Perspectives

The Respiratory Burst of Phagocytes

Bernard M. Babior
Department of Medicine, Tufts-New England Medical Center, Boston, Massachusetts 02111

When exposed to certain stimuli, phagocytes (neutrophils, eosinophils, and mononuclear phagocytes) undergo marked changes in the way they handle oxygen (1). Their rates of oxygen uptake increase greatly, sometimes more than 50-fold; they begin to produce large amounts of superoxide (O_2^-) and hydrogen peroxide (H_2O_2); and they start metabolizing large quantities of glucose by way of the hexose monophosphate shunt. Because of the sharp increase in oxygen uptake, this series of changes has come to be known as the "respiratory burst." The "respiration" associated with this burst, however, has nothing to do with energy production; rather, its purpose is to generate powerful microbicidal agents by the partial reduction of oxygen.

The respiratory burst results from the activation of an enzyme, dormant in resting cells, that catalyzes the one-electron reduction of oxygen to O_2^- at the expense of NADPH:

$$O_2 + NADPH \rightarrow O_2^- + NADP^+ + H^+.$$

Most of this O_2^- reacts rapidly with itself, dismuting to produce oxygen and H_2O_2:

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2.$$

At the same time, glucose is metabolized through the hexose monophosphate shunt in order to regenerate the NADPH that has been consumed by the O_2^--forming enzyme and by a glutathione-dependent H_2O_2-detoxifying system that is found in the cytoplasm of the phagocyte.

O_2^- and H_2O_2, the immediate products of the respiratory burst, are not used by the phagocytes for microbial killing because H_2O_2 is only weakly microbicidal and O_2^- is completely innocuous. These compounds are used instead as starting materials for the production of the true microbicidal oxidants of phagocytes. These microbicidal oxidants fall into two classes: oxidized halogens and oxidizing radicals. The production of the former starts with the myeloperoxidase-catalyzed oxidation of Cl^- to hypochlorite by H_2O_2 (2):

$$Cl^- + H_2O_2 \xrightarrow[\text{myeloperoxidase}]{} OCl^- + H_2O.$$

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Hypochlorite, the prototype of the oxidized halogens, is itself an exceedingly powerful microbicidal agent. It is also the precursor of the chloramines, a group of microbicidal oxidized halogens that are formed by the reaction between hypochlorite and ammonia or amines (3):

$$OCl^- + RNH_3 \rightarrow RNH_2Cl + OH^-$$

Oxidizing radicals, the other class of microbicidal oxidants, are formed in reactions involving O_2^-. The hydroxyl radical ($OH^\cdot$), the only member of this class to have been identified unequivocally as a product of stimulated phagocytes, is thought to be made by a metal-catalyzed reaction between O_2^- and H_2O_2 (the well-known Haber-Weiss reaction):

$$O_2^- + H_2O_2 \xrightarrow[\text{Fe or Cu}]{} OH^\cdot + OH^- + O_2.$$

Other microbicidal oxidizing radicals are undoubtedly produced as well, but (apart from the likelihood that O_2^- serves as a precursor) their nature and origin are presently obscure.

The respiratory burst was discovered in 1933 (4), but was completely ignored for the next quarter century. Interest in the burst was rekindled around 1960 by work from Karnovsky's and Quastel's laboratories indicating that its purpose was not to provide energy for phagocytosis, but to furnish lethal oxidants for microbial killing (5, 6). Since then, a growing number of scientists have become interested in the respiratory burst and its ramifications, and it is now the subject of investigation in many laboratories around the world. This burgeoning scientific activity has yielded a great deal of information about the respiratory burst, along with occasional controversies. In the following paragraphs, I discuss a few aspects of this field that are of particular current interest.

Properties of the O_2^--forming enzyme

Over the past two decades, some half dozen oxidases have been proposed as the enzyme responsible for the respiratory burst in phagocytes (1, 7). The candidacy of some of these oxidases was tentative and brief, but others remained in the running for some time. Within the past three or four years, however, a consensus has formed on this question, and it is now generally agreed that the enzyme responsible for the respiratory burst is the membrane-associated pyridine nucleotide oxidase that was first described by Rossi and Zatti in 1964 (8). This consensus view is supported by an overwhelming body of evidence, the most important piece of which is probably the observation of Hohn and Lehrer (9),

subsequently confirmed many times, that the activity of this oxidase is greatly reduced or absent in patients with chronic granulomatous disease, an inherited condition (actually, a group of conditions) in which the phagocytes are unable to express a respiratory burst.

The respiratory burst oxidase is an FAD-requiring enzyme that catalyzes the production of O_2^- from oxygen using a reduced pyridine nucleotide as electron donor (1). The physiological electron donor is NADPH (Michaelis constant [K_m] 0.025–0.05 mM), but the enzyme is also capable of using NADH, though much less efficiently (K_m 0.5–1.0 mM). The affinity of the enzyme for oxygen, its other substrate, is similar to its affinity for NADPH ($K_m \sim 0.03$ mM, equivalent to a P_{O_2} of 0.15 atm) (10). The oxidase resides in the plasma membrane with its NADPH binding site projecting into the cytosol (11). This location guarantees optimum delivery of oxidants onto ingested microorganisms because the walls of the vesicles that contain these organisms are composed of oxidase-bearing plasma membrane that was originally on the outer surface of the cell.

