Biological Oxidants: Generation and Injurious Consequences

EDITED BY

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Cellular and Molecular Mechanisms of Inflammation

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Preface

One of the major effector systems participating in a variety of human diseases appears to be that involving the generation of oxidant free radicals. In numerous forms of inflammation, the development of genetic mutations and malignant transformations, the generation of atherosclerotic plaques in arterial walls, and the process of aging all have in common the presence and participation of oxidant free radicals.

The past few years have witnessed a surge of information on the molecular events involved in the process of oxidant generation and its consequences in cells and tissues. In particular, knowledge of the molecules of the intracellular and plasma membrane generating system in leukocytes and other cells, the intracellular events involved in oxidant-induced cellular injury, mutational events and malignant transformation, and novel intracellular mechanisms of oxidant formation itself have taken a giant leap.

We have therefore thought it of value to bring together some of these important developments with selections from some of the most active and highly respected laboratories contributing to the new knowledge. In this volume, the reader will be exposed to the latest information from which a clear perception can be obtained of the fundamental bases of the several pathological processes mentioned above.

The information presented in this volume also contains the seeds of potential novel therapeutics that may be applied to oxidant systems. An understanding of the intracellular pathways responsible for the generation of oxidants, the formation of abnormal bases in the DNA, and the pathways leading to cell injury will be rewarding, if not essential, in the future development of specific therapies.

Under any circumstances, we are certain that the information in this volume will be of great interest to the reader.

Charles G. Cochrane
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CHAPTER 1

Composition and Function of the NADPH Oxidase of Phagocytic Cells with Particular Reference to Redox Components Located within the Plasma Membrane

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I. Introduction

Electron transport chains provide the biochemical basis for photosynthesis and respiration, and drug metabolism by the mixed-function oxidases. Oxidoreductases in the plasma membrane appear to be important for a wide range of cellular functions including the control of cell division,

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transmembrane signaling, regulation of intracellular pH, reduction of extracellular oxidants, and many others, in both plant and animal cells (1).

The NADPH oxidase present in "professional" phagocytic cells, neutrophils, monocytes, macrophages, and eosinophils, which generates superoxide and hydrogen peroxide in the endocytic vacuole, is the best characterized of the plasma membrane oxidoreductases. The "extra respiration of phagocytosis" produced by this NADPH oxidase was first observed in 1933 (2). The unusual nature of the process was only revealed in 1959 when it was discovered that it was not inhibited by classic mitochondrial poisons like cyanide and azide (3), indicating that it was not simply a reflection of the enhanced energy requirements of phagocytosis. The obvious importance of the oxidase for the efficient killing of ingested microbes is best demonstrated by the consequences of its absence in the syndrome of chronic granulomatous disease (CGD), which is characterized by a profound, often fatal, predisposition to infection associated with widespread granuloma formation (4).

The mechanism by which the products of the oxidase optimize killing have yet to be fully elucidated. They might have a direct microbicidal effect or produce substrate for myeloperoxidase-mediated halogenation. However, they also elevate the phagosomal pH by pumping electrons, unaccompanied by protons, across the vacuolar membrane, an alkalinization that is important for optimal digestion by neutral proteinases and other enzymes (5).

An extensive series of investigations have been undertaken in an attempt to define the nature of this oxidase and the molecular basis of CGD. This chapter complements recent reviews on the subject (6–9) and will outline our current understanding of the structure and function of this oxidase. Emphasis will be placed on those components that are located within the plasma membrane and in particular on our recent discovery of a flavocytochrome *b* as the central electron-transporting molecule of this system.

II. Early Attempts to Identify the Oxidase System in Cells, Organelles, and Extracts

The earliest experiments attempted to purify the oxidase from whole-cell homogenates and crude subcellular fractions. These were incubated with a variety of potential substrates in an attempt to demonstrate oxidase or

diaphorase activity (10). The dramatic increase in the activity of the hexose monophosphate shunt in association with the respiratory burst (11) indicated one of its products, NADPH (12), as the most physiological of these substrates. A variety of activities were detected, but the interpretation of these experiments was complicated by the lack of specificity of the oxidation of many of these substrates and the autocatalytic nature of many of the resulting reactions. A number of "enzymes" were discovered and described as defective in CGD (10).

The next advance in this approach came with the solubilization of an active oxidase from activated cells (13). Despite this achievement, complete purification of the source of the detectible activity was prevented by the instability of the system, particularly its sensitivity to salts, which prevented separations on most chromatographic media (14).

III. Identification of Cytochrome b_{-245} and Recognition of the Oxidase as an Electron Transport

In 1978 a cytochrome *b* was identified in human neutrophils (15), having been previously seen in animal cells (16). This discovery seemed significant as this was the sort of molecule that might be expected to accomplish the one-electron reduction of oxygen to form superoxide, and it pointed the way to the identification of the "NADPH oxidase" as an electron transport chain rather than a single enzyme. With few exceptions the cytochrome has been found by spectral analysis to be missing from cells of subjects having CGD with the commoner inheritance through a lesion on the X chromosome and normal in those with an autosomal recessive pattern (17–19).

In man this cytochrome was found in the "professional" phagocytic cells, neutrophils, monocytes, macrophages, and eosinophils, but not in a variety of other cell types (20). Its identity in these different cells was established by the determination of its midpoint redox potential ($Em_{7.0}$, see below). It is present in myeloid HL60 cells, a human promyelocytic cell line that can be induced to differentiate into cells resembling neutrophils, and in the U937 (21) monocyte/macrophage cell line. Uninduced cells are grossly deficient in all known specialized components of the oxidase, which accumulate on differentiation in association with the development of NADPH oxidase activity (22). In HL60 cells, the induction of differentiation with dimethyl sulfoxide was associated with the