

Editorial: Celebrating the 50th anniversary of the seminal discovery that the phagocyte respiratory burst enzyme is an NADPH oxidase

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As leukocyte biologists know well, stimulated phagocytes exhibit a burst of oxygen consumption, also known as the “respiratory burst,” which underlies the production of microbicidal reactive oxygen species. An immunology student, preparing in 2014 for an examination, will learn that oxidant generation relies on “the phagocyte oxidase system... a multisubunit enzyme that is assembled in activated phagocytes mainly in the phagolysosomal membrane. to reduce molecular oxygen into reactive oxygen species (ROS) such as superoxide radicals, with the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) acting as a cofactor” [1]. Albeit factually correct, the textbook description lacks acknowledgment of the scientific debate that raged for ~15 years over the identity of the electron donor-reducing molecular oxygen. Now, we recognize and celebrate the 50th anniversary of Filippo Rossi’s [2] demonstration that the phagocyte respiratory burst enzyme is an NADPH oxidase.

An appreciation of the significance of the Rossi finding requires recognition of the level of understanding of phagocyte biology in the early 1960s, when a young Filippo Rossi began his investigative pursuits (Fig. 1A). The earliest studies of oxygen metabolism by stimulated leukocytes go back to the early 1930s, when Baldrige and Gerard [3] first reported that dog leukocytes fed *Sarcina lutea* consumed oxygen, a phenomenon

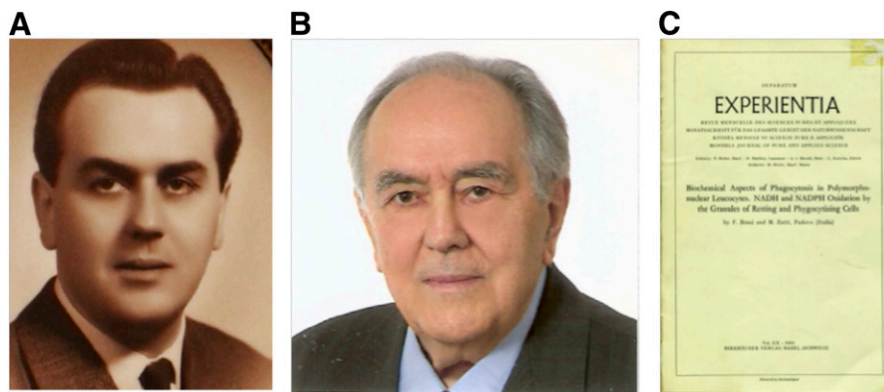
attributed to the “extra energy liberation of engulfment and ingestion,” and presumably of mitochondrial origin. However, more than 2 decades later, Karnovsky observed that oxygen consumption by phagocytosing guinea pig neutrophils was resistant to potassium cyanide, a mitochondrial poison, and thus, concluded that the cellular response that they observed was not mediated by mitochondria (see ref. [4] for a review of early studies by Karnovsky and colleagues). Iyer, Islam, and Quastel [5] confirmed the cyanide resistance of the oxidative burst and demonstrated a requirement for flavin and its linkage with hexose monophosphate shunt activity.

Elucidation of the biochemistry underlying the phagocyte oxidase exemplifies the notion that clinical relevance catalyzes biomedical research. Identification of the clinical syndrome of chronic granulomatous disease and the eventual links between phagocyte oxidase activity and efficient antimicrobial action of human neutrophils (reviewed in ref. [6]) provided the critical, clinical context to fuel pursuit of the enzymatic basis of the respiratory burst. The Karnovsky lab [7] had recovered reduced pyridine nucleotide oxidase activity in granules isolated from guinea pig leukocytes that was 10-fold more active toward NADH than NADPH under the low pH of the experimental conditions. Although Iyer and Quastel [8] demonstrated that stimulated guinea pig neutrophils oxidized

NADPH more readily than NADH, the system was manganese dependent, a feature later proved to be an experimental artifact (see refs. [4, 9, 10] for reviews). In 1964, Filippo Rossi and his colleague Mario Zatti [2], reported that a granule fraction (the 20,000 g pellet of a sucrose homogenate), obtained from guinea pig peritoneal neutrophils after phagocytosis of killed *S. lutea*, displayed NADPH-dependent oxidase activity, dramatically higher than that detected in granule fractions from nonstimulated cells (Fig. 1). An NADH oxidase activity was also present in this granule fraction, but the extent of its activation after phagocytosis was much lower than that detected for the NADPH oxidase. Despite these and other robust data (reviewed in refs. [9, 10]), the issue remained unsettled and associated with “some controversy, but, perhaps, little acrimony” [4], until studies of the particulate superoxide-forming fraction of neutrophils definitively revealed the reduced pyridine preference of the phagocyte oxidase; the Michaelis constant for NADPH was shown to be more than an order of magnitude lower than that for NADH (reviewed in refs. [9, 10]). Perhaps the final word on the electron source for the phagocyte oxidase came from Bernie

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Figure 1. Filippo Rossi (A) in 1963, at the beginning of his studies on the oxygen metabolism of phagocytes, and (B) now, ~50 years after the start of his struggle to convince the phagocyte biology community that the respiratory burst of phagocytes was a result of activation of an NADPH oxidase. (C) The cover of the extract of Rossi's paper [2] published in *Experientia* (now *Cellular and Molecular Life Sciences*) in 1964, reporting that a "granule fraction," obtained from guinea pig peritoneal neutrophils after phagocytosis of killed *S. lutea*, displayed an NADPH oxidase activity dramatically higher than that detected in a granule fraction of nonstimulated cells.



Babior, another pioneer in the field of phagocyte biology, in his *Medical Progress* review in 1978: "The activity that I believe to be responsible for respiratory burst oxygen consumption ... is an activity found in the particulate fraction of neutrophil homogenates that catalyzes a reaction between NADPH and oxygen and is written about by most workers in the field under the name 'NADPH oxidase'" [10].

With the electron source for the phagocyte oxidase settled, investigators turned their attention to defining its components and regulation, a pursuit with as many false leads, unanticipated complexity, and controversy (some with hints of acrimony) as accompanied the NADH versus NADPH debate. Nonetheless, advances in understanding the mechanisms underlying the phagocyte oxidase have accumulated over the past 2 decades. Furthermore, it has recently become clear that the NADPH oxidase is not an enzymatic system unique to

phagocytes but rather, a member of a family of proteins integral to normal biology of cells in animal and plant kingdoms [6].

When Filippo Rossi reflects on the early times in the NADPH oxidase field, he always highlights how challenging it was to present his data to audiences that were skeptical about the role of NADPH as a source of reducing equivalents to oxygen. With his feeling alone against giants in the biochemistry field, he labored to convince people that he was correct, and 50 years later, he can comfortably and confidently reflect on a job well done.

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