosis by erythrocytes remains the dominant mechanism of bacteria clearing from the bloodstream.

Oxycytosis was detected by phase contrast microscopy of the blood of patients with transient bacteremia [13]. The term “oxycytosis” was coined in analogy with the term “phagocytosis” after accumulation of data regarding the mechanisms of bacteria catching and

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killing by erythrocytes [14]. If reactive oxygen species (ROS) production in phagocytes and oxidative burst of macrophages that kill bacteria are more or less studied, then the destruction of bacteria by oxygen released from erythrocytes remains poorly understood. As for triboelectric charging and its role in human physiology and pathology, it is still little researched and almost not reported. So far, triboelectric charging and oxidation in oxycytosis has not been discussed enough in medical literature. For a better understanding of oxycytosis both triboelectric charge attraction and pathogen oxidation should be explained in detail.

Triboelectric charge attraction

Till now triboelectricity has been ignored as a fundamental phenomenon in the physiology of living systems. At the same time, any movement, secretion, interaction, flow and circulation of fluid and other phenomena of life generate triboelectricity, which is of great importance in the functioning of organisms. Triboelectricity (tribo- from "rubbing" in Greek) is generated by both friction and adhesion. Triboelectricity is the most powerful electric phenomenon in nature. Lightning is an example of triboelectricity discharge. Friction of water molecules in clouds may generate and accumulate a static charge more than 10,000 V per centimeter (10 kV/cm) [15, 16]. Friction to air also may generate triboelectricity. In aircraft fly triboelectricity is generated during air friction on the airframe. Friction generates so much electricity that NASA follows the "Triboelectrification Rule" that cancels a launch if the vehicle should pass through clouds that create triboelectricity around the vehicle [17]. Triboelectricicty can cause ignition and explosion of flammable vapors as it happened with Falcon 9 rocket [18]. Triboelectricity is a risk in refueling aircraft, cars, and others. Flowing liquids in a pipe generate triboelectricity that may cause explosion. Triboelectricity generated by friction in clouds of dusts and powders can ignite explosive mixtures [19]. Triboelectricity is generated during manipulations with liquids. In everyday life triboelectric charging accompanies human actions related to walking, removing synthetic and wool cloths, brushing, rubbing against different electricity-generating materials, etc. [20].

Triboelectricity plays an important role in human physiology. Blood flow causes friction between blood plasma, vessel walls, blood cells, and particles of any size available in the bloodstream. As a result, blood cells, vessel walls and moving particles are triboelectrically charged [4]. In bacteremia, bacteria become triboelectrically charged and start to interact with other triboelectrically charged objects. During movement, erythrocytes accumulate electrostatic charge on their membranes that attracts and captures bacteria [10]. In the bloodstream, erythrocytes are unique "hunter and killer of bacteria" because: a. Erythrocytes are the most numerous cell population in the blood; b. The total surface of all erythrocytes is the largest among all blood cells; c. The erythrocyte membrane has unusual properties that provide intense triboelectric electrification of the erythrocyte membrane; d. Red blood cells contain the highest amount of oxygen compared to other cells in the human body. These features of red blood cells are presented in more detail below.

One millimeter cube (microliter (μL)) of blood contains 5,100,000–5,800,000 (average 5,400,000) erythrocytes in men and 4,300,000–5,200,000 (average 4,800,000) erythrocytes in women. These numbers are dependent on age, gender, and height of place [21]. Humans have from 20 to 30 trillion (the average interval is $2.5–2.8 \times 10^{13}$) erythrocytes (that is 70% of all cells in the human body) [22]. The number of erythrocytes in the blood approximately 1,000 times exceeds the number of leukocytes. As a result, a leukocyte is surrounded by a thousand erythrocytes that restrict the leukocyte's access to bacteria. Moreover, leukocytes usually move slowly along the vascular walls while bacteria and erythrocytes are more concentrated toward the central axis of vessels with rapid blood flow (the Fåhræus effect) [23, 24]. As a consequence, the contact of leukocytes with bacteria in the bloodstream becomes almost impossible. In contrast, the surface area of all erythrocytes in the human body is so large that the contact of a bacterium with erythrocyte surface is inevitable. The surface area of a human erythrocyte is 136 micrometer^2 (μm^2) [25]. Taking into account the number of erythrocytes in the human body (from 2.5 to 2.8×10^{13}), a simple calculation (136 μm^2 $\times$ $2.5–2.8 \times 10^{13}$) shows that the surface area of all erythrocytes is from 3,400 to 3,800 m^2. At the same time, adult human skin has a surface area of 1.5–2.0 m^2 [26], the gut's surface area is 30 m^2 [27], the surface area of the lungs is 50 m^2 [28].

