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Forum Editorial

Redox Biology of Blood

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WHEN I was asked to put together a forum dealing with an emerging and complex area such as *Redox Biology of Blood*, the task seemed to be daunting. Rather than viewing blood as a complex tissue made up of enormous cellular, noncellular, redox-active, and non–redox-active components, the focus was placed instead on the two most important redox-active species in blood: hemoglobin (Hb), which is an oxygen carrier, and nitric oxide (NO), which has emerged as an important signaling molecule across physiologic systems of oxygen supply and demand (9). In the last decade, there has been an explosion in the research on NO biochemistry and physiology. In 1992, NO was crowned as the "molecule of the year" because of its key roles in a host of biochemical reactions (6). The rapid reaction of NO with Hb was central to the understanding of blood vessel function and circulation. This reaction formed the basis of early NO detection methodologies and helped elucidate the identity of endothelial relaxing factor as being indeed NO (11, 18). This reaction also accounts for some of the unique adverse effects of blood substitutes based on free Hb. Unlike red blood cells (RBCs), cell-free Hb can reach and scavenge endothelial NO, causing marked vasoconstriction and hemodynamic imbalances in humans infused with these products (1).

NO is produced enzymatically by endothelial cells lining the vascular system. It diffuses into the flowing blood, where it reacts with protein molecules in the plasma and finally reaches the RBCs. There exists a delicate balance between the synthesis and decomposition of NO in the two major compartments of blood, *i.e.*, plasma and RBCs. The mode and the rate of NO elimination depend on its concentration, diffusibility, and the concentration of other bioreactants (14). The major intermediate breakdown product of NO in human plasma is nitrite (NO_2^-). Plasma NO_2^- could be taken up by RBCs, where it is oxidized to nitrate (NO_3^-). Another pathway for NO decomposition is achieved through its reaction with superoxide anion ($O_2^{\cdot-}$) to form the extremely reactive peroxynitrite ($ONOO^-$). Redox-active thiols, which are abundantly present in plasma, can incorporate NO and transport it throughout the circulation in the form of bioactive *S*-nitrosothiol adducts (14). The second major compartment for NO metabolism in blood is represented by the RBCs. NO is metabolized in the RBCs by direct interaction with Hb entrapped within it. The consumption of NO by intraerthyrocytic Hb is, however, slowed to 1/1,000 the rate of consumption when Hb is free in solution. This was found to be primarily due to the existence of several physical barriers, including an RBC-free zone adjacent to the vessel wall in flowing blood, which provides a space that separates the RBC from the NO source (endothelial cells) (15).

A reaction between NO and RBCs involving *S*-nitrosylation of cysteine-93 of the β-chain of Hb has recently been suggested as a source of bioactive NO and a crucial component of the cardiorespiratory cycle (12). The so-called SNO-Hb (*S*-nitrosylated Hb), once formed in lungs, can then deliver NO in an allosteric-dependent manner to promote vasodilation of hypoxic tissues. Besides O_2 and CO_2 gases that are traditionally transported by RBCs, NO transport by RBCs has been advocated to occur as part of the normal physiology of blood. Moreover, it has been suggested that the well documented vasoconstrictive/hypertensive side effects of cell-free Hbs, due in large part to the removal of NO by Hb, can be attenuated through the nitrosylation of the free β93Cys, which can replenish the diminished blood levels of NO (10). Blood substitute developers, eager to resolve the vasoactivity problems associated with almost all Hb-based products, were not persuaded by the applicability of modified cell-free SNO-Hb, "he giveth, he taketh away," and opted instead for the removal of smaller molecular weight species believed to be partly responsible for the vasoactivity. The size of the Hb tetramer may be increased by chemical conjugation with other molecules, chemical polymerization, or encapsulation of cell-free Hb within the lipid bilayer, which represent common methods thought to reduce the NO/Hb interaction (20). Recent observations from several laboratories has cast some doubts about the allosteric regulation of blood flow by Hb, and instead a much clearer appreciation of the redox properties of Hb and

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its complex interaction with NO has emerged (8, 13), including possibly the involvement of nitrite reduction to NO by deoxyHb in the vasodilation of human circulation (17). Previous spectral changes suggestive of heme redox reactions (10, 12) may actually be, as recent electron paramagnetic resonance evidence suggests, due in part to changes in the NO-heme geometry (7).

The interplay between NO and Hb, free or inside RBCs, therefore presented and continues to present a unique opportunity for researchers to explore the dynamics and physiological consequences of such reactions on both signaling and oxidative inflammatory events in human physiology. With this in mind, the current forum, *Redox Biology of Blood*, focuses on several aspects of signaling in blood with some exciting insights into the role of Hb in its redox communications with the vasculature.

Yeh and Alayash (22) present data to show that cell-free Hb modulates key cell-signaling pathways by competing with biological peroxides that are required for the deactivation of the hypoxia-inducible factor, a transcriptional factor in endothelial cells subjected to hypoxia. These changes were shown to be more dependent on the protein redox rather than oxygen-carrying state. Reeder *et al*. (19) review the fundamentals of the radical and redox mechanisms of Hb and myoglobin "respiratory proteins" when these proteins are isolated from their normal reductant/antioxidant-rich environment with special emphasis on the prooxidant and pseudoperoxidase activity of these proteins under pathological conditions. Nagababu and Rifkind (16) remind us that heme, which is central to the hemoprotein functions in oxygen sensing, electron transport, signal transduction, and antioxidant defense mechanisms, is metabolically controlled by several enzymatic machineries. However, in the RBC, where the largest pool of heme proteins exists, no enzymatic degradative processes, such those outside RBCs, are operating. They showed that nonenzymatic heme degradation mechanisms are initiated within the red cell by the heme iron itself when it undergoes redox reactions in the presence of oxygen-producing reactive oxygen species. Bonaventura *et al*. (3) critically analyze the controversial "redox" and "allosteric" aspects of the physiological consequences of interactions of NO and Hb. The authors acknowledged that redox changes in the heme groups affect reactivity of the thiol groups on the protein and vice versa. In spite of the low levels of NO-Hb and SNO-Hb, these authors do not rule out participation of these species in NO-dependent signaling mechanisms. Crawford *et al*. (5) present a global role for the RBC in physiological mechanisms that may be controlling circulatory hemodynamics. They present current concepts surrounding this issue by addressing the question "How does the RBC control vascular function?" Buehler and Alayash (4) build on this concept and provide intriguing mechanisms by which RBCs can directly or indirectly communicate via redox intermediates with extravascular sites as part of a global oxygen-sensing mechanism. Tsai *et al*. (21) describe oxygen distribution and respiration by the microcirculation and provide data to show that tissue oxygenation requires the presence of oxygen delivery capacity and the control of oxygen consumption by the microvasculature. Baldwin (2) shows how, in microcirculation, this delicate balance can be disturbed when cell-free Hb, used as a "blood substitute" through heme-mediated redox reactions, can have far reaching consequences on both circulation and the integrity of the vascular system.

The editor hopes that this forum will serve as an up-to-date source of information on this emerging and exciting area of blood biology and the underlying redox chemistry. It is hoped that more research will be directed toward the physiological ramifications of these divers redox and signaling reactions, which may ultimately contribute to a better understanding of blood physiology. The editor would like to thank the contributing authors for their excellent contribution to this special forum.

ABBREVIATIONS

Hb, hemoglobin; NO, nitric oxide; RBC, red blood cell; SNO-Hb, *S*-nitrosylated Hb.

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