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# TRIGGERING AND REGULATION OF THE FREE RADICAL PRODUCTION BY PHAGOCYTES

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## INTRODUCTION

Phagocytic cells (neutrophils, eosinophils, monocytes and macrophages) are capable of converting oxygen into potentially toxic species such as superoxide anion, hydrogen peroxide and hydroxyl radical. This peculiar metabolic pathway, which is called respiratory burst, is turned on when a membrane-bound enzyme, the NADPH oxidase, is activated. Other reactions, such as those of the glutathione cycle and of the hexose monophosphate pathway, are secondary to the triggering of NADPH oxidase, having the function of continuous supply of reduced NADPH and of intracellular detoxification.[1-3]

The molecular structure of the NADPH oxidase has not completely clarified yet, but there is evidence that it is composed by an electron transport chain, where a flavoprotein, a cytochrome b with low potential (cytochrome $b_{558}$, or cytochrome $b_{-245}$) and possibly other proteins of unknown nature are assembled in a functional complex.[4-7] Membrane phospholipids give stability and possibly regulate the function of this complex.[8-9]

The free radical forming system is activated during phagocytosis and the generation of oxygen free radicals significantly contributes to the defensive (bactericidal and tumoricidal) function of neutrophils, eosinophils and macrophages. On the other hand, other agents that are not related to phagocytosis may trigger the respiratory burst. Toxic oxygen derivatives may diffuse into the extracellular space and damage connective tissue macromolecules, cell membranes and even cause DNA mutations.

In this brief review we will consider: I) the agents that are able of triggering the respiratory burst, especially in

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relation with lipid metabolism, II) the mechanisms of their action and III) the possibilities of regulation of the oxidative metabolism at cellular level.

## I. AGENTS THAT TRIGGER THE RESPIRATORY BURST OF PHAGOCYTES

As it can be seen in Table 1, besides the phagocytosable particles, a large series of substances with different chemical composition are able of interacting with the cell leading to its activation. The action of some of these agents may be related to lipid metabolism and vascular pathology. In fact, arachidonic acid, leucotriene $B_4$, platelet activating factor are potent stimulants of the burst and at the same time are produced and released by activated leukocytes, therefore acting as messengers and signals for further cell activation and amplification of the inflammatory process. The effect of acetylated LDL is noteworthy, because monocytes and macrophages exert a scavenger function into the vessel intima by taking up excess of modified lipoproteins. However, concomitant activation of oxygen free radical release could be one of the pathogenetic mechanisms of damage to the vessel wall,

Table 1. Some stimulants of phagocyte's metabolism

| | REF. |
|---|---|
| PARTICLES | |
| Opsonized bacteria, fungi, virus | 10,11 |
| Immunoglobulin aggregates | 12 |
| LIPIDS AND LIPID DERIVATIVES | |
| Arachidonic acid and other fatty acids | 13,14 |
| Leukotriene $B_4$ | 15 |
| Diacylglycerol | 16 |
| Platelet activating factor | 17 |
| Acetylated LDL | 18 |
| Cerebrosides | 19 |
| PROTEINS | |
| Concanavalin A | 20 |
| Anti-leukocyte antibodies | 21,22 |
| Complement fragments (C5a, C567) | 23,24 |
| Tumor necrosis factor | 25 |
| Phospholipase C | 26 |
| PEPTIDES | |
| N-formylated peptides | 27 |
| Substance P | 28 |
| OTHERS | |
| Calcium ionophores | 29 |
| Urate crystals | 30 |
| Sodium fluoride | 31 |
| Low-sodium solutions | 32 |
| Detergents | 33,34 |
| Cross-linking reagents | 35 |

inflammation, sclerosis and possibly cell transformation.

Among the proteins, the effect of C5a and of tumor necrosis factor (also called cachectin) are probably important in human pathology. By triggering leukocyte metabolism, the intravascular complement activation and the release of TNF by activated mononuclear phagocytes may be responsible for wasting systemic effects and damage to pulmonary microvasculature that often complicate sepsis, severe burns, shock.

The stimulatory effect of phospholipase C reveals the importance of membrane phospholipids in the triggering and regulation of oxidative metabolism. This is confirmed by the direct stimulatory effect of diacylglycerol, the main product of phospholipase C activity. Preliminary data from our laboratory indicate that also phosphatidic acid, that is formed in the cell both by phosphorylation of diacylglycerol and by action of phospholipase D, is able of activating $H_2O_2$ production by human neutrophils.

As far as the peptides are concerned, it has been recently discovered in our laboratory[28] that substance P (SP) is a stimulant of $H_2O_2$ production by human neutrophils. This undecapeptide is widely distributed in the nervous system and is particularly concentrated in the peripheral nerve terminals of small diameter unmyelinated sensory neurons, termed C-fibers, which terminate in the dorsal horn of spinal cord. SP containing fibers have been found also into the vessel wall. Although SP is considered to be a neurotransmitter at the central terminals of C-fibers, up to 90% of the peptide synhesized in the cell bodies of these neurons is transported to the peripheral terminals, from where it can be released by noxious stimuli. Fig. 1 provides a possible interpretation of

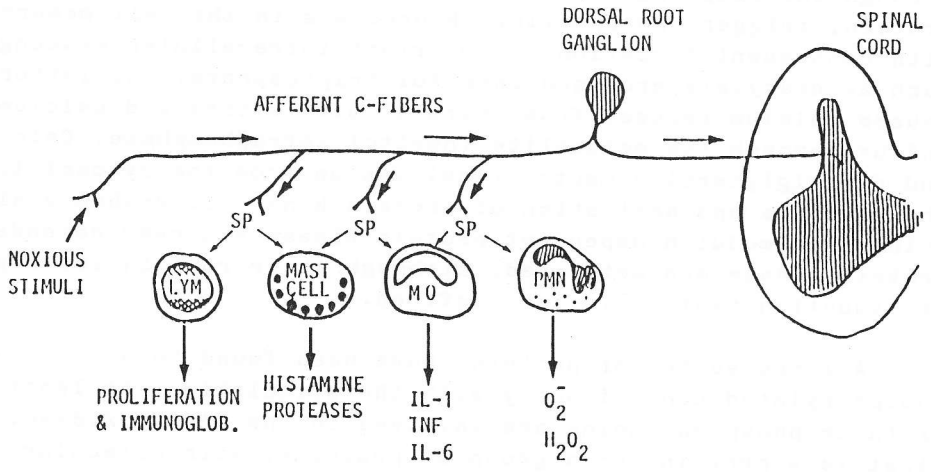


Fig. 1. Effects of substance P on the inflammatory cells

the physiological role of SP. Besides the stimulation of oxidative metabolism of neutrophils, it is known that SP produces vasodilatation, it acts as mitogen for lymphocytes, it degranulates mast cells and activates macrophages. This neuropeptide therefore meets many of the requirements for a mediator of the local inflammatory response and represents an important link between nervous and immunological systems.

