Oxygen Radicals – an Important Mediator of Sepsis and Septic Shock*

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Summary. There is considerable evidence to implicate aggressive species of oxygen in the pathogenesis of organ dysfunction consequent to sepsis and septic shock. The inflammatory process appears to participate ubiquitously in this setting. A characteristic of inflammation is the involvement of activated neutrophils and their generation of aggressive oxygen species. Such species may both directly injure cells proximal to the oxidant generating cells, and may inactivate any proteolytic mechanisms normally protective against proteolytic injury caused by neutrophil elastase and other proteolytic enzymes released during inflammation. The offending agent in sepsis is most commonly envisioned as bacterial lipopolysaccharide, or endotoxin. Infusion of endotoxin into animals can reproduce much of the pathophysiology of sepsis and septic shock. In addition, administration of endotoxin to cultured cells, particularly endothelial cells, can cause responses consistent with a sequence of events that occurs in intact animals and humans. In both experimental models, it appears that aggressive oxygen species are important actors in the scenario eventuating in cell or organ injury. Of importance, the toxic consequences of these free radicals probably occurs in relatively protected spaces, including microenvironments created by close adherence between inflammatory cells and endothelial cells and the cell interior. For those reasons, the potential for antioxidants as therapy should include consideration of the volume of distribution of such substances. It is probably important that antioxidants access excluded spaces including cell interiors in order to have their maximum effect in this setting. We have studied in a preliminary way the effects of n-acetyl-cysteine, a highly permeable free radical scavenger and antioxidant, in patients with established ARDS. The preliminary data suggest that this antioxidant may indeed be beneficial and a larger prospective study is underway. Novel, new therapies are on the horizon. The explosion of knowledge concerning the manipulation of DNA and the technologies for delivering functioning foreign genes to intact organisms promise completely new categories of therapies which may be applicable in sepsis and septic shock as well as the acute and subacute clinical situations. Eventual realization of the potential of such therapy must await additional basic and clinical investigation.

Key words: Oxygen radicals – Sepsis – Shock – ARDS

The past several years have witnessed a burgeoning of research related to aggressive species of oxygen as mediators of pathology. As understanding of the pathogenesis of the consequences of sepsis has accumulated, the notion that oxidative injury occurs and that aggressive oxygen species may be pivotal in the pathogenetic sequence has emerged. A broad spectrum of basic and clinical research suggests that mammalian systems have evolved elaborate processes for controlling redox state in cells, and that loss of control of this system is pathologic. Abundant evidence associates oxida-

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Abbreviations: LPS = lipopolysaccharide; DMSD = dimethyl sulfoxide; ARDS = acute respiratory distress syndrome; DNA = deoxyribonucleic acid

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tive injury of cells and organs with sepsis and septic shock. That fact creates a rationale for therapy although the precise role of oxidative injury in the syndromes accompanying sepsis may not yet be clear. In this chapter I will review some of the evidence suggesting that toxic oxidants generated as a consequence of the inflammatory response, was well as oxidants originating from other than inflammatory cells, may be important in the pathogenesis of injury consequent to sepsis and may provide a rationale for therapy.

**Inflammation and Sepsis**

It is certainly clear that in virtually every setting and virtually every organ in the body in which septic injury occurs, inflammation is an associated phenomenon. By inflammation I mean the influx of inflammatory cells and the triggering of the complex network of humoral and cellular mediators designed to protect the body from foreign invaders.

Much of the work describing mechanisms of injury consequent to sepsis has focused on the neutrophil. The polymorphonuclear neutrophil is the first inflammatory cell to respond to a foreign insult. That appears to be true in sepsis as well as in more localized infections. That process involves intimate association between the neutrophil and microvascular endothelial cells, activation of the neutrophil with release of proteolytic enzymes and generation of superoxide, and migration of neutrophils beyond the microvascular bed into the interstitial spaces. One way to think of sepsis is as a diffuse stimulus for the inflammatory response (as opposed to a localized response).

Presumably the toxic potential of activated neutrophils was designed to be aimed at bacteria or other foreign organisms. It appears in sepsis induced injury that these mechanisms are misdirected toward the body's own cells. The close adherence of neutrophils to microvascular endothelial cells creates a micro environment between the adherent cells where aggressive oxygen species generated by the activated neutrophil may injure endothelial cells. This injury would presumably occur only very near to the activated inflammatory cells. Because of the very short half life and therefore penetrability of the toxic oxygen species, these mediators would not explain injury remote from the generating system.

In addition to direct injury of parenchymal cells, aggressive oxygen species generated by inflammatory cells may also indirectly potentiate injury by inhibiting the normal antiproteolytic systems in the body. For example, the principal neutrophil elastase inhibitor in the body is alpha 1-antitrypsin. Alpha 1-antitrypsin can be oxidized to an inactive form by aggressive oxygen species generated by the inflammatory process. If proteolytic injury (neutrophil elastase) is a contributor to the pathogenesis of sepsis induced injury, then the proteolytic injury might well be exaggerated by the effects of free radicals to inhibit antiproteolytic proteins.

**Bacterial Lipopolysaccharide (LPS) as the Villain**

At least one, and perhaps the principal, stimulus to the complex set of responses which occurs in sepsis and septic shock appears to be the lipopolysaccharide component of cell membranes of gram negative bacteria usually called endotoxin. Much of the basic and clinical research aimed at defining the pathogenesis and identifying potential therapies for sepsis and septic shock has focused upon mechanisms triggered by endotoxin.

When either whole gram negative bacteria or bacterial endotoxin is infused into experimental animals, much of the pathophysiology of the sepsis syndrome in humans can be reproduced. This includes multi-organ injury, a generalized inflammatory response, and release of many of the mediators which are also present in humans with sepsis syndrome. Substantial evidence implicates oxygen radicals in the pathogenesis of the response to endotoxin and animal models. Products of lipid membrane peroxidation can be identified in body fluids following endotoxin infusion. Indirect measures of generation of aggressive oxygen species demonstrate such a process in animal models. The administration of some antioxidants and free radical scavengers can alter responses to endotoxin in intact animal preparations.

We have focused upon mechanisms by which gram negative bacterial endotoxins injure endothelial cells both in vivo and in culture. Electron microscopic studies of the sequence of events in the lung microcirculation following infusion of endotoxin in sheep implicates microvascular endothelium as an early target of activated neutrophils. Neutrophils adhere to endothelial cells and degranulate, and temporally coincident with these events there is ultrastructural evidence of injury to the microvascular endothelial cells. Timing of this response is consistent with physiologic evidence of microvascular injury.

Administration of antioxidants, particularly...