During the Respiratory Burst, Do Phagocytes Need Proton Channels or Potassium Channels, or Both?

Thomas E. DeCoursey (18 May 2004)

Sci. STKE **2004** (233), pe21. [DOI: 10.1126/stke.2332004pe21]

During the Respiratory Burst, Do Phagocytes Need Proton Channels or Potassium Channels, or Both?

Thomas E. DeCoursey*

(Published 4 May 2004)

The Respiratory Burst

Phagocytosis is an important defense mechanism of innate immunity, in which a macrophage, neutrophil, or eosinophil encounters a microbial invader, engulfs it, and kills it. NADPH oxidase mediates the "respiratory burst," a large (100-fold) increase in oxygen consumption that occurs during phagocytosis and results in microbe death. This multicomponent enzyme (Fig. 1) produces superoxide anion (O_2^-), which is a precursor to various reactive oxygen species (ROS). NADPH oxidase function is clearly essential to innate immunity, because a hereditary defect in any of the major NADPH oxidase components results in chronic granulomatous disease (CGD). Without medical intervention, CGD patients typically die in childhood of severe recurrent infections (*1*). The idea that ROS themselves are lethal to bacteria has been challenged by Segal's group (*2*, *3*). In their recent study, Ahluwalia *et al.* (*4*) not only conclude that ROS contribute little to microbe killing, but also cast doubt on the widespread belief that ROS contribute to various human diseases.

The discovery that NADPH oxidase is electrogenic (*5*) was pivotal to understanding its mechanism. The enzyme moves electrons from NADPH inside the cell across the cell membrane to reduce extracellular oxygen to superoxide (Fig. 1A). A practical consequence is that one can detect NADPH oxidase activity directly and quantitatively in real time as an electrical current. NADPH oxidase–generated electron current has been recorded in cells dialyzed with artificial cytoplasm in the whole-cell patch-clamp configuration (*6*, *7*) and in intact phagocytes studied using the perforated-patch approach (*8*, *9*). The latter approach has the advantage of being physiological in the sense that the cell is allowed to keep its own cytoplasm. Because the cytoplasm is retained, intracellular signaling pathways that are lost in whole-cell configuration are preserved and the respiratory burst can be triggered and studied at the single-cell level.

Electron translocation by NADPH oxidase will produce rapid, massive depolarization unless it is balanced by positive current flow across the membrane. This physical requirement, based on Maxwell's equations, was clearly stated in 1952 by Hodgkin *et al.* in regard to sodium and potassium currents in squid axons (*10*):

$$I_{total} = C_M(dV/dt) + I_{ionic}$$

Applying this equation to the respiratory burst, the total electron current I_{total} must equal the sum of the balancing ionic current I_{ionic} (which could be outward cation or inward anion flux) and the membrane capacity C_M term that gives the rate of membrane depolarization. During respiratory burst, the membrane potential of an eosinophil would depolarize at a rate of 18 V/s if no charge compensation mechanism existed (*11*). Within 20 ms, the membrane potential would reach +200 mV, which would prevent electron translocation, effectively shutting down NADPH oxidase function (*12*). Shortly thereafter, voltages that cause membrane rupture would be reached. It has been generally believed that practically all of the required charge compensation is mediated by H^+ efflux through proton channels (*1*, *5*, *12*–*15*) (Fig. 1A). Segal's group reported that ~6% of the charge compensation is mediated by K^+ efflux (*2*). When even a small fraction of charge compensation is mediated by K^+, the phagosome becomes hypertonic and its pH increases (Fig. 1B). In their model, both changes enhance bacterial killing by proteolytic enzymes that are released into the phagosome (*2*). Recently, Segal's group extended this proposal to suggest that most or all of the charge compensation is mediated by K^+ efflux, a scenario that challenges the necessity and even the existence of proton channels in phagocytes (*4*).

What Ion Channels Are Expressed in Phagocytes?