## II. MECHANISMS OF ACTIVATION OF THE RESPIRATORY BURST

The mechanisms by which agonist-stimulated receptors are coupled with the terminal effector systems such as phagocytosis, degranulation, movement, free radical production, gene expression, etc., are called transduction pathways (or systems). The matter is very complex because multiple pathways have been described, that may vary according to the stimulant used, and also inhibitory mechanisms may be operative in particular conditions. Clearly, elucidating the transduction systems is important because the intensity and the duration of the functional responses, including the respiratory burst, may be regulated at this level.

In the attempt to simplify the understanding of this point, three major hypothesis that provide an explanation of how the NADPH oxidase may be activated are here presented. More details may be found in other recent reviews.[3,36-39]

### II.a. Protein phosphorylation

Phosphorylation and dephosphorylation of specific proteins regulates a variety of cells responsive to external stimuli. There is increasing evidence that this mechanism operates also in neutrophils for the activation of NADPH oxidase and other functions. As shown in Fig. 2, the ligand-receptor interaction, through the coupling action of a guanine nucleotide binding protein, triggers phospholipid hydrolysis in the cell membrane, with consequent formation of important intracellular messengers such as diacylglycerol and inositol triphosphate. The latter causes calcium release from intracellular stores and calcium influx through its metabolite inositol tetraphosphate. Calcium and diacylglycerol promote translocation from the cytosol to the membrane and activation of protein kinase C. Probably also calcium/calmodulin dependent protein kinase and cAMP dependent protein kinase are activated, although their role in leukocyte transduction systems is less defined.

A large series of proteins have been found to be phosphorylated concomitantly with the stimulation. At least two of these phosphoproteins are involved in the NADPH oxidase. The first is a protein, or a group of proteins, with molecular weight of about 48 kDa. Although their nature is not known, the participation of these proteins is strongly suggested by the

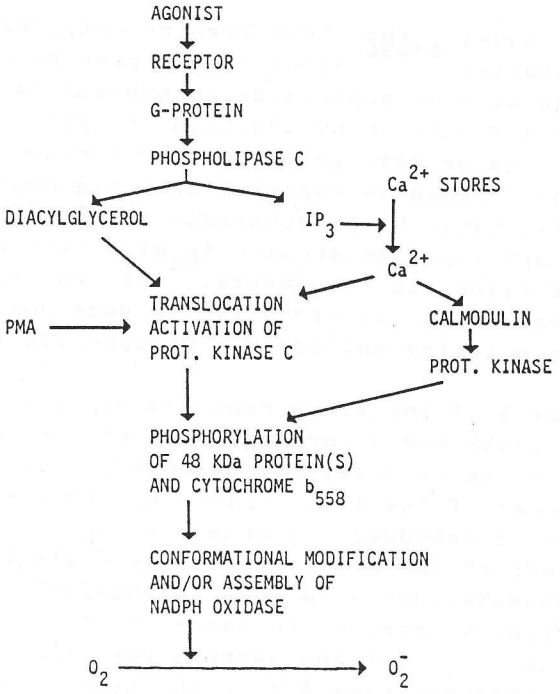


Fig. 2. NADPH oxidase activation by protein phosphorylation

observation that their phosphorylation is lacking in some forms of a disease - chronic granulomatous disease of childhood (CGD) - where the respiratory burst fails to be activated.[40-44] The second relevant protein that is phosphorylated is cytochrome $b_{558}$. This cytochrome is actually a component of the enzyme NADPH oxidase and its phosphorylation suggest a possible regulatory mechanism at this level. The kinase responsible for this modification of the protein is probably protein kinase C.[45]

II.b. Membrane lipid changes

The relationship between phosphorylation of cytochrome b and the enzymatic activation is still hypothetical, because there is no direct demonstration that phosphorylation directly triggers the enzyme. Studies carried out in our laboratory have shown that in cells stimulated with phorbol esters or opsonized zymosam there is marked phosphorylation and a proportional NADPH oxidase activation, while in cells stimulated with arachidonic acid a very little phosphorylation is accompanied by an high activation. We therefore concluded that phosphorylation is not the only activation mechanism[46] and this fact was confirmed by others.[44]

The existence of additional, or alternative, pathways of oxidase activation is also indicated by studies of activation mechanism carried out not in intact cells but in cell-free

systems. These models, that have been recently developed in several laboratories,[47-56] allow the triggering of the enzymatic production of superoxide in subcellular organelles or even in purified fractions by addition of cytosolic components and of fatty acids or detergents such as sodium dodecyl sulphate. We have recently reported that pig neutrophil NADPH oxidase is activatable by phosphatidic acid, an important product of phospholipid metabolism in stimulated cells, even in the absence of cytosolic components.[54] We and others have shown that the activation in cell-free system does not depend on the protein kinase activity and protein phosphorylation.

On the basis of the above reported data, it is possible to construct an hypothesis according to which the terminal modification of the oxidase, responsible for its activation, is caused by changes of the lipid milieu of the membrane where the enzyme complex is embedded. As shown in Fig. 3, the lipid changes that affect the oxidase activity could be either an increase of phosphatidic acid (due to phospholipase D and/or to diacylglycerol kinase) or an increase of arachidonic acid (due to calcium-dependent and perhaps receptor-dependent activation of phospholipase A2). Both these lipid changes have been documented in the membrane of stimulated cells. The alteration of lipid properties (fluidity, electric charges, melting point, etc.) in the enzyme microenvironment may cause conformational modifications and assembly of memebrane and cytosolic components of the oxidase. The electron-transport system can thus start to catalyse superoxide formation.