Erythrocyte membrane has unique viscoelastic properties that resemble those of both a fluid and a solid. The elasticity of the membrane provides its resistance to deformation, whereas the viscosity of the membrane determines its resistance to a rate of deformation [29]. Membrane resistance to changes and response to shear deformation is dependent on phospholipid bilayers and the proteins of cytoplasmic surface [30]. An erythrocyte membrane is 100-fold more elastic than a latex membrane of the same thickness, in 100 milliseconds responds to fluid stresses and is stronger than steel regarding structural resistance. Erythrocytes can deform with linear extensions of 250% [31, 32]. Having a diameter of 6–8 μm (average – 7 μm) [33], erythrocytes pass 500,000 times through capillaries 3 μm in diameter during their 120-day life span [34].

The erythrocyte membrane generates high levels of triboelectricity. On the contrary, the leukocyte membrane generates weak triboelectric charge because of softness, numerous surface wrinkles and folds, and leukocyte lower speed of floating [35]. Having a weak electrostatic charge, leukocytes do not participate in bacteria "catching" by electric charge attraction. During move in the bloodstream, erythrocytes generate a very large summary electrostatic charge. The latter is determined by the surface of all erythrocytes and the speed of blood flow. If take the average size of erythrocyte 7 μm and put side by side (make a chain) all erythrocytes of human body (2.8×10^{13}), it is easy to calculate that this "erythrocyte train" would be 196 km long.

In arterial blood this "train" moves especially rapidly. Arterial blood flow velocities are 4.9–19 cm/s whereas venous blood flow is slower: 1.5–7.1 cm/s [36]. In aorta blood flow velocity is 40 cm/s [37, 38]. Oxycytosis occurs in arterial (non-venous) blood for the following reasons: a. Arterial erythrocytes contain the maximum amount of oxygen; b. Arterial blood moves rapidly and erythrocytes become the most triboelectrically charged in arterial blood. The generation of ultra-high power triboelectric charges on the surface of erythrocytes becomes obvious when one considers that a 196 km long "train of erythrocytes" or a red blood cell area of 3,800 m^2 moves at a speed of 4.9–19 cm/s in the arteries and 40 cm/s in the aorta. Triboelectric charge of erythrocytes with extraordinary force attracts and fixes triboelectrically charged bacteria.

Thus, in the bloodstream, the physical phenomenon of attraction of opposite electrostatic charges provides a quick and effective mechanism for "trapping" bacteria by erythrocytes. To trap and hold bacteria, red blood cells also form filaments similar to pili. Multiple filaments (similar to leukocyte pseudopods) firmly fix bacteria on the surface of erythrocytes [39]. If bacteria are resistant to oxidation on the surface of the erythrocyte membrane, the erythrocytes engulf them [40]. Bacteria that cause sepsis usually penetrate the erythrocyte membrane and enter the erythrocytes using hemolytic pore-forming enzymes [4]. Encapsulated bacteria and bacterial L-forms are weakly charged in the bloodstream and often escape from triboelectric capture by erythrocytes [10].