The electrophysiological data of Ahluwalia *et al.* (*4*) differ markedly from those obtained in previous studies of neutrophils and eosinophils. They report "small" currents of 250 pA at +140 mV in resting neutrophils, and describe development of "a large outwardly rectifying current" [p. 854 in (*4*)] upon stimulation with the phorbol ester, phorbol myristate acetate (PMA). The selectivity of the currents in resting or stimulated cells was not studied; however, they were reversibly inhibited by iberiotoxin, an inhibitor of "maxi-K^+" channels (high-conductance, voltage- and calcium-activated K^+ channels). On the basis of this pharmacology, Ahluwalia *et al.* (*4*) conclude that maxi-K^+ channels are responsible for the currents seen in neutrophils and in eosinophils. In neither cell were PMA-activated currents inhibited by 3 mM Zn^{2+}, a classical inhibitor of proton channels (*16*). If one views these data in isolation, one would conclude that the main and possibly only important ionic conductance in phagocytes is maxi-K^+ current. Iberiotoxin and paxilline (another maxi-K^+ channel blocker) inhibited Rb^+ fluxes, phagosomal alkalinization, and killing of several pathogens (*Staphylococcus aureus*, *Candida albicans*, and *Serratia marcescens*). The implication is that maxi-K^+ channels and not proton channels are necessary for effective microbe killing.

Among many previous electrophysiological studies of human eosinophils and neutrophils, none has reported outward currents (other than proton currents) that are within an order of magnitude of the 1000-pA putative maxi-K^+ currents reported in neutrophils by Ahluwalia *et al.* (*4*). In human neutrophils, Krause and Welsh (*17*) reported Ca^{2+}-activated K^+ and Cl^- currents that combined were <50 pA at +90 mV. Volume-regulated

Department of Molecular Biophysics and Physiology, Rush University Medical Center, 1750 West Harrison, Chicago, IL 60612, USA.

