WHAT IS NEW? WHAT IS HOT?

Immune function? Consult me too, RBCs' authentic claim!

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It has been presumed for long that ferrying oxygen is the only function of red blood cells (RBCs) and the same has been taught in schools and through standard textbooks. However, a new research published in October this year by Lam *et al*. has revealed that RBCs play a much more significant role in the immune system and function through their DNA-binding capability, a newly discovered mechanism.[1]

RBC functions beyond oxygen transport

RBCs, variedly referred to as red cells/red blood corpuscles/hematids/erythroid cells or erythrocytes, are the most common abundant type of blood cells and the vertebrates' principal means of delivering oxygen (O_2) to the body tissues via circulatory system. RBCs play a key role in the CO_2 transport and they ensure that most of the CO_2 is transported as bicarbonate. RBCs also perform various secondary functions. ATP release by RBCs, when they undergo shear stress in constricted vessels, has been shown to cause the vessel walls to relax and dilate to promote normal blood flow.[2] When the hemoglobin molecules are deoxygenated, RBCs release S-nitrosothiols, which also act to dilate blood vessels, thus directing more blood to areas of the body depleted of oxygen.[3] Like endothelial cells, RBCs can also synthesize nitric oxide enzymatically using L-arginine as substrate.[4] Exposure of RBCs to physiological levels of shear stress has been known to activate nitric oxide synthase and export of nitric oxide, which is linked to the regulation of vascular tone.[5] Interestingly, RBCs can also produce hydrogen sulfide, a gaseous signaling second messenger that acts to relax vessel walls. It is believed that the cardioprotective effects of garlic are due to RBCs that convert its sulfur compounds into hydrogen sulfide.[6]

Revisiting red cell biology in the context of immune function

Despite the assumption of their primary service as oxygen carriers, RBCs are now re-visited and emerging as important modulators of the innate immune response. The possibility that RBCs could participate in the defensive mechanism of the human body was postulated long ago by Bishlawy, as RBCs serve as both mechanical and biochemical barriers against infections, bacteria, and blood parasites.[7] Mounting evidence suggests that RBCs also play an important role in the innate immune system, as these cells, in an evolutionary perspective, have shown to retain the ability to bind and interact with a variety of inflammatory molecules including chemokines, nucleic acids, and pathogens, thereby regulating and modulating immune responses.[8-10] As reviewed by Anderson *et al.*, studying the evolutionary conservation of innate immunity role of RBCs in humans is a thrust area of research, as we already knew that the nucleated erythrocytes of birds, amphibians, and fishes actively participate in the immune response via production of cytokine-like factors, upregulation of viral response genes, and sequestration of pathogens through surface binding or phagocytosis.[11]

In the above context, the recent work led by Mangalmurti and colleagues titled 'DNA binding to TLR9 expressed by red blood cells promotes innate immune activation and anemia', received greater attention as the finding has clinical translation and utility.[1] Earlier, this group has pioneered and reported that human erythrocytes express TLR9 and found that TLR9-positive erythrocytes bind and sequester circulating cell-free mtDNA, and *in vivo* mouse studies demonstrated that loss of this function resulted in enhanced lung injury during states of inflammation.[10] RBCs use TLR9 to scavenge cell-free mitochondrial DNA, which is present at low levels during normal cellular turnover and circulates at an increased level during illness or extensive cell death. It appears that depending on the conditions of the microenvironment, erythrocytes may either promote immune activation or maintain immune quiescence.

COVID-19 infection and RBCs' confession

COVID-19 research has unraveled novel new-biology

findings across the medicine fields, a real 'blessing in disguise' outcomes. While dysregulation of the host immune response is a critical mediator of increased morbidity and mortality due to COVID-19, the autopsy and pre-clinical evidence implicate aberrant complement activation in endothelial injury and organ failure. In this connection, Lam *et al.* have reported the presence of complement activation products on circulating erythrocytes from hospitalized COVID-19 patients, and these findings provide directionality for novel erythrocyte-based diagnostics to identify patients with dysregulated complement activation.[12] This group has also reported an enhanced C3b and C4d deposition on erythrocytes in COVID-19 sepsis patients and non-COVID sepsis patients compared to healthy controls.[13] This study also supports the role of complement in sepsis-associated organ injury and points towards a precision medicine approach to sepsis with a diagnostic value in monitoring complement dysregulation.

