Respiratory Burst Oxidase of the Neutrophil

Bioenergetics Group Colloquium Organized and Edited by M. N. McKillen (Trinity College, Dublin) at the 636th Meeting, held at Trinity College, Dublin, 4–7 September 1990

Components of the microbicidal oxidase of phagocytes
Anthony W. Segal
Department of Medicine, University College London, Rayne Institute, University Street, London WC1E 6JJ, U.K.

Engulfment of microbes and other particles by the 'professional' phagocytic cells, neutrophils, monocytes and macrophages, and eosinophils, is associated with a burst of oxygen consumption that is important for efficient killing and digestion. This respiratory burst is not accomplished by mitochondrial respiration, but by a unique electron transport chain called the NADPH oxidase [1]. The composition of this system and its regulation have been of considerable interest, in particular because defects of the different components result in failure of the system as a whole, causing a clinical picture of chronic granulomatous disease (CGD), associated with an unusual susceptibility to infection [2].

The NADPH oxidase lies between its substrate, NADPH, in the cytoplasm and the terminal electron acceptor, oxygen, on the external side of the plasma membrane which invaginates to form the lining of the phagocytic vacuole. The only component of this electron transport chain that has been clearly identified is a very unusual cytochrome, with a particularly low mid-point potential, called cytochrome b-245. This cytochrome is located in the plasma membrane and the membrane of the specific granules, and comes to lie in the wall of the phagocytic vacuole when this forms [1]. This cytochrome is composed of a large heavily glycosylated subunit with a molecular mass of 76–92 kDa, and a 23 kDa protein [3, 4], to which the haem is attached [5]. Defects of these molecules are responsible for most of the cases of CGD. The gene for the large subunit [6, 7] was discovered by reverse genetics to be located on the X chromosome [8], and is defective in the approximately two-thirds of patients with CGD whose cells lack the cytochrome. The cytochrome is also missing in a few of the patients with an autosomal recessive pattern of inheritance where the defect is in the gene on chromosome 16 coding for the 23 kDa subunit (M. C. Dinauer, E. A. Pierce, G. A. P. Bruns, J. T. Curnette & S. H. Orkin, unpublished work). A ras-like protein, rap-1 appears to be attached to the cytochrome [9] and is probably important for its regulation.

Two other proteins have been identified as vital to the function of this oxidase system because their absence results in its dysfunction and the syndrome of CGD [10–12]. These proteins with molecular masses of 47 kDa and 67 kDa are located in the cytosol of resting cells and translocate to the plasma membrane upon activation of the cell [13]. The mechanism responsible for this transfer is unknown, but it is associated with intense phosphorylation of the 47 kDa protein which appears to link with the cytochrome in the membrane. Defects in the gene for the 47 kDa protein are responsible for the vast majority of cases of the third of patients with CGD with an autosomal recessive inheritance (C. M. Casimir & A. W. Segal, unpublished work). Most of these patients share a common mechanism, the loss of a dinucleotide from a tandem repeat at the first splice junction of the gene, probably due to slippage of the polymerase at this particular point in the message. We are ignorant as to the function of these two proteins. Both have been cloned and sequenced [14–17] and both contain two regions of homology to an amino acid sequence found in the non-catalytic domain of the src superfamily of protein kinases, the SH3 domain [18], that is also seen in a number of cytoplasmic proteins that become associated with the inner surface of the plasma membrane, including phospholipase C, fodrin, GAP, myosin, and yeast proteins cdc-25 and fus-1 [15]. These domains are probably important for the translocation of these and other associated proteins from the cytosol to the membranes upon activation.

Abbreviation used: CGD, chronic granulomatous disease.
Many other proteins have been implicated as components of this system, but in none has a clear association been established [1]. We still do not know the identity of the NADPH binding protein involved.

Phosphorylation and dephosphorylation are involved in the activation of this oxidase. We have recently found that the phosphatase inhibitor okadaic acid has interesting effects (R. Garcia & A. W. Segal, unpublished work). It strongly inhibits activation by phorbol-12-myristate-13-acetate by depleting cellular calcium which diminishes translocation of protein kinase C and phosphorylation of the cytochrome and other proteins. In contrast, superoxide generation after exposure to the chemotactic tetrapeptide N-formyl-methionyl-leucyl-phenylalanine, which normally results in a brief response, is greatly enhanced and prolonged, indicating the importance of dephosphorylation in terminating activity.

The function of this NADPH oxidase is also unclear. The discovery that it produces superoxide coincided with a flurry of interest in free radicals and seemed to provide a direct toxic mechanism. It has been subsequently established that this radical is not itself sufficiently reactive to be directly toxic and there is little evidence for the generation of the highly reactive hydroxyl radical. Superoxide dismutates to hydrogen peroxide which acts as substrate for myeloperoxidase to oxidize halides to hypochlorous acids and chloramines. However, myeloperoxidase deficiency is not all that uncommon and is not associated with an unusual predisposition to infection, indicating that this system plays an ancillary role.

Activation of this oxidase does have a pronounced effect upon the pH within the phagocytic vacuole [19]. The initial concept that this was an acid response has been shown to be incorrect and the respiratory burst is associated with an alkaline phase produced by the pumping of electrons unaccompanied by protons across the wall of the phagosome. The vacuole remains acid in CGD cells and in normal cells under anaerobic conditions. This alkaline phase is important for the antimicrobial and digestive functions of the neutral hydrolases released from the cytoplasmic granules into the vacuole upon phagocytosis. This failure of digestion because of the excessively acid conditions in the vacuole provides one explanation for the genesis of the diffuse granulomata that gives CGD its descriptive name.

I thank the Wellcome Trust for supporting this work.


Received 28 August 1990