***Corresponding author. Telephone, 312-942-3267; fax, 312-942-8711; e-mail, tdecours@rush.edu**

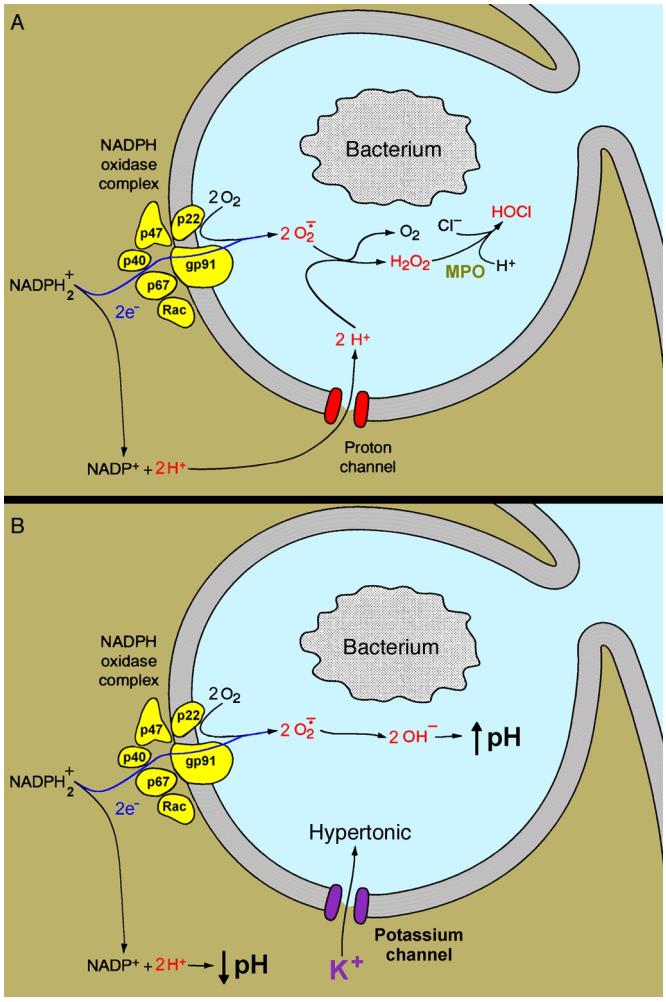

Fig. 1. The "respiratory burst" in a phagocyte is triggered when a bacterium is phagocytosed. During the phagocytosis of bacteria by macrophages and neutrophils, the phagosome membrane pinches off and the microbe is endocytosed along with a small volume of extracellular fluid. Eosinophils attack helminths too large to endocytose, and NADPH oxidase assembles in their plasma membrane. The main components of NADPH oxidase are shown in yellow. The cytoplasmic components are at a remote location inside resting phagocytes; stimulation results in their migration to the membrane, where the entire complex assembles. Electrons are removed from NADPH in the cytoplasm and transferred through the gp91^{phox} component (which includes flavin adenine dinucleotide and two hemes) across the membrane, where they reduce extracellular (or intraphagosomal) O_2 to O_2^-. Protons left behind in the cell are extruded through voltage-gated proton channels (red). Some of the ROS derived from O_2^- are indicated. Spontaneous or superoxide dismutase–catalyzed disproportionation of O_2^- produces hydrogen peroxide (H_2O_2), which may be converted to HOCl (hypochlorous acid, or household bleach) by myeloperoxidase (MPO). (**A**) Traditional view of the respiratory burst with charge compensation by proton channels. A perfect match of one proton per electron results in no change in membrane potential, intracellular pH (pH_i), or external pH (pH_o) and little change in ionic strength (*2*, *28*). Because proton channels are separate molecules and for the most part operate independently of NADPH oxidase, perfect 1:1 stoichiometry is not obligatory. The large depolarization that occurs during the respiratory burst in intact neutrophils and eosinophils (*41*, *42*) is likely the most important factor that causes proton channels to open (*11*), although both pH_i and pH_o tend to change in a direction that causes proton channels to open. The fact that depolarization occurs demonstrates unequivocally that proton efflux initially lags behind electron efflux, as was first pointed out by Henderson *et al.* (*5*, *28*). (**B**) If any fraction of the total charge compensation were mediated by K^+ efflux, pH_i would fall, pH_o (or phagosomal pH) would increase, and the osmolality of the phagosomal contents would increase. In this model (*2*, *4*), the elevated pH and osmolality of the phagosomal contents are crucial to activating proteolytic enzymes that actually kill bacteria, as opposed to ROS, which are said to be inert (*3*). An extreme version of this model obviates the contribution of proton channels and ascribes the entire charge compensation function to maxi-K^+ channels (*4*). [Adapted by permission from (*11*).]

Cl^- currents in neutrophils studied by Stoddard *et al.* (*18*) were ~70 pA at +80 mV. In previous studies of human eosinophils, outward K^+ currents were small or absent: Using KCl-containing pipette solutions, Tare *et al.* (*19*) and Gordienko *et al.* (*20*) saw little outward current (<10 pA). Schwingshackl *et al.* (*21*) reported that after PMA enhancement, the total current was ~65 pA at +80 mV, including both Cl^- and K^+ currents. Calcium-activated K^+ channels reported in stimulated eosinophils by Saito *et al.* (*22*) were not voltage-gated, and their conductance was only 10 to 22 pS, one-tenth that of maxi-K^+ channels. Finally, H^+ current was the only detectable outward current in PMA-stimulated human eosinophils with active NADPH oxidase in perforated-patch studies with K^+-containing pipette solutions (*23*).

Ahluwalia *et al.* (*4*) report that Zn^{2+}, a classical inhibitor of voltage-gated proton channels (*24*), "at concentrations three orders of magnitude greater than those causing almost complete blockage of proton channels, was also without effect on the currents from neutrophils and eosinophils" [p. 854 in (*4*)]. In contrast, 42 electrophysiological studies of phagocytes and related leukocytes (eosinophils, neutrophils, macrophages, microglia, mast cells, osteoclasts, basophils, lymphocytes, and related cell lines) have described Zn^{2+}-sensitive, voltage-gated proton current (*11*). H^+ currents in human neutrophils are small (*25*) and difficult to detect, but the H^+ current density in human eosinophils (~200 pA/pF) is the largest known in any cell (*20*, *11*, *26*); thus, the failure of Ahluwalia *et al.* (*4*) to detect H^+ currents is puzzling.