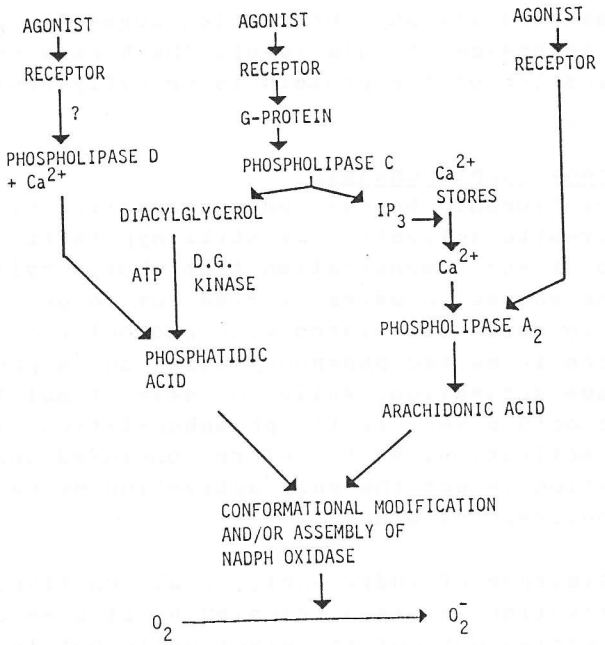


Fig. 3. NADPH oxidase activation by membrane lipid changes

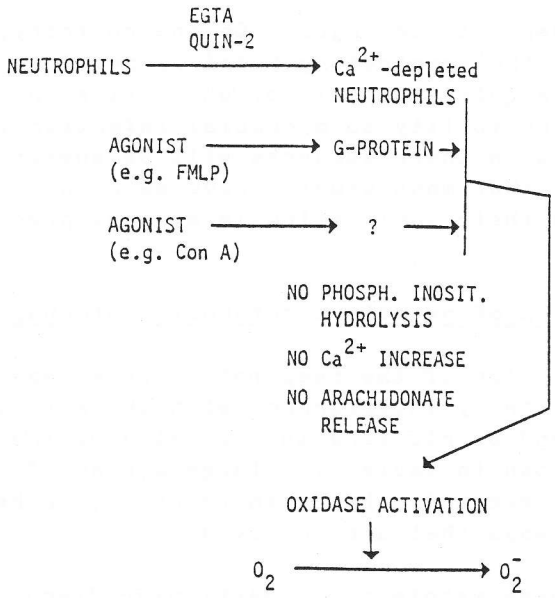


Fig. 4. A third existing pathway of NADPH oxidase activation

II.c. Calcium and phosphoinositide-independent pathway

The existence of a third mechanism that is independent of calcium and lipid changes may be postulated on the basis of recent work from the group of F. Rossi in our laboratory.[57-59] This is schematically represented in Fig. 4. An experimental model has been developed where the neutrophils are completely depleted of intracellular free calcium by the addition of chelators. In these conditions no modifications of free calcium, no phospholipid hydrolysis, no arachidonic acid and phosphatidic acid formation occur. When these cells are challenged with two different agents, either given in sequence or contemporaneously, they undergo to marked metabolic stimulation. Therefore a further and unknown activation mechanism exists and is currently investigated in our laboratory. It remains to be established whether this mechanism, that is operative in calcium depleted cells, is alternative or is additional to the other pathways previously described.

## III. REGULATION OF THE RESPIRATORY BURST

On the basis of the knowledge of the structure and the activation mechanism of the NADPH oxidase it is possible to deal with the possible ways of regulating the respiratory burst. This subject is of great interest because it would be useful to decrease, or increase, the intensity and the duration of free radical production when required. An inhibition, or down dregulation of the burst is theoretically desirable during pathologic inflammatory processes in order to decrease free

radical dependent tissue injury. On the contrary, an enhancement of the respiratory burst is required in the case of congenital or acquired defects of phagocytes that often cause increased susceptibility to microbial infections. Here the main literature data on these subjects will be summarized. it should be pointed out that most studies have been done on isolated leukocytes and their application in medical practice is still hypothetical.

## III.a Inhibition of oxidative metabolism of phagocytes

The inhibition of the respiratory burst may be accomplished both by interference with the activation mechanism(s) and by blocking the activity of the terminal oxidase. As shown in Table 2, a large series of inhibitors of the activation mechanism has been reported, in keeping with the multiform pathways that are involved.

Some of these agents merit particular discussion. The homologous pre-stimulation causes de-sensitization of the

Table 2. Inhibitors of the respiratory burst that act on some step of the activation mechanism

| Agent | Possible mechanism | Ref. |
|---|---|---|
| Albumin (on arach. acid) | Binding to stimulant | 60 |
| $H_2O_2$ + peroxidase + halide | Inactivation of stimulant | 61 |
| -met-mannopyr.(on Con A) | Displacement of ligand | 20 |
| Homologous pre-stimulation | Receptor desensitization | 62,63 |
| Agonist-coated surfaces | Receptor down-regulation | 64,65 |
| Tumor-conditioned medium | ? | 66 |
| PDGF | Post-recept. deactivation | 67 |
| Pertussis toxin | G-protein inactivation | 68 |
| Bromophenacylbromide | Phospholipase inhibitor | 69 |
| Quinacrine | Membrane perturbation | 70 |
| Corticosteroids | Inhibition of Ph.lipase A2 | 71 |
| Non-ster. antiinfl. agents | Various | 72,73 |
| Prostaglandins (E2,D2) | cAMP increase | 74,75 |
| Adenosine | cAMP increase | 76,77 |
| Nifedipine, Verapamil | Calcium antagonists | 78-80 |
| Trifluoperazine | Calmodulin inhibitor | 81,82 |
| TPCK, DFP | Protease inhibitors | 83,84 |
| Sphinganine, H-7, C-I, etc. | Prot. kinase C inhibitors | 85 |
| Nordihydroguaiaretic acid | Lipooxygenase inhibitor | 86 |
| Disuccinimidyl suberate | Crosslinking reagent | 87 |
| Opioids, benzodiazepines | ? | 88,89 |
| Anaesthetics (halotane, lidocaine) | ? | 90,91 |
| Bee venom melittin | ? | 92 |

receptor. When the cells come in contact with low concentrations of a stimulant they do not activate the burst, on the contrary they become unresponsive to a second challenge with the same stimulant. This is an important mechanism that inhibits the triggering of the burst in phagocytes that are exposed to a gradient of chemotactic agents, that is during their movement from vessels to the centre of the inflammatory site.

Some tumors produce inhibitory factors, whose nature has to be determined. Recent data suggest that one of these factors may be transforming-growth factor ß.[93] This mechanism could protect the tumor cells from the oxidative attack by phagocytes and therefore could allow them to escape host defence systems.

The effect of platelet-derived growth factor (PDGF) may have physiological relevance. PDGF inhibits the respiratory burst at concentrations that are present in serum during the hemostatic process. PDGF does not inhibit phagocytosis and chemotaxis. This factor may therefore play an important regulatory role during hemostasis and wound healing, because it prevents unsuitable activation of the burst while it does not affect the scavenger function of these cells.

Phospholipase inhibitors are important tools for investigating the role of phospholipid hydrolysis in the activation mechanism, but their specificity is not well established. Powerful inhibitors such as bromophenacyl bromide are too toxic for use in vivo. On the other hand, corticosteroids are poor inhibitors of the respiratory burst, probably because they do not influence phospholipase C activity.

A second possibility for down-regulating the respiratory burst is the inhibition of the NADPH oxidase. The list of inhibitors is reported in table 3. Most of these agents have interest for research purposes only. They have been useful for exploring the participation of individual components in the catalysis.

A recent advance in the knowledge of the nature of the oxidase has been provided by the production of antibodies that inhibit the enzymatic activity. By this way proteins, with molecular weight of 65 kDa,[105] 70 kDa[106] and a heterodimer of 16/18 and 14 kDa,[107] that participate in the activity of the oxidase have been identified.

