Review

The superoxide-generating oxidase of phagocytic cells
Physiological, molecular and pathological aspects

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Professional phagocytes (neutrophils, eosinophils, monocytes and macrophages) possess an enzymatic complex, the NADPH oxidase, which is able to catalyze the one-electron reduction of molecular oxygen to superoxide, $O_2^-$. The NADPH oxidase is dormant in non-activated phagocytes. It is suddenly activated upon exposure of phagocytes to the appropriate stimuli and thereby contributes to the microbicidal activity of these cells. Oxidase activation in phagocytes involves the assembly, in the plasma membrane, of membrane-bound and cytosolic components of the oxidase complex, which were disassembled in the resting state. One of the membrane-bound components in resting phagocytes has been identified as a low-potential $b$-type cytochrome, a heterodimer composed of two subunits of 22-kDa and 91-kDa. The link between NADPH and cytochrome $b$ is probably a flavoprotein whose subcellular localization in resting phagocytes remains to be determined. Genetic defects in the cytochrome $b$ subunits and in the cytosolic factors have been shown to be the molecular basis of chronic granulomatous disease, a group of inherited disorders in the host defense, characterized by severe, recurrent bacterial and fungal infections in which phagocytic cells fail to generate $O_2^-$ upon stimulation. The present review is focused on recent data concerning the signaling pathway which leads to oxidase activation, including specific receptors, the production of second messengers, the organization of the oxidase complex and the molecular defects responsible for granulomatous disease.

Introduction

Professional phagocytes play a crucial role in host protection by combating infections. For this purpose, they are equipped with a molecular machinery which is able, upon activation, to generate toxic oxygen derivatives. Among phagocytic cells, neutrophils have attracted the most attention because of their efficient capacity to kill and digest bacteria. Compared to the long-lived macrophages, which reside in tissues and acquire their cytotoxic potency after some delay, circulating neutrophils are short-lived cells which are readily mobilized by exposure to a variety of stimuli. Formylmethionyl peptides are among the best-known stimuli. They are released from bacteria at the site of infection and diffuse into the blood vessels located in close proximity. There, at nanomolar concentrations, they bind to specific receptors distributed on the surface of the circulating neutrophils. This binding process governs the chemotactic response, which consists of a series of sequential events including the adhesion of the circulating neutrophils to the endothelium of neighboring capillaries, transport across the endothelium, migration along the formylpeptide gradient towards the infected tissue where the formylpeptide concentration is in the micromolar range, and finally engulfment of the bacteria by an endosomal vacuole. Neutrophil motility involves cytoskeletal activation, including actin polymerization. These different steps are orchestrated by a number of chemical signals which are released from the site of infection. For instance, certain inflammatory cytokines such as interleukin 1-$\alpha$ and tumor necrosis factor $\alpha$ (TNF-$\alpha$) stimulate the expression of several cellular adhesion molecules in the endothelial cells, such as the endothelial leukocyte adhesion molecule 1 (Bevilacqua et al., 1987). This facilitates the attachment of circulating neutrophils to the vascular endothelium, prior to emigration into the extravascular space.

Concomitantly with engulfment of bacteria, two important processes are brought into play. The first process, known as the respiratory burst, consists of a sudden and marked activation of oxidative metabolism, resulting in the production of reactive oxygen species, including superoxide anion radical and hydrogen peroxide, which further react to produce hydroxyl radical, nitric oxide and more toxic species. These oxygen radicals are intended to kill bacteria and fungi. For this purpose, they are involved in the killing of bacteria by the generation of toxic oxygen intermediates (in particular, superoxide anion radical) which in turn react with cytochrome $b$ to produce reactive oxygen species.

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production of O$_2^-$ (Halliwell and Gutteridge, 1989) and derived toxic species. The second process, called degranulation, corresponds to the release of the contents of cytoplasmic granules into the endosomal vacuole. The combination of these processes results in the killing and digestion of the engulfed bacteria and the development of an inflammatory response. Secondary recruitment of circulating neutrophils and, at a later stage, of monocytes, is triggered by a number of additional chemotactic factors released from the site of inflammation, including products of complement activation and lipid derivatives.

The respiratory burst is an event central to phagocytosis. The failure of phagocytes to mount a respiratory burst is the functional basis for chronic granulomatous disease (CGD), an inherited disease characterized by recurrent and severe infections and a granulomatous tissue reaction. The increase in O$_2$ consumption by neutrophils during the course of phagocytosis was first reported by Baldridge and Gerard (1933). The insensitivity of neutrophils to cyanide enabled the extra respiration, linked to phagocytosis, to be identified as different in nature from mitochondrial respiration (Sbarra and Karnovsky, 1959). The superoxide anion is the initial product of the respiratory burst (Babior et al., 1973). It is formed by the mono-electronic reduction of O$_2$, with NADPH as the specific electron donor (Rossi and Zatti, 1964), according to the overall reaction:

\[
\text{NADPH} + 2 \text{O}_2 \rightarrow \text{NADP}^+ + \text{H}^+ + 2 \text{O}_2^-.
\]

The active enzyme complex which catalyzes the generation of O$_2^-$ from O$_2$ is called the respiratory-burst oxidase or NADPH oxidase.