Possible Artifacts in the Standard Superoxide Assay

An important piece of evidence supporting the charge compensation model depicted in Fig. 1A is the inhibition of NADPH oxidase function by H^+ channel inhibitors such as Cd^{2+} and Zn^{2+} (*12*, *27–32*). Ahluwalia *et al.* (*4*) challenge this evidence, which is based on a widely used assay in which O_2^- produced by NADPH oxidase reduces cytochrome c, resulting in a color change that is measured spectroscopically. Using a cell-free assay in which O_2^- was generated by xanthine plus xanthine oxidase, they showed that 3 mM Zn^{2+} or Cd^{2+} accelerated the spontaneous conversion of O_2^- into H_2O_2 (dismutation). The implication is that these metals do not inhibit NADPH oxidase activity in phagocytes by inhibiting proton channels, but instead

interfere with the measurement of O_2^-. Several previous studies have shown that these metals can interact with various ROS, although interpretation of the data can be complex (*33*–*35*). If we accept that metals may catalyze the transfer of electrons from O_2^- to H_2O_2, then the question is whether and to what extent this mechanism contributes to the apparent inhibition of NADPH oxidase activity in phagocytes.

Three observations indicate that direct catalytic conversion of O_2^- to H_2O_2 is not responsible for the observed inhibition of NADPH oxidase activity by metals. First, in previous studies, Zn^{2+} and Cd^{2+}, at concentrations that inhibit O_2^- generation by phagocytes (*12*, *30*, *32*), did not interfere with the cytochrome c assay, whether O_2^- was generated by photoreduction of riboflavin using methionine as the electron donor (*30*) or by the xanthine plus xanthine oxidase method (*33*). Second, in the presence of Zn^{2+} concentrations that largely abolish O_2^- generation (measured as cytochrome c reduction) in phagocytes, addition of the protonophore CCCP (carbonyl cyanide *m*-chlorophenyl hydrazone) restored part or all of the response (*12*, *29*). Third, COS-7 cells transfected with the four main components of NADPH oxidase produced O_2^- (measured as cytochrome c reduction) in the presence of $ZnCl_2$ at concentrations up to 1 to 3 mM—conditions in which O_2^- production is inhibited by 90% in eosinophils and neutrophils (*12*). In summary, although metals may scavenge ROS under some circumstances, the reported inhibition of NADPH oxidase function by Zn^{2+} and Cd^{2+} (*12*, *28*–*32*) is most likely mediated through their effects on proton channels.

How Is Charge Compensated?

If not protons, then what compensates charge? NADPH oxidase is electrogenic: It deposits the equivalent of 4 M electrons into the phagosome during the respiratory burst (*2*). If this electron flux were not compensated, the phagosome membrane in a neutrophil would depolarize by 6400 V during the respiratory burst (*11*), roughly 25,000 times the amount of depolarization required to shut off NADPH oxidase completely (*12*). K^+ influx into the phagosome may compensate ~6% of the required charge (*2*); however, full compensation by K^+ would result in 4 M K^+ in the phagosome, with intolerable osmotic consequences. Chloride efflux from the phagosome could compensate <4% of the electron current before depleting phagosomal Cl^- concentration to nil. Ahluwalia *et al*. (*4*) reported that Zn^{2+} had no effect on the currents in PMA-activated neutrophils and eosinophils, but that iberiotoxin completely abolished all currents in stimulated neutrophils without affecting NADPH oxidase activity. These data indicate that the only detectable conductance in phagocytes during the respiratory burst is maxi-K^+, yet inhibiting the putative maxi-K^+ channel did not inhibit O_2^- production by neutrophils (*4*). The results of Ahluwalia *et al*. (*4*) thus seem to have eliminated all possible routes of compensating the charge translocated during NADPH oxidase activity. Should we conclude that charge compensation is unnecessary?

Charge compensation is necessary (*12*), and in all likelihood it is mediated by proton flux. Other than protons, no ion is capable of compensating the massive electron translocation. In contrast, the proton can combine with O_2^- and, after peregrinating in the guise of various ROS such as H_2O_2 and HOCl, can essentially disappear as membrane-permeable H_2O. Voltage-gated proton channels are observed universally in phagocytes and related cells (*11*). The level of H^+ channel expression is roughly proportional to the amplitude of the respiratory burst in various phagocytes, and in all cells there are at least 10 times as many H^+ channels as necessary to compensate maximal NADPH oxidase activity completely (*11*, *36*). Thus, kinetic competence has been demonstrated. Finally, protons are extruded from phagocytes during the respiratory burst stoichiometrically with O_2 consumed (*37*–*40*). In fact, the original discovery that NADPH oxidase was electrogenic (*5*) was based on pH changes that indicated massive H^+ efflux proton efflux during the respiratory burst (*5*, *28*, *41*). These observations all indicate that the bulk of charge compensation is mediated by protons.