Practical application could have vitamin E, gold salts (that in rheumatoid arthritis are used as antiinflammatory agents) and possibly diphenylene iodonium. Imidazole is an

Table 3. Agents tat inhibit the terminal oxidase of the respiratory burst

| Agent | Possible mechanism | Ref. |
|---|---|---|
| Cibacron blue | NADPH analogue | 94 |
| %-carba-deaza FAD | Flavin analogue | 95 |
| Diphenylene iodonium | Flavoprotein inhibitor | 96 |
| Pyridine, imidazole | Cytochrome b inhibitor | 97 |
| Quinones, vitamin E | Interference with electron transport | 98-100 |
| EDTA | $Ca^{2+}$ and $Mg^{2+}$ chelation | 101 |
| Batophenanthroline sulfonate | $Fe^{3+}$ chelation | 5,102 |
| P-chloromercuribenzoate | Sulfhydryl reagent | 103,104 |
| Antibodies against proteins of 65-70, 18, 14 kDa | Binding to oxidase | 105-107 |
| Strong detergents and salts | Dissociation of complex | 108 |
| $H_2O_2$ + peroxidase + halide | Oxidative inactivation | 109 |
| Heat shock | ? | 110 |
| Gold salts | ? | 111 |

inhibitor of NADPH oxidase, but at too high concentration for to be used in vivo.

III.b. Enhancement of the respiratory burst

The response of the phagocyte to a stimulant may be potentiated essentially according to two mechanisms: one is the priming effect, the other is the activation by cytokines. These are physiological phenomena that serve for the enhancement of resistance to infection and there is the hope that in near future they may be utilized also for the pharmacological treatment of immunocompromised host. The priming effect is observed when the cells are exposed either to chemoattractants or to several other compounds (see table V-A) and become more responsive to a second different stimulant. The precise modification that is responsible for the priming is not clear. Theeffect takes place very rapidly in treated cells, but it is not permanent and the increased responsiveness disappears after a few minutes.

These features distinguish priming from the up-regulation of the burst induced by cytokines. This effect was initially thought to be a property of mononuclear phagocytes only but it has been recently described also in neutrophils.[125-127] The activation requires several hours of treatment of the cells and consists in a permanent modification of the responsiveness that probably involves new gene expression.

The cytokines that are able of augment the respiratory burst are interferon-$\gamma$, granulocyte-macrophage colony stimulating factor, tumor necrosis factor and interleukin 1.

Table 4. Agents that potentiate the respiratory burst

| | | | |
|---|---|---|---|
| **A. Priming effect** | | | |
| Chemotactic factors | (112,113) | Lypopolysaccharide | (114,115) |
| Phorbol esters | (116) | Leukotriene B4 | (117) |
| Diacylglycerol | (118,119) | Platelet activ. factor | (120) |
| Concanavalin A | (57) | ATP | (121,122) |
| Muramyl peptide | (114) | | |
| **B. Cytokines** | | | |
| Interleukin-1 | (123) | Interferon-$\gamma$ | (124-127) |
| GM-Colony stim. factor | (128) | Tumor necrosis factor | (129) |

The mechanism of the potentiating effect of cytokines is under active investigation. It has been shown that interferon-$\gamma$ increases membrane receptors for immunoglobulins,[130] increases gene expression for several proteins including cytochrome $b_{558}$[131,132] and induces a shift of the oxidase from a form with low affinity for NADPH to a form with high affinity.[133] The availability of human recombinant cytokines has given new support to these studies. Interferon-$\gamma$ has been already employed in vivo with promising results. This cytokine has been shown to increase $H_2O_2$ production by phagocytes in patients with tumors[134] and to improve oxidative metabolism of phagocytes in a variant of CGD.[135,136] The study of the effects in vivo and in vitro of cytokines will be one of the most important research fields for leukocytologists in near future.

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REFERENCES

1. S. J. Klebanoff and R. A. Clark, "The Neutrophil: Function and Clinical Disorders", North Holland Publ. Comp., Amsterdam (1978).
2. B. M. Babior, R. S. Kipnes and J. T. Curnutte, Biological defense mechanisms. The production by leukocytes of superoxide, a potential bactericidal agent, J. Clin. Invest. 52: 7414 (1973).
3. F. Rossi, The $O_2^-$ forming NADPH oxidase of the phagocytes: nature, mechanisms of activation and function, Biochim. Biophys. Acta 853: 65 (1986).
4. P. Bellavite, O. T. G. Jones, A.R. Cross, E. Papini and F. Rossi, Composition of partially purified NADPH oxidase from pig neutrophils, Biochem. J. 223: 639 (1984).
5. G. Berton, E. Papini, M. Cassatella, P. Bellavite and F. Rossi, Partial purification of the superoxide-generating

system of macrophages. Possible association of the NADPH oxidase activity with a low potential cytochrome b, Biochim. Biophys. Acta 810: 164 (1985).

6. J. Doussiere and P. V. Vignais, Purification and properties of $O_2^-$-generating oxidase from bovine polymorphonuclear neutrophils, Biochemistry 24: 7231 (1985).
7. G. A. Glass, D. M. DeLisle, P. DeTogni, T. G. Gabig, B. H. Magee, M. Markert and B. M. Babior, The respiratory burst oxidase of human neutrophils. Further studies of the purified enzyme, J. Biol. Chem. 261: 13247 (1986).
8. T. G. Gabig and B. M. Babior, The $O_2^-$ forming oxidase responsible for the respiratory burst in human neutrophils. Properties of the solubilized enzyme, J. Biol. Chem. 254: 9070 (1979).
9. M. L. Karnovsky, Comparative aspects of the production of oxygen radicals by phagocytic cells, and aspects of other effector substances, Int. J. Tiss. Reac. 8: 91 (1986).
10. R. B.Jr. Johnston, Oxygen metabolism and the microbicidal activity of macrophages, Fed. Proc. 37: 2759 (1978).
11. C. F. Nathan, Regulation of macrophage oxidative metabolism and parasitic activity, In:"Mononuclear Phagocytes. Characteristics, Physiology and Function", R. Van Furth ed., Martinus Niijhoff Publishers, Dordrecht, 411 (1985).
12. P. M. Henson and Z. G. Oades, Stimulation of human neutrophils by soluble and insoluble immunoglobulin aggregates. Secretion of granule constituents and increased oxidation of glucose, J. Clin. Invest. 56: 1053 (1975).
13. K. Kakinuma, Effects of fatty acids on the oxidative metabolism of leukocytes, Biochim. Biophys. Acta 348: 76 (1974).
14. T. Yamaguchi, M. Kaneda and K. Kakinuma, Effect of saturated and unsaturated fatty acids on the oxidative metabolism of human neutrophils. The role of calcium ion in the extracellular medium, Biochim. Biophys. Acta 861: 440 (1986).
15. H. Sumimoto, K. Takeshige and S. Minakami, Superoxide production of human polymorphonuclear leukocytes stimulated by leukotriene B4, Biochim. Biophys. Acta 803: 271 (1984).
16. I. Fujita, K. Irita, K. Takeshige and S. Minakami, Diacylglycerol, 1-oleoyl-2-acetyl-glycerol, stimulates superoxide generation from human neutrophils, Biochem. Biophys. Res. Commun. 120: 318 (1984).
17. H. P.Hartung, M.J. Parnham, J. Winkelman, W. Englberger and U. Hadding, Platelet activating factor (PAF) induces the oxidative burst in macrophages, Int. J. Immunopharmacol. 5: 115 (1983).
18. H. P. Hartung, R. G. Kladetzky, B. Melnik and M. Hennerici, Stimulation of the scavenger receptor on monocytes-macrophages evokes release of arachidonic acid

metabolites and reduced oxygen species, Lab. Invest. 55: 209 (1986).