Bacteria oxidation by oxygen released from oxyhemoglobin

The blood flow in the vessels is fast, and all cells move quickly [41]. Not only catching pathogens but also their killing should be fast and effective [42]. Oxidation of bacteria by atomic oxygen and reactive oxygen species (ROS) is the fastest tool for bacteria-killing [43]. Erythrocytes kill pathogens by atomic oxygen and highly toxic ROS, such as superoxide anion $O_2\bullet$-) [44]. Erythrocytes release atomic oxygen and ROS immediately after "catching" bacteria and fixing them on their surface [10, 14]. Atomic oxygen and ROS kill bacteria by oxidation which is the process of taking away electrons from bacterial body molecules. Oxidation decomposes bacterial cell wall, stops trans membrane transport of molecules, affects all structures inside bacterial cells [45]. DNA is the most important target of active oxygen and ROS [46]. Base oxidation, particularly guanine oxidation, is fatal for bacterial cells [47].

Oxygen transport by erythrocytes needs the reversible binding of oxygen to hemoglobin. During oxygen binding to hemoglobin hydrogen peroxide, superoxide anion and other ROS may be produced [48]. Superoxide anion dismutases into hydrogen peroxide (H_2O_2) and is a potential source for subsequent oxidative reactions [49, 50].

During auto-oxidation of hemoglobin Hb-Fe^{2+} transforms to Hb-Fe^{3+} (methemoglobin). Superoxide radical (O_2-.) after dismutating into H_2O_2 can further oxidize Hb-Fe^{3+} to transient $HbFe^{4+}$-OH (ferryl-Hb). Bacterial extracellular proteases and pathogen-associated molecular patterns (PAMPs) binding to Hb may stimulate pseudoperoxidase and generate ROS [51]. MetHb activated by microbial cellular factors may catabolize into transient ferryl-Hb by elevating the production of superoxide radicals [51]. The greater is the amount of PAMPs or proteases, the more is the cleavage of metHb proteolytic profiles [52, 53].

Thus, erythrocytes rapidly kill bacteria in the bloodstream by releasing atomic oxygen and ROS.

Summary of the oxycytosis concept in bacteria-host interaction

Human innate immunity has cellular and humoral components. The cellular component includes non-specific immune cells: tissue-resident macrophages, leukocytes, erythrocytes, and platelets [54]; the humoral component consists of the complement, the naturally occurring antibodies (NAb), pentraxins, contact cascades, host defense peptides (antimicrobial peptides) and others [55]. Innate immunity humoral factors in the tissues and the bloodstream are almost the same because of interaction and partial mixing of blood plasma, lymph, and interstitial fluid [56]. Bacteremia may be caused by direct entering of the infectious agent into the bloodstream (for example, in contaminated intravenous injections) or by bacterial invasion from the tissue site of infection. In the majority of bacteremia cases, the infectious agent first proliferates in the tissues and only after overcoming tissue cellular and humoral immunity enters the bloodstream [57]. As a result, an infection that enters the bloodstream from the tissues is resistant to plasma innate humoral immunity. In this situation, oxycytosis remains the only prompt and effective mechanism of bacteria clearing from the bloodstream. Being triboelectrically charged in the bloodstream, both erythrocytes and bacteria are attracted to each other and get in contact by the electric charge. Bacterial electrical charge, PAMP, proteases, enzymes, etc. irritate the surface of red blood cells and cause the release of atomic oxygen and ROS from erythrocytes, which kill bacteria by oxidation and inactivate bacterial factors that irritate the red blood cell membrane. Killed bacteria are released to plasma and get recycled there by lysis: cell membranes disintegrate, the bacterium's innards – the cytoplasm, ribosomes, and DNA spill out and degrade to simpler substances (amino acids, lipids, proteins, etc.) that are absorbed and digested by the liver and the spleen [4]. If short-term exposure to oxygen and ROS is not enough to kill bacteria, the irritated erythrocyte membrane forms pili-like pseudopodia that fix (hold) bacteria on the surface of the erythrocyte and prolong the effect of atomic oxygen and ROS on bacteria. If bacteria survive longer exposure to oxygen and ROS, they can be absorbed and destroyed by more intense oxidation within the red blood cells. Sepsis-causing bacteria produce potent antioxidant enzymes (such as catalase, superoxide dismutase, glutathione peroxidase) and usually survive oxidation on the surface of erythrocytes; moreover, they can penetrate

and enter erythrocytes by pore-forming hemolytic enzymes [58]. Proliferating inside erythrocytes, sepsis-causing bacteria form bacterial reservoir inside erythrocytes that is inaccessible and resistant to host immune factors and antibiotics [42]. Thus, oxycytosis can be a two-stage or multi-stage process, depending on the resistance of the pathogen to oxidation. The resistance of bacteria to oxidation determines the modes of action of oxycytosis, which can be as follows:

1. Two-stage oxycytosis: erythrocytes "catch" bacteria by triboelectric charge attraction and kill on the surface of their membrane by released atomic oxygen and ROS. This scenario is available in most cases of bacteremia if the bacterium is susceptible to oxidation. Two-step oxycytosis provides rapid and complete clearing of bacteria from the bloodstream.
2. Three-stage oxycytosis: erythrocytes "catch" bacteria by triboelectric charge attraction but cannot immediately kill them because of bacterial resistance to short-term oxidation. Erythrocytes form pili-like pseudopodia to retain bacteria on the surface of their membranes and prolonged exposure to atomic oxygen and ROS.
3. Four-stage oxycytosis: erythrocytes "catch" a bacterium by triboelectric charge attraction, form pili-like pseudopodia, and expose bacteria for a relatively long time to released atomic oxygen and ROS, but bacteria survive oxidation. Erythrocytes engulf bacteria for killing them inside by higher concentrations of oxygen and ROS. The absorption results can be different: a. The bacterium dies during more intense (than on the surface of the erythrocyte) oxidation; b. The bacterium goes into a dormant state and remains inside the erythrocyte; c. The bacterium "consumes" and neutralizes atomic oxygen and ROS inside the erythrocyte, producing antioxidant enzymes (including catalase, superoxide dismutase, glutathione peroxidase), and begins to multiply using hemoglobin as a food source. This situation is common in sepsis; in addition, bacteria that cause sepsis usually quickly invade red blood cells, producing hemolytic enzymes that form pores.

The phenomenon of oxycytosis shows that innate human immunity in the bloodstream can successfully use elementary physicochemical reactions of electrification and oxidation as a bactericidal tool. Oxycytosis also demonstrates that red blood cells not only carry oxygen to tissues, but also use oxygen to kill bacteria, that is, red blood cells also play an important antibacterial role in blood flow. Oxycytosis allows re-evaluating the role of erythrocytes in the human body. Oxycytosis explains why bacteremia in some cases turns into sepsis. It is known that bacteremia can be a frequent daily occurrence in many people and usually rarely causes sepsis [59–61]. The situation changes if the bacteria that cause bacteremia have a number of features that allow them to resist oxycytosis [42]. It is the ineffectiveness of oxycytosis that is one of the main reasons for the development of sepsis in bacteremia. The ineffectiveness of oxycytosis leads to an abundant release of oxygen from the erythrocytes, which stimulates the blood coagulation system and causes disseminated intravascular coagulation DIC [54]. Oxycytosis-resistant bacteria also invade erythrocytes, creating a reservoir of infection in red blood cells with all the ensuing consequences. The most unfavorable consequences are the multiplication of bacteria in erythrocytes, their dissemination from erythrocytes with the formation of metastatic foci in the tissues and an extremely persistent course of infection. A big therapeutic problem is the inaccessibility of bacteria inside erythrocytes for antibiotics, immune complexes, and other antibacterial factors.

CONCLUSION

Oxycytosis is a fast and effective mechanism of cellular innate immunity that kills bacteria and removes them from the bloodstream. Oxycytosis is based on the physical phenomenon of attraction of a triboelectric charge and the chemical reaction of oxidation of pathogens by atomic oxygen and ROS released from erythrocytes. The main advantage of oxycytosis is its independence from a complex long-term immune response, which requires (in the case of phagocytosis) recognition, chemotaxis, absorption and digestion of pathogens. Oxycytosis is the only cellular mechanism that traps and kills bacteria in the bloodstream. This mechanism allows a new look at the role of erythrocytes in human physiology and immunity and helps both to identify some important factors that determine the development of bacteremia in sepsis and to understand the problems that occur in the treatment of sepsis.

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