The Huge Putative K^+ Conductance Would Prevent the Depolarization Observed in Phagocytes During the Respiratory Burst

To compensate the electrogenic activity of NADPH oxidase, cation current must match the electron current. The electron efflux generated by NADPH oxidase (adjusted to body temperature) corresponds to 8 to 16 pA in neutrophils (*25*) and 30 to 40 pA in eosinophils (*9*, *23*). During the respiratory burst, the membrane depolarizes to +58 mV in human neutrophils (*42*) and +17 mV in human eosinophils (*43*). The observed depolarization of 120 mV or 50 mV, respectively, from the resting potential is sufficient to activate precisely the H^+ efflux necessary for full compensation in both cells. In contrast, the putative maxi-K^+ currents reported by Ahluwalia *et al*. (*4*) are so large that proton currents, which are ubiquitous in phagocytes (*11*), were not even detected. Activation of such a large K^+ conductance in a living cell would clamp the membrane potential within 10 to 20 mV of the potassium equilibrium potential (E_K, roughly –70 mV), thus preventing the massive, sustained depolarization that has been observed to occur during the respiratory burst in intact phagocytes (*42*, *43*). Indeed, this depolarization must occur in order to activate the H^+ efflux required for charge compensation (*11*).

In conclusion, the original Segal model (*2*)—in which a small fraction of charge is compensated by K^+, resulting in biologically important increases in pH and ionic strength of the phagosome—is intriguing and worth pursuing, although several questions have been raised (*15*, *44*). The recently proposed exclusive involvement of maxi-K^+ channels in microbe killing by phagocytes (*4*) is inconsistent with a large body of existing data, and hence must be viewed with skepticism. H^+ efflux through proton channels thus remains the most viable mechanism for the charge compensation that is necessary to ensure continuous O_2^- production in phagocytes during the respiratory burst. Finally, the toxicity of ROS in phagocytes and in other cells, which has long been taken for granted, remains a widespread assumption that deserves critical study.

References

1. M. Geiszt, A. Kapus, E. Ligeti, Chronic granulomatous disease: More than the lack of superoxide? *J. Leukoc. Biol.* **69**, 191–196 (2001).
2. E. P. Reeves, H. Lu, H. L. Jacobs, C. G. M. Messina, S. Bolsover, G. Gabella, E. O. Potma, A. Warley, J. Roes, A. W. Segal, Killing activity of neutrophils is mediated through activation of proteases by K^+ flux. *Nature* **416**, 291–297 (2002).
3. E. P. Reeves, M. Nagl, J. Godovac-Zimmermann, A. W. Segal, Reassessment of the microbicidal activity of reactive oxygen species and hypochlorous acid with reference to the phagocytic vacuole of the neutrophil granulocyte. *J. Med. Microbiol.* **52**, 643–651 (2003).
4. J. Ahluwalia, A. Tinker, L. H. Clapp, M. R. Duchen, A. Y. Abramov, S. Page, M. Nobles, A. W. Segal, The large conductance Ca^{2+}-activated K^+ channel is essential for innate immunity. *Nature* **427**, 853–858 (2004).