19. T. Chiba, Y. Nagai and K. Kakinuma, Cerebroside sulfuric ester (sulfatide) induces oxygen radical generation in guinea-pig leukocytes, Biochem. Biophys. Acta 930: 10 (1987).
20. D. Romeo, G. Zabucchi and F. Rossi, Reversible metabolic stimulation of polymorphonuclear leukocytes and macrophages by concanavalin A, Nature 243: 111 (1973).
21. F. Rossi, M. Zatti, P. Patriarca and R. Cramer, Stimulation of the respiration of polymorphonuclear leucocytes by antileucocyte antibodies, Experientia 26: 491 (1970).
22. G. Berton, H. Rosen, R. A. B. Ezekowitz, P. Bellavite, M. C. Serra, F. Rossi and S. Gordon, Monoclonal antibodies to a particulate superoxide-forming system stimulate a respiratory burst in intact guinea pig neutrophils, Proc. Natl. Acad. Sci. USA 83: 4002 (1986).
23. F. Tedesco, S. Trani, M.R. Soranzo and P. Patriarca, Stimulation of glucose oxidation in human polymorphonuclear leucocytes by C3-sepharose and soluble C567, FEBS Lett. 51: 232 (1975).
24. R. Gennaro, T. Pozzan and D. Romeo, Monitoring of cytosolic free $Ca^{2+}$ in C5a-stimulated neutrophils: Loss of receptor-modulated $Ca^{2+}$ stores and $Ca^{2+}$ uptake in granule-free cytoplasts, Proc. Natl. Acad. Sci. USA 81: 1416 (1984).
25. M. Tsujimoto, S. Yokota, J. Vilcek and G. Weissmann, Tumor necrosis factor provokes superoxide anion generation from neutrophils, Biochem. Biophys. Res. Commun. 137: 1094 (1986).
26. P. Patriarca, M. Zatti, R. Cramer and F. Rossi, Stimulation of the respiration of polymorphonuclear leucocytes by phospholipase C, Life Sci. 9: 841 (1970).
27. L. C. McPhail and R. Snyderman, Activation of the respiratory burst enzyme in human polymorphonuclear leukocytes by chemoattractants and othe soluble stimuli. Evidence that the same oxidase is activated by different transductional mechanisms, J. Clin. Invest. 72: 192 (1983).
28. M. C. Serra, F. Bazzoni, V. DellaBianca, M. Grzeskowiak and F. Rossi, Activation of human neutrophiuls by substance P: Effect on oxidative metabolism, exocytosis, cytosolic $Ca^{2+}$ concentration and inositol phosphates formation, J. Immunology 141: 2118 (1988).
29. E. L. Becker, M. Sigman and J. M. Oliver, Superoxide production induced in rabbit polymorphonuclear leukocytes by synthetic chemotactic peptides and A23187, Am. J. Pathol. 95: 81 (1979).
30. C. Salerno, Urate crystal-induced superoxide radical production by human neutrophils, Adv. Exp. Med. Biol. 165: 184 (1984).

31. J. T. Curnutte, B. M. Babior and M. L. Karnovsky, Fluoride-mediated activation of the respiratory burst in human neutrophils. A reversible process, J. Clin. Invest. 63: 637 (1979).
32. F. Rossi, V. Della Bianca and A. Davoli, A new way for inducing a respiratory burst in guinea pig neutrophils. Change in the $Na^+$, $K^+$ concentration of the medium, FEBS Lett. 132: 273 (1981).
33. R. C. Graham, M. J. Karnovsky, A. W. Shafer, E. A. Glass and M. L. Karnovsky, Metabolic and morphological observations on the effect of surface-active agents on leukocytes, J. Cell. Biol. 32: 629 (1967).
34. F. Rossi and M. Zatti, Mechanism of the respiratory stimulation in saponine-treated leukocytes. The KCN insensitive oxidation of NADPH, Biochim. Biophys. Acta 153: 296 (1968).
35. A. Aviram and I. Aviram, Activation of guinea-pig and bovine neutrophil NADPH oxidase by N,N'- dicyclohexylcarbodiimide, Biochim. Biophys. Acta 844: 224 (1985).
36. L. C. McPhail and R. Snyderman Mechanisms of regulating the respiratory burst in leukocytes, In:" Regulation of Leukocyte Function" R. Snyderman, ed., Plenum Press, New York 247 (1984).
37. A. I. Tauber, Protein kinase C and the activation of the human neutrophil NADPH-oxidase, Blood 69: 711 (1987).
38. P. Bellavite, The superoxide-forming enzymatic system of phagocytes, Free Radical Biol. Med. 4: 225 (1988).
39. R. R. Sandborg and J. E. Smolen, Biology of disease. Early biochemical events in leukocyte activation, Lab. Invest. 59: 300 (1988).
40. A.W. Segal, P.G. Heyworth, S. Cockroft and M.M. Barrowman, Stimulated neutrophils from patients with autosomal recessive chronic granulomatous disease fail to phosphorylate a Mr-44,000 protein, Nature 316: 547 (1985).
41. T. Hayakawa, K. Suzuki, S. Suzuki, P. C. Andrews and B. M. Babior, A possible role for protein phosphorylation in the activation of the respiratory burst in human neutrophils. Evidence for studies with cells from patients with chronic granulomatous disease, J. Biol. Chem. 261: 9109 (1986).
42. I. M. Kramer, A. J. Verhoeven, R. vanderBend, R.S. Weening and D. Roos, Purified protein kinase C phosphorylates a 47-kDa Protein in control neutrophil cytoplasts but not in neutrophil cytoplasts from patients with the autosomal form of chronic granulomatous disease, J. Biol. Chem. 263: 2352 (1988).
43. R.C. Garcia and A.W. Segal, Phosphorylation of the subunits of cytochrome b-245 upon triggering of the respiratory burst of human neutrophils and macrophages, Biochem. J. 252: 901 (1988).
44. N. Okamura, J. T. Curnutte, R. L. Roberts and B. M. Babior,

Relationship of protein phosphorylation to the activation of the respiratory burst in human neutrophils. Defects of the phosphorylation of a group of closely related 48-kDa proteins in two forms of chronic granulomatous disease, J. Biol. Chem. 263: 6777 (1988).

