The Respiratory Burst and Endothelial Cells

ALDO DOBRINA and PIERLUIGI PATRIARCA

1. INTRODUCTION

Neutrophils are the first-line defense of the host against foreign invaders once they have reached the tissues. When at work, however, the neutrophils behave as a double-edged sword. In fact, the reactive oxygen intermediates (ROI) generated by the respiratory burst and the granular components released during degranulation contribute to killing of ingested microorganisms on one side, but, on the other they may damage tissues in both their cellular and extracellular components. Several cell types, including the neutrophils themselves, may be the target of ROI produced by the neutrophil respiratory burst. Endothelial cells, a crucial cell type in the interface phenomena between blood and tissues, are among those targets. This chapter first reviews evidence, from both in vitro and in vivo studies, of the involvement of the neutrophil respiratory burst in endothelial cell damage, followed by a discussion of the possible role of such a damage in human pathology.

2. EFFECT OF ACTIVATED NEUTROPHILS ON ENDOTHELIAL CELLS

2.1. In Vitro Studies

The experimental model generally employed is based on the use of monolayers of cultured endothelial cells exposed to neutrophils stimulated by different agents. Endothelial cell injury is then evaluated by several methods, including release of $^{51}$Cr or $^{111}$In or other radioactive tracers, from prelabeled cells, as well as detachment of endothelial cells from the culture dish.
Sacks et al. first reported that neutrophilic leukocytes (PMN) stimulated by either zymosan-activated serum or purified C5a induced a small but statistically significant release of \(^{51}\text{Cr}\) from subconfluent cultures of endothelial cells derived from human umbilical vein. \(^{51}\text{Cr}\) release was abolished by pretreatment of PMN with cytochalasin B, a fungal metabolite that prevents granulocyte spreading and adherence to endothelial cells, suggesting that a close contact between target and effector cells was necessary for injury to take place. Neutrophils from a patient with chronic granulomatous disease (CGD), which are unable to respond with a respiratory burst to stimulation, were 50% less effective in producing endothelial cell injury than normal granulocytes, suggesting that at least two mechanisms underlie injury: one dependent on ROI generated by the respiratory burst and another that is oxygen independent. The role of oxygen metabolites as injurious agents was further supported by inhibition of \(^{51}\text{Cr}\) release by addition of catalase to the incubation medium, whereas addition of superoxide dismutase (SOD) alone afforded variable and inconstant protection. These data also suggest that \(\text{H}_2\text{O}_2\), but not \(\cdot\text{O}_2\), was the most destructive species in that particular study.

The occurrence of catalase-inhibitable injury to endothelial cells by stimulated PMN has been subsequently confirmed using other stimuli such as opsonized zymosan, endotoxin, or its lipid A moiety and phorbol myristate acetate (PMA). It was also shown that the severity of endothelial injury correlated with the amount of \(\text{H}_2\text{O}_2\) produced by stimulated PMN. \(\text{H}_2\text{O}_2\) seems to act as the toxic agent per se rather than in combination with myeloperoxidase (MPO) and an oxidizable substrate, as it occurs in the well-known microbicidal and cytotoxic MPO–\(\text{H}_2\text{O}_2\)–halide system. This conclusion is based on several observations: (1) there was no correlation between release of MPO by PMN and extent of injury; (2) MPO-deficient neutrophils were as active as normal neutrophils in mediating cell damage; (3) scavengers of hypochlorous acid, the final mediator of the cytotoxic effects produced by the MPO–\(\text{H}_2\text{O}_2\)–halide system, did not inhibit PMN-induced damage to endothelial cells, and (4) MPO inhibitors were likewise ineffective in protecting endothelial cells from PMN-mediated injury.

What emerges from these studies is that lytic injury of endothelial cells caused by the metabolic burst of neutrophils is attributable mostly to \(\text{H}_2\text{O}_2\). The capacity of \(\text{H}_2\text{O}_2\) to lyse endothelial cells has been also confirmed using either reagent-grade \(\text{H}_2\text{O}_2\) or enzymatically generated \(\text{H}_2\text{O}_2\) Some workers have also attributed a role in endothelial cell injury to the hydroxyl radical (OH•), on the basis of protection against damage by PMA-stimulated PMN afforded by hydroxyl radical scavengers such as mannitol and dimethylsulfoxide, and the iron chelator deferoxamine, which inhibits the (OH•)-generating reactions. In some studies, however, the ability of PMN to cause endothelial cell lysis could not be demonstrated using several stimuli including PMA. It should be pointed out, however, that in four of those studies, no control is available on the degree of PMN activation, since the amount of \(\text{H}_2\text{O}_2\) or \(\cdot\text{O}_2\) produced by neutrophils was not indicated, while in the fifth study, inhibition of \(\cdot\text{O}_2\) production in PMA-stimulated neutrophils by endothelial cells has been demonstrated. Further-