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Sepsis:

Redox Mechanisms and Therapeutic Opportunities

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DESPITE ADVANCEMENTS IN THE treatment of critically ill patients, sepsis remains the leading cause of death in the ICU—an estimated 175,000 deaths per year (2). The incidence of sepsis in the United States ranges from 400,000 to 750,000 cases per year. Mortality due to sepsis is around 30% and increases with age from 10% in children to 40% in the elderly; mortality is 50% or greater in patients with the more severe syndrome, septic shock, and a recent study reported a 70% increase in the number of severe sepsis cases from 1993 to 2003 (2,5).

Sepsis is a complex syndrome characterized by hyperinflammation, oxidative damage, hypercoagulation, tissue hypoperfusion and hypoxia, immune suppression, as well as multiorgan dysfunction. The pathogenesis of sepsis involves cumulative dysfunction of immune cells (macrophages, neutrophils, and lymphocytes), endothelial cells, and epithelial cells. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) significantly contribute to the dysfunction of these cells during sepsis.

ROS/RNS play a key role in physiologic cellular functions, including signal transduction involved in expression of several cytokines, growth factors, and hormones. Additionally, ROS produced by the NADPH oxidase complex during phagocytosis is essential for microbicidal activity. However, excess production of ROS/RNS represents a key element in the cascade of deleterious processes in sepsis. Recent studies indicated that ROS can affect the pathogenesis of sepsis by two mechanisms: (a) modulating the innate immune signaling cascade, and (b) causing pathologic damage to cells and organs (1,4,6,11,13,16). ROS can alter LPS-TLR4 signaling at multiple levels and prime the innate immune cell for increased responsiveness to subsequent stimuli (14,17). ROS, such as superoxide and hydrogen peroxide, enhance NF- κ B activation (3). More recently, ROS were shown to mediate trafficking of the TLR4 receptor to lipid rafts (16,17). Oxidative stress generated during hemorrhagic shock caused increase translocation of TLR4 receptor to the lipid rafts in the plasma membrane of macrophages that increased responsiveness to subsequent stimuli (17). Choi's group (16) also showed that carbon monoxide generated from heme oxygenase inhibits LPS-induced translocation of TLR4 to lipid rafts, as well as its downstream signaling adapter molecules (MYD88, TRIF, TRAF6, IRAK) through suppression of NADPH oxidase-dependent ROS generation (16).

Superoxide anion and peroxynitrite play key roles in the pathogenesis of hemodynamic instability and organ dysfunction during septic shock. A growing body of evidence relates neutrophil dysfunction with the severity of sepsis and is linked with end-organ failure and mortality (8). Excessive release of proinflammatory mediators, ROS, and proteases by activated neutrophils exacerbates sepsis by increasing inflammation, oxidative tissue damage,

vascular permeability, and organ injury (8). Interestingly, depletion of neutrophils after CLP in mice model has been shown significantly to reduce bacteremia, reduce liver and renal dysfunction, as well as decrease serum levels of proinflammatory cytokines, but the timing of neutrophil depletion was important to achieve these effects (9). Several clinical studies have demonstrated low antioxidants and elevated levels of oxidative stress markers, such as lipid hydroperoxides and plasma nitrite, in septic patients (6,15). Recently, Kaufmann *et al.* (12) reported neutrophil dysfunction in patients with severe sepsis. Neutrophils from patients with severe sepsis exhibited compromised phagocytic function; however, they produced higher amounts of ROS on activation by soluble stimuli (such as fMLP, TNF- α , and TPA) compared with healthy subjects (12). ROS/RNS can cause DNA-strand breakage, triggering the activation of poly(ADP-ribose) polymerase (PARP). PARP plays a role in the repair of strand breaks in DNA, and its activation results in a substantial depletion of nicotinamide adenine dinucleotide, thus leading to cell dysfunction. This field is beginning to unfold, and better understanding of molecular mechanisms will help in developing novel therapeutic intervention to improve survival in sepsis.

Nitron spin traps to catch oxygen free radicals have been used for measuring oxygen free radical generation by using electron spin-resonance spectrometry. Tawadros *et al.* (19) exploit this potential and report that stilbazulenyl nitron (STAZN), a novel second-generation azulenyl nitron, protects from lung injury in a model of hemorrhagic shock/resuscitation and subsequent intratracheal LPS treatment. STAZN confers protection by attenuating NF κ B activation and secretion of proinflammatory cytokines by decreasing oxidative stress.