45. E. Papini, M. Grzeskowiak, P. Bellavite and F. Rossi, Protein kinase C phosphorylates a component of NADPH oxidase of neutrophils, FEBS Lett. 190: 204 (1985).
46. P. Bellavite, S. Dusi and M. A. Cassatella, Studies on the nature and activation of $O_2^-$-forming NADPH oxidase of leukocytes II. Relationships between phosphorylation of a component of the enzyme and oxidase activity, Free Rad. Res. Commun. 4: 83 (1987).
47. Y. Bromberg and E. Pick, Unsaturated fatty acids stimulate NADPH-dependent superoxide production by cell-free system derived from macrophages, Cell. Immunol. 88: 213 (1984).
48. R. A. Heyneman and R. E. Vercauteren, Activation of a NADPH oxidase from horse polymorphonuclear leukocytes in a cell-free system, J. Leukocyte Biol. 36: 751 (1984).
49. I. Maridonneau-Parini and A. I. Tauber, Activation of NADPH oxidase by arachidonic acid involves phospholipase A2 in intact human neutrophils but not in the cell-free system, Biochem. Biophys. Res. Commun. 138: 1099 (1986).
50. J. T. Curnutte, Activation of human neutrophil nicotinamide adenine dinucleotide phosphate, reduced (triphosphopyridine nucleotide, reduced) oxidase by arachidonic acid in a cell-free system, J. Clin. Invest. 75: 1740 (1985).
51. L. C. McPhail, P. S. Shirley, C. C. Clayton and R. Snyderman, Activation of the respiratory burst enzyme from human neutrophils ina cell-free system. Evidence for a soluble cofactor, J. Clin. Invest. 75: 1735 (1985).
52. R. A. Clark, K. G. Leidal, D. W. Pearson and W. M. Nauseef, NADPH oxidase of human neutrophils: Subcellular localization and characterization of an arachidonate-activatable superoxide-generating system, J. Biol. Chem. 262: 4065 (1987).
53. R. Seifert, W. Rosenthal and G. Schultz, Guanine nucleotides stimulate NADPH oxidase in membranes of human neutrophils, FEBS Lett. 205: 161 (1986).
54. P. Bellavite, F. Corso, S. Dusi, M. Grzeskowiak, V. Della Bianca and F. Rossi, Activation of a NADPH-dependent superoxide production in plasma-membrane extracts of pig neutrophils by phosphatidic acid, J. Biol. Chem. 263: 8210 (1988).
55. E. Ligeti, J. Doussiere and P. V. Vignais, Activation of the $O_2^-$-generating oxidase in plasma membrane from bovine polymorphonuclear neutrophils by arachidonic acid, a cytosolic factor of protein nature, and nonhydrolyzable

analogues of GTP, Biochemistry 27: 193 (1988).

56. I. Fujita, K. Takeshige and S. Minakami, Characterization of the NADPH-dependent superoxide production activated by sodium dodecyl sulfate in a cell-free system of pig neutrophils, Biochim. Biophys. Acta 931: 41 (1987).
57. F. Rossi, M. Grzeskowiak and V. Della Bianca, Double stimulation with FMLP and Con A restores the activation of the respiratory burst but not of the phosphoinositide turnover in $Ca^{2+}$-depleted human neutrophils. A further example of dissociation between stimulation of the NADPH oxidase and phosphoinositide turnover, Biochem. Biophys. Res. Commun. 140: 1 (1986).
58. M. Grzeskowiak, V. Della Bianca, M. Cassatella and F. Rossi, Complete dissociation between the activation of phosphoinositide turnover and of NADPH oxidase by Formyl-methionyl-leucyl-phenylalanine in human neutrophils depleted of $Ca^{2+}$ and primed by subtreshold doses of phorbol myristate acetate, Biochem. Biophys. Res. Commun. 135: 785 (1986).
59. F. Rossi, V. Della Bianca, M. Grzeskowiak and F. Bazzoni, Studies on molecular regulation of phagocytosis in neutrophils. Con A-mediated ingestion and associated respiratory burst independent of phosphoinositide turnover, rise in $Ca^{2+}$ i and arachidonic acid release. J. Immunol. (in press)
60. J. A. Badwey, J. T. Curnutte, J. M. Robinson, C. B. Berde, M. J. Karnovsky and M. L. Karnovsky, Effects of free fatty acids on release of superoxide and on change of shape by human neutrophils. Reversibility by albumin, J. Biol. Chem. 259: 7870 (1984).
61. P. De Togni, P. Bellavite, V. Della Bianca, M. Grzeskowiak and F. Rossi, Intensity and kinetics of the respiratory burst of human neutrophils in relation to receptor occupancy and rate of occupation by formyl-methionyl-leucyl-phenylalanine, Biochim. Biophys. Acta 838: 12 (1985).
62. L. Simkowitz, J. P. Atkinson and I. Spilberg, Stimulus-specific deactivation of chemotactic factor-induced cyclic AMP response and superoxide generation by human neutrophils, J. Clin. Invest. 66: 736 (1980).
63. L. A. Sklar, A. J. Jesaitis, R. G. Painter and C. G. Cochrane, The kinetics of neutrophil activation. The response to chemotactic peptides depends upon whether ligand-receptor interaction is rate-limiting, J. Biol. Chem. 256: 9909 (1981).
64. G. Berton and S. Gordon, Desensitization of macrophages to stimuli which induce secretion of superoxide anion. Down-regulation of receptors for phorbol myristate acetate, Eur. J. Immunol. 13: 620 (1983).
65. E. A. Valletta and G. Berton, Desensitization of macrophage oxygen metabolism on immobilized ligand: different effect of immunoglobulin G and complement. J. Immunology 138: 4366 (1987).

66. S. Tsunawaki and C. F. Nathan, Macrophage deactivation. Altered kinetic properties of superoxide-producing enzyme after exposure to tumor cell-conditioned medium, J. Exp. Med. 164: 1319 (1986).
67. E. Wilson, S. M. Laster, L. R. Gooding and J. D. Lambeth, Platelet-derived growth factor stimulates phagocytosis and blocks agonist-induced activation of the neutrophil oxidative burst: a possible cellular mechanism to protect against oxygen radical damage, Proc. Natl. Acad. Sci. U.S.A. 84: 2213 (1987).
68. M. W. Verghese, C. D. Smith, L. A. Charles, L. Jakoi and R. Snyderman, A guanine nucleotide regulatory protein controls polyphosphoinositide metabolism, $Ca^{2+}$ mobilization, and cellular responses to chemoattractants in human monocytes, J. Immunol. 137: 271 (1986).
69. R. E. Duque, Inhibition of neutrophil activation by p-bromophenacyl bromide and its effects on phospholipase A2, Br. J. Pharmacol. 88: 463 (1986).
70. A. I. Tauber and E. R. Simons, Dissociation of human neutrophil membrane depolarization, respiratory burst stimulation and phospholipid metabolism by quinacrine, FEBS Lett. 156: 161 (1983).
71. I. M. Goldstein, D. Roos, G. Weissmann and H. B. Kaplan, Influence of corticosteroids on human polymorphonuclear leukocyte function in vitro: reduction of lysosomal enzyme release and superoxide production, Inflammation 1: 305 (1976).
72. J. C. Gay, J. N. Lukens and D. K. English, Differential inhibition of neutrophil superoxide generation by nonsteroidal antiinflammatory drugs, Inflammation 8: 209 (1984).
73. J. O. Minta and M. D. Williams, Interactions of antirheumatic drugs with the superoxide generation system of activated human polymorphonuclear leukocytes, J. Rheumatol. 13: 498 (1986).
74. R. J. Gryglewsky, A. Szczeklik and M. Wandzilak, The effect of six prostaglandins, prostacyclin and iloprost on generation of superoxide anions by human polymorphonuclear leukocytes stimulated by zymosan or formyl-methionyl-leucyl-phenylalanine, Biochem. Pharmacol. 36: 4209 (1987).
75. P. DeTogni, G. Cabrini and F. Di Virgilio, Cyclic AMP inhibition of fMet-Leu-Phe-dependent metabolic responses in human neutrophils is not due to its effects on cytosolic $Ca^{2+}$, Biochem. J. 224: 629 (1984).
76. B. N. Cronstein, S. B. Kramer, G. Weissmann and R. Hirshhorn, Adenosine: A physiological modulator of superoxide anion generation by human neutrophils, J. Exp. Med. 158: 1160 (1983).
77. P. A. Ward, T. W. Cunningham, K. K. McCulloch and K. J.