5. L. M. Henderson, J. B. Chappell, O. T. G. Jones, The superoxide-generating NADPH oxidase of human neutrophils is electrogenic and associated with an H^+ channel. *Biochem. J.* **246**, 325–329 (1987).
6. J. Schrenzel, L. Serrander, B. Bánfi, O. Nüsse, R. Fouyouzi, D. P. Lew, N. Demaurex, K.-H. Krause, Electron currents generated by the human phagocyte NADPH oxidase. *Nature* **392**, 734–737 (1998).
7. B. Bánfi, J. Schrenzel, O. Nüsse, D. P. Lew, E. Ligeti, K.-H. Krause, N. Demaurex, A novel H^+ conductance in eosinophils: Unique characteristics and absence in chronic granulomatous disease. *J. Exp. Med.* **190**, 183–194 (1999).
8. T. E. DeCoursey, V. V. Cherny, W. Zhou, L. L. Thomas, Simultaneous activation of NADPH oxidase-related proton and electron currents in human neutrophils. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 6885–6889 (2000).
9. T. E. DeCoursey, V. V. Cherny, A. G. DeCoursey, W. Xu, L. L. Thomas, Interactions between NADPH oxidase-related proton and electron currents in human eosinophils. *J. Physiol.* **535**, 767–781 (2001).
10. A. L. Hodgkin, A. F. Huxley, B. Katz, Measurement of current-voltage relations in the membrane of the giant axon of *Loligo*. *J. Physiol.* **116**, 424–448 (1952).
11. T. E. DeCoursey, Voltage-gated proton channels and other proton transfer pathways. *Physiol. Rev.* **83**, 475–579 (2003).
12. T. E. DeCoursey, D. Morgan, V. V. Cherny, The voltage dependence of NADPH oxidase reveals why phagocytes need proton channels. *Nature* **422**, 531–534 (2003).
13. A. R. Cross, O. T. G. Jones, Enzymic mechanisms of superoxide production. *Biochim. Biophys. Acta* **1057**, 281–298 (1991).
14. B. M. Babior, J. D. Lambeth, W. Nauseef, The neutrophil NADPH oxidase. *Arch. Biochem. Biophys.* **397**, 342–344 (2002).
15. R. E. Harrison, N. Touret, S. Grinstein, Microbial killing: Oxidants, proteases and ions. *Curr. Biol.* **12**, R357–R359 (2002).
16. V. V. Cherny, T. E. DeCoursey, pH-dependent inhibition of voltage-gated H^+ currents in rat alveolar epithelial cells by Zn^{2+} and other divalent cations. *J. Gen. Physiol.* **114**, 819–838 (1999).
17. K.-H. Krause, M. J. Welsh, Voltage-dependent and Ca^{2+}-activated ion channels in human neutrophils. *J. Clin. Invest.* **85**, 491–498 (1990).
18. J. S. Stoddard, J. H. Steinbach, L. Simchowitz, Whole cell Cl^- currents in human neutrophils induced by cell swelling. *Am. J. Physiol.* **265**, C156–C165 (1993).
19. M. Tare, S. A. Prestwich, D. V. Gordienko, S. Parveen, J. E. Carver, C. Robinson, T. B. Bolton, Inwardly rectifying whole cell potassium current in human blood eosinophils. *J. Physiol.* **506**, 303–318 (1998).
20. D. V. Gordienko, M. Tare, S. Parveen, C. J. Fenech, C. Robinson, T. B. Bolton, Voltage-activated proton current in eosinophils from human blood. *J. Physiol.* **496**, 299–316 (1996).
21. A. Schwingshackl, R. Moqbel, M. Duszyk, Involvement of ion channels in human eosinophil respiratory burst. *J. Allergy Clin. Immunol.* **106**, 272–279 (2000).
22. M. Saito, R. Sato, N. M. Munoz, A. Herrnreiter, M. Oyaizu, H. Kasugai, T. Narahashi, A. R. Leff, Association of granular exocytosis with Ca^{2+}-activated K^+ channels in human eosinophils. *Am. J. Physiol.* **273**, L16–L21 (1997).
23. V. V. Morgan, Cherny, R. Murphy, W. Xu, L. L. Thomas, T. E. DeCoursey, Temperature dependence of NADPH oxidase in human eosinophils. *J. Physiol.* **550**, 447–458 (2003).
24. V. V. Cherny, T. E. DeCoursey, pH-dependent inhibition of voltage-gated H^+ currents in rat alveolar epithelial cells by Zn^{2+} and other divalent cations. *J. Gen. Physiol.* **114**, 819–838 (1999).
25. T. E. DeCoursey, V. V. Cherny, Potential, pH, and arachidonate gate hydrogen ion currents in human neutrophils. *Biophys. J.* **65**, 1590–1598 (1993).
