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Losing control over ATP release: Implications for the RBC storage lesion

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While transfusion with banked blood is essential for the critically ill patient, it is increasingly being recognized that storage itself may result in a product that contributes to various transfusion related toxicities[1]. Recent evidence, using leukodepleted banked blood suggests that this effect is mediated at least in part by storage dependent changes to the red blood cell (RBC) itself. The positive association between the age of transfused RBC with an increased incidence of transfusion related pathologies is referred to as the RBC storage lesion, key features of which are with microcirculatory dysfunction and inflammatory tissue injury [2]. How storage induced changes in RBC promote these toxicities remains unclear however [3], with both gain of toxic functions (e.g. accelerated nitric oxide scavenging[4, 5]) or loss of protective mechanisms (e.g. pro-inflammatory cytokine scavenging and inhibition of inflammation[6]) being reported. In the current issue, Zhu et al[7] provide evidence for a novel mechanism involving dysfunctional ATP release from stored RBC.

Controlled release of ATP from RBC in response to mechanical deformation or hemoglobin desaturation is a key physiological process for matching oxygen supply with demand in both pulmonary and systemic tissues[8]. Released ATP binds to endothelial purinergic receptors and initiates signaling events that ultimately lead to increased vasodilator and/or decreased vasoconstrictor activity[9]; the net effect being increased blood flow. Seminal studies over the last decade have mapped out elements of the signaling pathway in RBC that regulates ATP release and involves Gi-proteins, cAMP activation of PKA and key roles for CFTR and Pannexin proteins[8] and have done much to dispel the notion that RBC are 'dead' cellular bags whose sole function is to compartmentalize hemoglobin. Furthermore, dysfunction in this signaling pathway has been demonstrated in diabetic and pulmonary hypertensive patients with the concomitant loss of ATP dependent regulation of blood flow discussed as a possible mechanism underlying vascular complications that characterize these diseases[10, 11]. The study presented by Zhu et al adds RBC storage lesion and development of transfusion induced lung injury and pulmonary hypertension into this emerging list of diseases associated with compromised ATP release from RBC.

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Zhu et al confirm previous studies showing roles for CFTR, K⁺ATP channels and pannexin 1 in mediating ATP release and show that this is impaired in stored RBC. Importantly, from using a combination of experimental approaches that either blocked ATP release from young cells, that inhibited extracellular ATP dependent effects by enzymatic degradation of this nucleotide, or by performing ATP add back studies to stored RBC that were unable to release ATP, Zhu et al show that RBC derived ATP is i) important in preventing stored RBC dependent increases in pulmonary arterial pressure in isolated perfused lungs, and, ii) important in preventing RBC adhesion to endothelial cells in vitro and in the lung after transfusion. These data begin to establish a cause-effect relationship for inhibited ATP release from stored RBC and transfusion related toxicity in the lung specifically.

Zhu et al also show that transfusing stored RBC into nude mice results in a relatively rapid (within mins) RBC adhesion and migration across the pulmonary vasculature that correlates with poor lung function. Adhesion was shown to be mediated by RBC ICAM-4 ligation of endothelial $\alpha_v\beta_3$ and moreover the only stimulus required for this was the lack of ATP release. This observation suggests that under physiologic conditions, RBC adhesion is tonically suppressed by RBC derived ATP with the pathologic consequences in the lung only manifesting when ATP release is inhibited. As discussed by the authors, an important consideration with respect to implications for storage lesion mechanisms is that the current studies did not incorporate the two-hit concept [6] whereby addition of stored RBC to critically ill patients, who are already under an inflammatory burden, provides the second hit to exacerbate organ damage. Testing how compromised ATP release contributes to transfusion related toxicity in relevant injury models is clearly an important next step.

Additional questions that arise from the present studies include how does RBC storage lead to inhibited ATP release? Does storage compromise RBC responsiveness to ATP releasing stimuli and if so, does this involve alterations in the amount and/or activity of proteins or molecules that regulate the ATP release signaling pathway? Additionally, how does ATP prevent ICAM-1 - $\alpha_v\beta_3$ interactions and does this involve endothelial purinergic receptors and why are the proadhesive effects of inhibiting ATP release from RBC only observed in the lung? These questions are not only of academic interest, but their elucidation, have the potential to provide novel therapeutic targets and strategies for preventing RBC storage lesion related effects. Pursuing this line of research is critical since the need for RBC transfusions is not waning and coupled with limitations in donor blood supplies, and the current lack of alternative resuscitation agents necessitates the use of older RBC units first in the clinic. Relying on use of only young RBC is not feasible therefore, underscoring the importance of developing strategies that prevent detrimental changes in RBC during storage and / or preventing detrimental effects of transfusing with stored RBC.

In summary, the data presented by Zhu et al expand on an emerging paradigm for the RBC storage lesion in which aging of cells under blood banking conditions results in molecular alterations in the RBC that lead to either loss of, or gain of function which ultimately leads to RBC that are pro-inflammatory, pro-coagulative and hypertensive [12]. Interestingly, sustaining ATP levels is one primary goal in current RBC storage protocols. The current study suggests protocols that maintain RBC responsiveness to stimuli that release ATP may be equally important.

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