MCP-1 is a chemoattractant that is secreted by monocytes, fibroblasts, vascular endothelial cells, and smooth muscle cells, and attracts monocytes, T lymphocytes, natural killer cells, and neutrophils to the site of infection/inflammation. MCP-1 has been implicated in ARDS-associated respiratory failure. The article by Xing and Remick (23) focuses on the role of ROS as secondary messengers in the secretion of chemokine, MCP-1. The authors demonstrate by promoter analysis that the antioxidant DMSO inhibited cytokine-stimulated MCP-1 production at the transcriptional level through suppressing NF- κ B by decreasing ROS generation.

Nrf2 is a key transcription factor that regulates antioxidant defense in the host. Disruption of Nrf2 has been shown to enhance sensitivity to both endotoxin-induced shock and cecal ligation and puncture (CLP) of murine sepsis in a study by Thimmulappa *et al.* (21). More recently, the activation of the Nrf2 pathway by CDDO-Im [imidazole derivative of 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid] attenuates LPS-induced ROS generation and protects from the exaggerated expression of proinflammatory mediators in macrophages and neutrophils, as well as mortality in the mouse model (22). In the current study (20), the authors used neutrophils and peripheral blood mononuclear cells (PBMCs) isolated from normal subjects as surrogate cells to demonstrate the efficacy of CDDO-Im and CDDO-Me [methyl ester derivative of 2-cyano-3,12-di-oxooleana-1,9(11)-dien-28-oic acid (CDDO)] activate the Nrf2 pathway and protect from lipopolysaccharide (LPS)-induced inflammatory response in humans. The data demonstrate the potency of CDDO-Im to upregulate a network of Nrf2-dependent antioxidative genes in neutrophils and PBMCs and suppress LPS-induced ROS generation and cytokine expression. Thus, targeting host factors such as Nrf2 that upregulate antioxidant defense pathways may provide a novel strategy for intervening sepsis.

Carbon monoxide (CO) is emerging as a therapy for sepsis. The review by Hoetzel *et al.* (10) presents an in-depth overview of the beneficial effects of CO in systemic inflammation and sepsis, with an emphasis on animal studies with clinical relevance. The authors comprehensively discuss the endogenous sources of CO and the protective effects of CO on septic organs: lungs, liver, heart, kidney, and gut. The antioxidant, antiinflammatory, and

antiapoptotic effects of CO, the mechanism of action of CO, and finally prospective therapeutic studies with CO in human sepsis also are presented.

Mechanical ventilation (MV) and oxygen supplementation remain cornerstones of therapy for the treatment of critically ill patients with severe lung injury associated with sepsis. Reddy *et al.* (18) review the role of oxidative stress in the pathogenic mechanisms underlying ventilator-induced lung injury (VILI). In this review, the authors extensively discuss the potential mechanism of ROS/RNS generation by cyclic stretch associated with mechanical ventilation and the involvement of antioxidant defense systems regulating VILI. In addition, a perspective on antioxidant therapy for acute lung injury is discussed.

Finally, Guo and Ward (7) provide a comprehensive review on the role of ROS/RNS in sepsis-induced lung injury. The authors describe the cellular source of ROS and RNS, mechanisms of ROS/RNS-mediated tissue damage, markers of oxidative/nitrosative stress, molecular regulators of oxidative and nitrosative stress and inflammation, and finally, prospective therapeutic strategies directed at the improvement of net proinflammatory, prooxidant, and cytotoxic imbalances that develop in ARDS.

In summary, this forum focuses on the redox regulation of inflammatory mediators, oxidative/nitrosative pathogenic mechanisms, and molecular targets for reducing oxidative/inflammatory stress that play an important role in pathophysiology of sepsis. We are grateful to the Editor of *Antioxidants and Redox Signaling* for the opportunity to organize this forum and the enjoyable experience of interacting with several groups actively working in the field of oxidative stress in sepsis. We hope that this forum will help in stimulating ideas to develop creatively novel antioxidant strategies for the therapy of sepsis.

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