26. J. Schrenzel, D. P. Lew, K.-H. Krause, Proton currents in human eosinophils. *Am. J. Physiol.* **271**, C1861–C1871 (1996).
27. P. H. Beswick, P. C. Brannen, S. S. Hurles, The effects of smoking and zinc on the oxidative reactions of human neutrophils. *J. Clin. Lab. Immunol.* **21**, 71–75 (1986).
28. L. M. Henderson, J. B. Chappell, O. T. G. Jones, Internal pH changes associated with the activity of NADPH oxidase of human neutrophils: Further evidence for the presence of an H^+ conducting channel. *Biochem. J.* **251**, 563–567 (1988).
29. L. M. Henderson, J. B. Chappell, O. T. G. Jones, Superoxide generation by the electrogenic NADPH oxidase of human neutrophils is limited by the movement of a compensating charge. *Biochem. J.* **255**, 285–290 (1988).
30. L. Simchowitz, M. A. Foy, E. J. Cragoe, A role for Na^+/Ca^{2+} exchange in the generation of superoxide radicals by human neutrophils. *J. Biol. Chem.* **265**, 13449–13456 (1990).
31. A. Kapus, K. Szászi, E. Ligeti, Phorbol 12-myristate 13-acetate activates an electrogenic H^+-conducting pathway in the membrane of neutrophils. *Biochem. J.* **281**, 697–701 (1992).
32. A. Lowenthal, R. Levy, Essential requirement of cytosolic phospholipase A_2 for activation of the H^+ channel in phagocyte-like cells. *J. Biol. Chem.* **274**, 21603–21608 (1999).
33. A. W. Girotti, J. P. Thomas, J. E. Jordan, Inhibitory effect of zinc(II) on free radical lipid peroxidation in erythrocyte membranes. *J. Free Radic. Biol. Med.* **1**, 395–401 (1985).
34. P. J. Thornalley, M. Vašák, Possible role for metallothionein in protection against radiation-induced oxidative stress. Kinetics and mechanism of its reaction with superoxide and hydroxyl radicals. *Biochim. Biophys. Acta* **827**, 36–44 (1985).
35. J. P. Thomas, G. J. Bachowski, A. W. Girotti, Inhibition of cell membrane lipid peroxidation by cadmium- and zinc-metallothioneins. *Biochim. Biophys. Acta* **884**, 448–461 (1986).
36. C. Eder, T. E. DeCoursey, Voltage-gated proton channels in microglia. *Prog. Neurobiol.* **64**, 277–305 (2001).
37. R. van Zwieten, R. Wever, M. N. Hamers, R. S. Weening, D. Roos, Extracellular proton release by stimulated neutrophils. *J. Clin. Invest.* **68**, 310–313 (1981).
38. N. Borregaard, J. H. Schwartz, A. I. Tauber, Proton secretion by stimulated neutrophils: Significance of hexose monophosphate shunt activity as source of electrons and protons for the respiratory burst. *J. Clin. Invest.* **74**, 455–459 (1984).
39. T. G. Gabig, B. A. Lefker, P. J. Ossanna, S. J. Weiss, Proton stoichiometry associated with human neutrophil respiratory-burst reactions. *J. Biol. Chem.* **259**, 13166–13171 (1984).
40. K. Takanaka, P. J. O'Brien, Proton release associated with respiratory burst of polymorphonuclear leukocytes. *J. Biochem. (Tokyo)* **103**, 656–660 (1988).
41. A. Nanda, S. Grinstein, Protein kinase C activates an H^+ (equivalent) conductance in the plasma membrane of human neutrophils. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 10816–10820 (1991).
42. A. Jankowski, S. Grinstein, A noninvasive fluorimetric procedure for measurement of membrane potential. *J. Biol. Chem.* **274**, 26098–26104 (1999).
43. J. L. Bankers-Fulbright, G. J. Gleich, G. M. Kephart, H. Kita, S. M. O'Grady, Regulation of eosinophil membrane depolarization during NADPH oxidase activation. *J. Cell Sci.* **116**, 3221–3226 (2003).
44. D. Roos, C. C. Winterbourn, Lethal weapons. *Science* **296**, 669–671 (2002).

Citation: T. E. DeCoursey, During the respiratory burst, do phagocytes need proton channels or potassium channels, or both? *Sci. STKE* **2004**, pe21 (2004).