Take-home message

RBCs serve as DNA sensors through the surface expression of TLR9, which appears to be beneficial during quiescent states, as it promotes scavenging of trace levels of CpG to prevent non-specific inflammation. However, during conditions of excess circulating CpG, such as sepsis (also seen in COVID19), binding of CpG by RBC-TLR9 leads to accelerated clearance and inflammation. Although this innate immune mechanism may primarily be beneficial in the clearance of microbial infection and damaged RBCs, the phenomenon of increased CpG binding by RBCs would contribute to systemic inflammation and development of anemia during pathologic states where cell-free DNA is overwhelmingly elevated. Thus, DNA recognition by TLR9 on RBCs provides a bona fide certificate for red cells acting as immune sentinels. Therefore, RBCs can be used to scan for signs of infection and injury; they could be used to snare suspect DNA from microbial invaders or damaged tissue so as to warn the immune system of danger. As a strong immune role of RBCs is now linked to the anemia that often afflicts people with sepsis, COVID-19, and other conditions, there is an imperative demanding need for more clinical research in this field. Drugs that prevent the wayward DNA from adhering to TLR9 on red blood cells could become a potential treatment for anemia and other sepsis-related critical illnesses. If researchers could detect an increase in the nucleic acid-positive population of RBCs and track its levels over time, particularly in states of subclinical inflammation, it appears that this could signal the need for intervention without the need for more complex diagnostic tests. As always, **red (cell) alerts should not be neglected!**

Competing interests

The authors declare that they have no competing interests.

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References

1. Lam LKM, Murphy S, Kokkinaki D, Venosa A, Sherrill-Mix S, Casu C, et al. DNA binding to TLR9 expressed by red blood cells promotes innate immune activation and anemia. Sci Transl Med. 2021 Oct 20;13(616):eabj1008.
2. Wan J, Ristenpart WD, Stone HA. Dynamics of shear-induced ATP release from red blood cells. Proc Natl Acad Sci U S A. 2008 Oct 28;105(43):16432–7.
3. Diesen DL, Hess DT, Stamler JS. Hypoxic vasodilation by red blood cells: evidence for an s-nitrosothiol-based signal. Circ Res. 2008 Aug 29;103(5):545–53.
4. Kleinbongard P, Schulz R, Rassaf T, Lauer T, Dejam A, Jax T, et al. Red blood cells express a functional endothelial nitric oxide synthase. Blood. 2006 Apr 1;107(7):2943–51.
5. Ulker P, Sati L, Celik-Ozenci C, Meiselman HJ, Baskurt OK. Mechanical stimulation of nitric oxide synthesizing mechanisms in erythrocytes. Biorheology. 2009;46(2):121–32.
6. Benavides GA, Squadrito GL, Mills RW, Patel HD, Isbell TS, Patel RP, et al. Hydrogen sulfide mediates the vasoactivity of garlic. Proc Natl Acad Sci U S A. 2007 Nov 13;104(46):17977–82.
7. Bishlawy IM. Red blood cells, hemoglobin and the immune system. Med Hypotheses. 1999 Oct;53(4):345–6.
8. Darbonne WC, Rice GC, Mohler MA, Apple T, Hébert CA, Valente AJ, et al. Red blood cells are a sink for interleukin 8, a leukocyte chemotaxin. J Clin Invest. 1991 Oct;88(4):1362–9.
9. Neote K, Darbonne W, Ogez J, Horuk R, Schall TJ. Identification of a promiscuous inflammatory peptide receptor on the surface of red blood cells. J Biol Chem. 1993 Jun 15;268(17):12247–9.
10. Hotz MJ, Qing D, Shashaty MGS, Zhang P, Faust H, Sondheimer N, et al. Red Blood Cells Homeostatically Bind Mitochondrial DNA through TLR9 to Maintain Quiescence and to Prevent Lung Injury. Am J Respir Crit Care Med. 2018 Feb 15;197(4):470–80.
11. Anderson HL, Brodsky IE, Mangalmurti NS. The Evolving Erythrocyte: Red Blood Cells as Modulators of Innate Immunity. J Immunol. 2018 Sep 1;201(5):1343–51.
12. Lam LM, Murphy SJ, Kuri-Cervantes L, Weisman AR, Ittner CAG, Reilly JP, et al. Erythrocytes Reveal Complement Activation in Patients with COVID-19 [Internet]. 2020 May [cited 2021 Oct 25] p. 2020.05.20.20104398
13. Lam LKM, Reilly JP, Rux AH, Murphy SJ, Kuri-Cervantes L, Weisman AR, et al. Erythrocytes identify complement activation in patients with COVID-19. Am J Physiol Lung Cell Mol Physiol. 2021 Aug 1;321(2):L485–9.