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Commentary

Fanning the Fire: Can Methemoglobin Enhance Neutrophil Activation?

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Sepsis is a common, costly and lethal condition with high mortality and substantial long-term disability in survivors. With high rates of sepsis at the extremes of age (premature infants and the elderly), growth of both of these populations will lead to an increase in sepsis burden in years to come. Development of targeted sepsis therapies, of which there are currently none, is a priority. In recent years, our group and others have described high levels of circulating cell-free hemoglobin (Hb) in the majority of patient with sepsis (Janz et al., 2013; Adamzik et al., 2012) and have shown that higher levels are associated with poor clinical outcomes. Pre-clinical studies have begun to dissect the molecular mechanisms of the damaging effects of cell-free Hb in sepsis, most of which have focused on the endothelium (Lisk et al., 2013) or macrophages (Lin et al., 2010) and not on neutrophils. In this issue of the journal, the study by Lee et al. (2015) brings us one step closer to understanding the pathophysiologic role of circulating cell-free Hb in sepsis. The authors show that oxidized Hb, specifically methemoglobin (metHb), is a damage-associated molecular pattern (DAMP) that signals through the Toll-like receptor (TLR) 2/NF-κB pathway to generate neutrophil reactive oxygen species, induce neutrophil apoptosis and increase expression of proinflammatory cytokines. Importantly, the effects of metHb were synergistic with lipoteichoic acid (LTA), a cell wall component of *Staphylococcus aureus* that also signals through TLR2. The effects of metHb in neutrophils were attenuated in the presence of other immune cells indicating modulation of metHb effects in a complex immune environment.

These findings are important for several reasons. First, the identification of TLR2 as a receptor for cell-free Hb is novel, although it must be noted that the authors did not make direct measurements of metHb-TLR2 binding so this conclusion is inferred. Second, the observation that LTA modulates the effects of metHb highlights the need to consider how interactions of Hb with bacterial products may affect the immune system during sepsis. Third, although the group focused on the interaction between metHb and LTA, the fact that metHb alone affects neutrophil function and survival has broad implications for sepsis in general, not just sepsis caused by *S. aureus*. Finally, the demonstration that metHb acts as a pseudoperoxidase, although not a new finding, has important therapeutic implications. In a highly pro-oxidant environment, such as sepsis, ferrous (2+) Hb can become oxidized to met (3+) and ferryl (4+) Hb through reaction with H_2O_2. Acetaminophen (APAP) is a specific hemoprotein reductant that can reduce ferryl Hb to metHb by virtue of the pseudoperoxidase activity of ferryl Hb (Boutaud et al., 2010). Whether APAP could further reduce metHb to unoxidized ferrous Hb is unknown but theoretically possible based on the pseudoperoxidase activity of metHb. Supporting the concept of APAP as a cell-free Hb targeted therapy, we recently completed a pilot randomized, placebo controlled trial of APAP in severe sepsis. Subjects treated with APAP had improved renal function and lower levels of oxidative injury compared to placebo after 3 days of treatment (Janz et al., 2014). The current study by Lee adds further biologic rationale for targeting cell-free Hb in patients with severe sepsis and suggests that such a therapy could have added benefit in sepsis caused by *S. aureus*. Finally, these interesting findings in neutrophils add to the growing body of literature showing cell-specific effects of metHb. MetHb induces proinflammatory pathways in both lung epithelial cells (Mumby et al., 2014) and astrocytes (Gram et al., 2013) but not in vascular endothelial cells (Silva et al., 2009). These distinct, cell specific responses to metHb highlight the need to study the independent effects of metHb in multiple clinically relevant cell types.

The study by Lee has some limitations that should be commented on. First, all of the effects of metHb on neutrophils were studied in vitro; whether they are biologically relevant in vivo is unknown. Second, the studies were limited to metHb, so the effects of non-oxidized Hb or ferryl Hb on neutrophil function are unknown. Along these lines, although there is ample evidence that non-oxidized Hb is elevated in sepsis, direct evidence that oxidized (met or ferryl) Hb is elevated in plasma in patients with sepsis is surprisingly lacking. Detailed analysis of the extent of oxidation of cell-free Hb in sepsis is needed to guide future mechanistic studies. Third, the authors show that the effects of metHb are only partially mediated by TLR2, which begs the question: What other mechanisms mediate metHb effects? Potential mechanisms include other cell surface receptors such as TLR4 which mediates the effects of cell-free Hb in endothelial cells (Lisk et al., 2013) and macrophages (Lin et al., 2010) as well as non-receptor mediated effects through generation of lipid peroxidation products such as isoprostanes that are known to be associated with poor clinical outcomes in sepsis (Janz et al., 2014). Future studies will be needed to uncover these alternate mechanisms and test their relevance in vivo.

Disclosure

The authors have no relevant conflicts of interest to disclose.

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