Johnson, Regulatory effects of adenosine and adenine nucleotides on oxygen radical responses of neutrophils, Lab. Invest. 58: 438 (1988).

78. T. Matsumoto, K. Takeshige and S. Minakami, Inhibition of phagocytotic metabolic changes of leukocytes by an intracellular calcium antagonist 8-(N,N-diethylamine)-octyl-3,4,5-trimethoxybenzoate, Biochem. Biophys. Res. Commun, 88: 974 (1979).
79. V. Della Bianca, M. Grzeskowiak, P. DeTogni, M. Cassatella, and F. Rossi, Inhibition by verapamil of neutrophil responses to formyl-methionyl-leucyl- phenylalanine and phorbol myristate acetate. Mechanism involving $Ca^{2+}$ changes, cAMP and protein kinase C, Biochim. Biopys. Acta 845: 223 (1985).
80. Y. Azuma, T. Tokunaga, Y. Takeda, T. Ogawa and N. Takagi, The effect of calcium antagonists on the activation of guinea pig neutrophils, Japan J. Pharmacol. 42: 243 (1986).
81. J. M. Robinson, J. A. Badwey, M. L. Karnovsky and M. J. Karnovsky, Release of superoxide and change in morphology by neutrophils in response to phorbol esters: Antagonism by inhibitors of calcium-binding proteins, J. Cell. Biol. 101: 1052 (1985).
82. K. Takeshige and S. Minakami, Involvement of calmodulin in phagocytotic respiratory burst of leukocytes, Biochem. Biophys. Res. Commun. 99: 484 (1981).
83. B. D. Goldstein, G. Witz, M. Amoruso and W. Troll, Protease inhibitors antagonize the activation of polymorphonuclear leukocyte oxygen consumption, Biochem. Biophys. Res. Commun. 88: 854 (1979).
84. K. M. K. Rao and V. Castranova, Phenylmethyl sulphonyl fluoride (PMSF) inhibits chemotactic peptide-induced actin polymerization and respiratory burst activity in human neutrophils, Fed. Proc. 46: Abs. 3907 (1987).
85. E. Wilson, M. C. Olcott, R. M. Bell, A. H. Merrill and D. J. Lambeth, Inhibition of the oxidative burst in human neutrophils by sphingoid long-chain bases. Role of protein kinase C in activation of the burst, J. Biol. Chem. 261: 12616 (1986).
86. F. Rossi, V. DellaBianca and P. Bellavite Inhibition of the respiratory burst and of phagocytosis by nordihydroguaiaretic acid in neutrophils. FEBS Lett. 127: 183 (1981).
87. I. Aviram and Y. I. Henis, Activation of human neutrophil NADPH oxidase and lateral mobility of membrane proteins, Biochim. Biophys. Acta 805: 227 (1984).
88. G. Goldfarb, J. Belghiti, H. Gautero and P. Boivin, In vitro effect of benzodiazepines on polymorphonuclear leukocyte oxidative activity. Anesthesiol. 60: 57 (1984).
89. P. K. Peterson, B. Sharp, G. Gekker, C. Brummit and W. F.

Keane, Opioid-mediated suppression of cultured peripheral blood mononuclear cell respiratory burst activity, J. Immunol. 138: 3907 (1987).

90. J. W. C. White, A. W. Gelb, H. R. Wexler, C. R. Stiller and P. A. Keown, The effects of intravenous anaesthetic agents on human neutrophil chemiluminescence, Can. Anaesth. Soc. J. 30: 506 (1983).
91. M. Nakagawara, K. Takeshige, J. Takamatsu, S. Takahasi, J. Yoshitake and S. Minakami, Inhibition of superoxide production and Ca2+ mobilization in human neutrophils by halotane, enflurane, and isoflurane, Anesthesiol. 64: 4 (1986).
92. S. D. Somerfield, G. L. Stach, C. Mraz, F. Gervais and E. Skamene, Bee venom melittin blocks neutrophil O2- production, Inflammation 10: 175 (1986).
93. S. Tsunawaki, M. Sporn, A. Ding and C. Nathan, Deactivation of macrophages by transforming growth factor-beta, Nature 334: 260 (1988).
94. T. Yamaguchi and K. Kakinuma, Inhibitory effect of cibacron blue F3GA on the $O^{2-}$ generating enzyme of guinea pig polymorphonuclear leukocytes, Biochem. Biophys. Res. Commun. 104: 200 (1982).
95. D. R. Light, C. Walsh, A. M. O'Callaghan, E. J. Goetzl and A. I. Tauber, Characteristics of the cofactor requirements for the superoxide-generating NADPH oxidase of human polymorphonuclear leukocytes, Biochemistry 20: 1468 (1981).
96. A. R. Cross and O. T. G. Jones, The effect of the inhibitor diphenylene iodonium on the superoxide-generating system of neutrophils. Specific labelling of a component polypeptide of the oxidase, Biochem. J. 237: 111 (1986).
97. T. Iizuka, S. Kanegasaki, R. Makino, T. Tanaka and Y. Ishimura, Pyridine and imidazole reversibly inhibit the respiratory burst in porcine and human neutrophils: evidence for the involvement of cytochrome b558 in the reaction, Biochem. Biophys. Res. Commun. 130: 621 (1985).
98. F. Rossi and G. Zoppi, Effect of menadione on the phagocytic activity of guinea pig polymorphonuclear leukocytes, Experientia 22: 433 (1966).
99. C. J. Butterick, R. L. Baehner, L. A. Boxer and R. A. Jersild, Vitamin E-a selective inhibitor of the NADPH oxidoreductase enzyme system in human granulocytes. Am. J. Pathol. 112: 287-293 (1983).
100. D. R. Crawford and D. L. Schneider, Evidence that a quinone may be required for the production of superoxide and hydrogen peroxide in neutrophils, Biochem. Biophys. Res. Commun. 99: 1277 (1981).
101. T. Yamaguchi, M. Kaneda and K. Kakinuma, Essential requirement of magnesium ion for optimal activity of the NADPH oxidase of guinea pig polymorphonuclear leukocytes, Biochem. Biophys. Res. Commun. 115: 261 (1983).

