**Oxidative Stress and Endothelial Dysfunction during Sepsis**

O. Huet, A. Harrois, and J. Duranteau

**Introduction**

The endothelium is an active tissue that plays a pivotal role in maintaining cardiovascular homeostasis. The endothelium ensures the quality of both the global and microcirculation. It forms an interface between blood and tissues. The human body contains approximately $10^{13}$ endothelial cells, an area of 4000 to 7000 m². This size is one of the reasons why endothelium must be considered an organ. Physiological functions of endothelial cells are: 1) to control vascular tone and blood flow by a local balance between vasodilators (paracrine release of diffusible vasodilator mediators, such as nitric oxide [NO], prostacyclin) and vasopressors (endothelin-1 [ET-1]); 2) to keep blood in a fluid state by preventing thrombosis; 3) to control the exchange of fluid and macromolecules between the blood and the tissues; and 4) to control the local balance between pro- and anti-inflammatory mediators.

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) have several potentially important effects on endothelial function and are implicated both in physiological regulation and in disease pathophysiology [1–3]. The effects of ROS on endothelial cells are dependent on the amount and the sites of production of ROS, but also on the processes that degrade or scavenge ROS. The imbalance between the production of ROS and their effective removal by non-enzymatic and enzymatic antioxidant systems could induce endothelial dysfunction with alteration of vascular tone, increase in cell adhesion properties (leukocyte and platelet adhesion), increase in vascular wall permeability and a pro-coagulant state.

Endothelial dysfunction appears to be critical during septic shock, and its activation is involved in microcirculatory impairment and organ dysfunction. During sepsis, the endothelium becomes both a target and a source of ROS and RNS. Exogenous sources of ROS and RNS are mainly phagocytes; however, endothelial cells can also generate ROS and RNS which may help to initiate and perpetuate the development of a systemic inflammatory response and subsequently cause organ dysfunction.

**Sources and Actions of ROS and RNS in the Endothelium during Sepsis**

The endothelium represents both a source and a target for ROS released in the vasculature in sepsis, although other cells in the vessel wall as well as the inflammatory cells also play important roles. During sepsis, stimulated inflammatory cells, such as neutrophils and macrophages, produce large amounts of ROS and RNS [4, 5]. This production of ROS and RNS is a crucial mechanism for neutrophils and macrophages to damage or kill microorganisms and contributes to part of the host defense
against bacterial spread. Oxygen burst can also cause cell damage, and the endothelium is one of the first targets of ROS.

During sepsis, a large number of components (e.g., pro- and anti-inflammatory cytokine balance, degree of leukocyte activation, oscillatory shear stress) and conditions (hypoxia, reperfusion injury) are responsible for endothelial superoxide (O$_2$·-) production. Endothelial sources of O$_2$·- that are implicated in endothelial dysfunction include mitochondria, xanthine oxidase (XO), uncoupled NO synthases (NOS), cytochrome P450 enzymes, and NADPH oxidases. In addition, enzymes such as lipoxygenases may also generate O$_2$·-. Increasing evidence supports the idea that ROS generated from mitochondria contribute significantly to endothelial cell dysfunction. The mitochondrial respiratory chain can be a major source of O$_2$·- [6, 7]. During oxidative phosphorylation, 1–4 % of oxygen may be incompletely reduced in the mitochondrial respiratory chain, resulting in O$_2$·- formation, mainly at complex I (NADH coenzyme Q reductase) and complex III (ubiquinol Cyt c reductase) of the mitochondrial respiratory chain. Increased mitochondrial O$_2$·- generation appears to be particularly prominent in situations of metabolic perturbation. For example, hyperglycemia, hypoxia, and ischemia/reperfusion induce O$_2$·- production. Moreover, pro-inflammatory cytokines (tumor necrosis factor [TNF]-α) may directly induce mitochondrial O$_2$·- production [8].

Our group has tested the capacity of the plasma of patients in septic shock as a whole to induce ROS production in naïve human umbilical vein endothelial cells (HUVEC) [9]. For this purpose, we used a fluorescence technique which has been widely used by our group and others to quantify ROS production in HUVEC. We found that plasma from patients treated for septic shock induced ROS formation in naive HUVEC, and that the extent of ROS production was higher in non-survivors than in survivors and was correlated with mortality and with criteria of the severity of septic shock as assessed by the sequential organ failure assessment (SOFA) score and simplified acute physiology score (SAPS) II (Fig. 1) [9]. This experiment therefore demonstrated the ability of the plasma to induce ROS production independent of any direct effect of sepsis mediated by circulating cells on the endothelial cells. This observation may be of clinical relevance because during septic shock, infection and

![Fig. 1. Patients in septic shock. Changes in reactive oxygen species (2'7'DCFH levels) are significantly higher at Day 1, Day 3, and Day 5 in non-survivors than in survivors (ANOVA, p = .0015). Values are means ± SD. From [9] with permission.](image-url)