A continuous supply of NADPH in phagocytic cells is ensured by the hexose monophosphate shunt. The respiratory burst also generates hydrogen peroxide, a dismutation product of O$_2^-$. In neutrophils myeloperoxidase, released from azurophil granules during degranulation, catalyzes the reaction of hydrogen peroxide with the abundant chloride anions present in the cells, to generate hypochlorous acid, a potent anti-bacterial compound. A major reaction involving hypochlorous acid is the N-chlorination of nitrogen-containing compounds, yielding chloramines. Other reactive oxygen products occurring by secondary reactions include the OH$^-$ radical and oxygen singlet, O$_3$. The killing of bacteria depends on these oxygen metabolites and, more particularly, on hypochlorite and chloramines. However, the killing of bacteria also involves the participation of cationic proteins and defensins and their digestion is achieved with different hydrolyases, including proteinases, all of which belong to the antimicrobial arsenal of the phagocytic cell.

The production of O$_2^-$ and derived metabolites is a highly controlled process. Under adverse circumstances, these oxidizing agents may be released by phagocytes in an uncontrolled manner. The extensive release of O$_2^-$ derivatives is probably involved in the pathogenesis of rheumatoid arthritis, in tissue damage following ischemia, in stroke and myocardial infarction and in the respiratory distress syndrome.

Interest in the O$_2^-$ generating oxidase complex of phagocytic cells is reflected in the increasing number of papers which have been published on this subject. In this article, we shall focus on the more recent data in the literature with respect to the signal pathway controlling the respiratory burst, the nature of the components of the oxidase and its activation mechanism and specific pathological aspects relating to CGD.

The signaling pathway leading to oxidase activation

Most studies on NADPH oxidase and the mechanisms of its activation have been performed with neutrophils, which represent the most abundant cell type amongst the so-called professional phagocytes. The capacity to generate significant amounts of O$_2^-$, via a similar type of NADPH oxidase, has been observed with other professional phagocytes, namely eosinophils (Segal et al., 1981a; Petreccia et al., 1987), macrophages (Segal et al., 1981a) and monocytes (Segal et al., 1981a; Chaudry et al., 1982). Oxidase activity is also found in neutrophil-like cells obtained by differentiation of human leukemia cells (HL60 line) grown in the presence of dimethylsulfoxide, dimethylformamide, retinoic acid or N$^6$,2'-O-dibutyryl-adenosine 3',5'-phosphate (Collins et al., 1979).

Although much less intense than in neutrophils, a measurable oxidase activity is expressed in some non-phagocytic cells. This is the case in B lymphocytes, transformed by the Epstein-Barr virus, when they are treated with phorbol esters (but not by fMet-Leu-Phe or the Ca$^{2+}$ ionophore A23187; Volkman et al., 1984) and also in some non-transformed B lymphocytes, for example human tonsillar B lymphocytes (Maly et al., 1989) and B lymphocytes from different Burkitt cell lines (Hancock et al., 1989). The function of the oxidase system in B lymphocytes remains enigmatic. Cytotoxic, immunomodulatory, mutational effects (Maly et al., 1989) and the contribution of oxidation of susceptible amino acids to antigenic processing (Jones et al., 1991), have been discussed. Fibroblasts (Meier et al., 1989) and endothelial cells (Matsubara and Ziff, 1986) can release small amounts of O$_2^-$. An overproduction of O$_2^-$ due to deregulation of the activated oxidase is possibly responsible for the participation of fibroblasts in the joint-damaging processes in rheumatoid arthritis (Meier et al., 1989) and in the alteration of the basement membrane of blood vessels and the surrounding connective tissue in the case of endothelial cells (Matsubara and Ziff, 1986).

The early steps in the signaling pathway

Unstimulated human neutrophils consume relatively little O$_2$ (less than 1 nmol O$_2$ $\cdot$ min$^{-1}$ $\cdot$ 10$^7$ cells at 37 $^\circ$C). Within a few seconds after contact with specific stimuli, the rate of O$_2$ consumption abruptly increases by a factor of 50–100. The most effective stimuli are present at sites of inflammation and consist of opsonized microorganisms, the complement fragment C5a which is formed upon complement activation after interaction of microorganisms with antibodies, N-formylated methionylpeptides that are secreted by bacteria or released by lysis of dead microorganisms, two bioactive lipids produced by activated cells, namely platelet activating factor (PAF) and leukotriene B$_4$ (LTB$_4$) (for review see Snyderman and Uehling, 1988), and several recently discovered neutrophil activating proteins having structural similarities, namely neutrophil activating protein (NAP) 1 or interleukin 8 (Bagnoli et al., 1989), melanoma growth-stimulatory activator (Moser et al., 1990) and NAP 2 (Waltz et al., 1989). In contrast to formylpeptides and C5a, which induce the release of PAF and LTB4 from neutrophils, NAP 1/interleukin 8 is unable to promote the release of these bioactive lipids (Wirthmueller et al., 1991). In contrast to NAP 1/interleukin 8, whose production is induced by inflammatory stimuli in monocytes and a variety of tissue cells (Bagnoli et al., 1989; Moser et al., 1990), NAP 2 is generated by proteolysis of connective-tissue-activating peptide III and platelet basic protein released from stimulated platelets (Waltz and Bagnoli,