A lot of attention is currently being paid to the question whether the oxidase contains a cytochrome as a second electron carrier. There is a b-type cytochrome found uniquely in phagocytes whose association with the respiratory burst oxidase is well documented (12). What is not known is whether this cytochrome is on the electron transport path between NADPH and oxygen (it has been proposed as the terminal component of a short electron transport chain) or whether it plays some other role, participating, say, in the conversion of the oxidase from its resting to its activated form. Kinetic studies have provided strong evidence that the cytochrome is off the electron transport path (13, 14), but these studies have not yet been performed with purified enzyme, so their interpretation may be debated. Further work is clearly needed to settle this question.

Activation of the oxidase

More is known and less is understood about the mechanism of activation of the O_2^--forming oxidase than about any other aspect of the respiratory burst. Observations abound, some contradicting others. Among these observations are the following: (*a*) a remarkably wide variety of agents, from F^- through detergents to inflammatory mediators such as C5a and leukotriene B_4, are able to activate the oxidase; (*b*) activation is energy-dependent and reversible; (*c*) activation requires neither degranulation nor phagocytosis (15); (*d*) it is generally preceded by a change in the transmembrane potential, though there is dispute as to whether this change is in the direction of depolarization (16, 17) or hyperpolarization (18); (*e*) it appears to occur by more than one route, since depending upon the stimulus, it is or is not associated with a lag (19), does or does not require exogenous calcium (20), and is or is not accompanied by an increase in cytosolic calcium or the release into the cytoplasm of a poorly understood "membrane-bound calcium pool" (21–23). It is not surprising that little has been concluded from this confusing welter of findings beyond the idea that biochemical pathways for activating the oxidase are multiple and complex.

On the other hand, a number of biochemical events have been seen to occur on exposure of various types of cells to external stimuli, and some of these may provide clues to the molecular basis for respiratory burst activation. Nishizuka and associates, for instance, have shown that the powerful respiratory burst activator, phorbol myristate acetate, binds and activates a membrane-associated phosphorylating enzyme known as protein kinase C (24), raising the possibility (sought but not yet found) that the activation of the oxidase might be the consequence of a change in the state of phosphorylation of an activator protein. Rapid and extensive alterations in membrane phospholipids which are induced by stimulation in many kinds of cells (25–27) suggest additional biochemical mechanisms by which the oxidase might be activated. Still other ways to vary activity have emerged from studies with cell-free systems: interconversion between 3-Fe and 4-Fe iron-sulfur centers (28), ADP-ribosylation of arginine residues (29), and complex formation between catalytic and regulatory subunits (30) are only a few examples. A clear connection, however, between any of these biochemical activating mechanisms and the onset of the respiratory burst has yet to be established.

There is a second meaning to the term "activation" as applied to phagocytes that pertains solely to mononuclear phagocytes. "Activation" in this second sense refers to a series of changes leading to a generalized increase in destructive power that are elicited in these cells by exposure to endotoxin or the lymphokine γ-interferon. Included among these changes are alterations in the respiratory burst activity of maximally stimulated phagocytes. These alterations are most clearly seen with resident peritoneal macrophages, which exhibit almost no respiratory burst when freshly isolated but acquire a substantial capacity to make O_2^- after 2 or 3 days of culture in the presence of a suitable activator (31), only to lose this capacity on further culture (32). The mechanism of this second form of activation has received little attention; in all likelihood, it will turn out to involve major changes in gene expression induced by the activating agent.

Bacterial killing mechanisms

Studies on the nature of the lethal injury inflicted on bacteria by microbicidal oxidants are just beginning to get under way. Oxidized halogens have been shown to destroy many bacterial components, including nucleotides and redox enzymes, at a very rapid rate (33), but they kill bacteria even more quickly (34), indicating that the bacterial Achilles' heel is something even more susceptible to oxidant damage than the components hitherto examined. Oxidizing radicals, which were recognized as the source of most of the harmful effects of ionizing radiation long before their production by phagocytes was discovered, inflict damage on many types of biological molecules, particularly proteins and nucleic acids (35). The radical-mediated lesions that kill ingested bacteria, however, have not yet been identified.

Although there is uncertainty regarding the biochemical na-

ture of the lesion (or lesions) responsible for the death of an organism under attack by microbicidal oxidants, there is one action of these oxidants in which a biochemical and a functional effect can be unequivocally tied together. This action is that of genetic mutation, and it has been shown to occur in both bacteria and mammalian cells after exposure to phagocyte-generated oxidants. In bacteria, it has been demonstrated in the form of a positive Ames test (36), while mammalian fibroblasts that have been exposed to these oxidants in tissue culture show an increased number of sister chromatid exchanges compared with cells that have not been so exposed (37). Phagocytes, with their respiratory burst, must therefore be regarded as potential carcinogens according to current federal regulations, and should be added to the long list of natural hazards that have been proposed to constitute the major carcinogenic threat in the environment (38).

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