102. P. Bellavite, M. C. Serra, A. Davoli, J. V. Bannister and F. Rossi, The NADPH oxidase of guimea pig polymorphonuclear leucocytes. Properties of the deoxycholate extracted enzyme, Molec. Cell. Biochem. 52: 17 (1983).
103. A. I. Tauber and E. J. Goetzl, Structural and catalytic properties of the solubilized superoxide-generating activity of human polymorphonuclear leukocytes. Solubilization, stabilization in solution and partial characterization, Biochemistry 18: 5576 (1979).
104. G. L. Babior, R. E. Rosin, B. J. McMurrich, W. A. Peters and B.M. Babior, Arrangement of the respiratory burst oxidase in the plasma membrane of the neutrophil, J. Clin. Invest. 67: 1724 (1981).
105. J. Doussiere and P. V. Vignais, Immunological properties of $O^{2-}$ generating oxidase from bovine neutrophils, FEBS Lett. 234: 362 (1988).
106. Y. Fukuhara, Y. Ise and K. Kakinuma, Immunological studies on the respiratory burst oxidase of pig neutrophils, FEBS Lett. 229: 150 (1988).
107. G. Berton, S. Dusi, M. C. Serra, P. Bellavite and F. Rossi, Studies on the NADPH oxidase of phagocytes. Production of a monoclonal antibody which blocks the enzymatic activity of pig neutrophils NADPH oxidase, J. Biol. Chem. (submitted for publication).
108. P. Patriarca, R. E. Basford, R. Cramer, P. Dri and F. Rossi, Studies on the NADPH oxidizing activity in polymorphonuclear leukocytes: The mode of association with the granule membrane, the relationship to myelo peroxidase and the interference of hemoglobin with NADPH oxidase determination, Biochim. Biophys. Acta 362: 221 (1974).
109. R. C. Jandl, J. Andre'-Schwartz, L. Borges-Dubois, R. S. Kipnes, B. J. McMurrich and B. M. Babior, Termination of the respiratory burst in human neutrophils . J. Clin. Invest. 61: 1176 (1978).
110. I. Maridonneau-Parini, J. Clerk and B. S. Polla, Heat shock inhibits NADPH oxidase in human neutrophils, Biochem. Biophys. Res. Commun. 154: 179 (1988).
111. M. Miyahara, Watanabe, E. Okimasu and K. Utsumi, Charge-dependent regulation of NADPH oxidase activity in guinea-pig polymorphonuclear leukocytes. Biochem. Biophys. Acta 929: 253 (1987).
112. D. E. VanEpps and M. L. Garcia, Enhancement of neutrophil function as a result of prior exposure to chemotactic factor. J. Clin. Invest. 66: 167 (1980).
113. D. English, J. S. Roloff and J. M. Lukens, Chemotactic factor enhancement of superoxide release from fluoride and phorbol myristate acetate stimulated neutrophils. Blood 58: 129 (1981).
114. M. Kaku, K. Yagawa, S. Nagao and A. Tanaka, Enhanced superoxide anion release from phagocytes by muramyl

dipeptide or lipopolysaccharide, Infect. Immun. 39: 559 (1983).

115. L. A. Guthrie, L. C. McPhail, P. M. Henson and R. B. Johnston, Priming of neutrophils for enhanced release of oxygen metabolites by bacterial lipopolysaccharide. Evidence for increased activity of the superoxide-producing enzyme, J. Exp. Med. 160: 1657 (1984).
116. L. C. McPhail, C. C. Clayton and R. Snyderman, The NADPH oxidase of human polymorphonuclear leukocytes. Evidence for regulation by multiple signals, J. Biol. Chem. 259: 5768 (1984).
117. J. C. Gay, J. K. Beckman, A. R. Brash, J. A. Oates and J. N, Lukens Enhancement of chemotactic factor-stimulated neutrophil oxidative metabolism by leukotriene B4, Blood 64: 780 (1984).
118. B. Dewald, T.G. Payne and M. Baggiolini, Activation of NADPH oxidase of human neutrophils. Potentiation of chemotactic peptide by a diacylglycerol, Biochem. Biophys. Res. Commun. 125: 367 (1984).
119. R. J. Smith, L. M. Sam and J. M. Justen, Diacylglycerols modulate human polymorphonuclear neutrophil responsiveness: Effects on intracellular calcium mobilization, granule exocytosis, and superoxide anion production, J. Leukoc. Biol. 43: 411 (1988).
120. B. Dewald and M. Baggiolini, Activation of NADPH oxidase in human neutrophils. Synergism between fMLP and the neutrophil products PAF and LTB4, Biochem. Biophys. Res. Commun. 128: 297 (1985).
121. P. A. Ward, T. W. Cunningham, B. A. M. Walker and K. J. Johnson, Differing calcium requirements for regulatory effects of ATP, ATPgammaS and adenosine on $O^{2-}$ responses of human neutrophils, Biochem. Biophys. Res. Commun. 154: 746 (1988).
122. D. B. Kuhns, D. G. Wright, J. Nath, S. Kaplan and R. E. Basford, ATP induces transient elevations of $(Ca^{2+})i$ in human neutrophils and primes these cells for enhances $O^{2-}$ generation, Lab. Invest. 58: 448 (1988).
123. Y. Ozaki, T. Ohashi and S. Kume, Potentiation of neutrophil function by recombinant DNA-produced interleukin 1a. J. Leukoc. Biol. 42: 621 (1987).
124. C. F. Nathan, H. W. Murray, M. E. Wiebe and B. Y. Rubin, Identification of interferon gamma as the lymphokine that activates human macrophages oxidative metabolism and antimicrobial activity, J. Exp. Med. 158: 670 (1983).
125. G. Berton, L. Zeni, M. A. Cassatella and F. Rossi, Gamma interferon is able to enhance the oxidative metabolism of human neutrophils, Biochem. Biophys. Res. Commun. 138: 1276 (1986).
126. B. Perussia, M. Kobayashi, M. E. Rossi, I. Anegon and G. Trinchieri, Immune interferon enhances functional properties of human granulocytes: role of Fc receptors

and effect of lymphotoxin, tumor necrosis factor, and granulocyte-macrophage colony-stimulating factor, J. Immunol. 138: 765 (1987).

127. I. C. Kowanko and A. Ferrante, Stimulation of neutrophil respiratory burst and lysosomal enzyme release by human interferon-gamma, Immunology 62: 149 (1987).

128. R. H. Weisbart, L. Kwan, D. W. Golde and J.C. Gasson, Human GM-CSF Primes neutrophils for enhanced oxidative metabolism in response to the major physiological chemoattractants, Blood 69: 18 (1987).

129. A. Ferrante, M. Nandoskar, E. J. Bates, D. H. B. Goh and L. J. Beard Tumor necrosis factor beta (lymphotoxin ) inhibits locomotion and stimulates the respiratory burst and degranulation of neutrophils, Immunology 63: 507 (1988).

130. B. Perussia, E. T. Dayton, R. Lazarus, V. Fanning and G. Trinchieri, Immune interferon induces the receptor for monomeric IgG1 on human monocytic and myeloid cells, J. Exp. Med. 158: 1092 (1983).

131. P. W. Andrew, A. K. Robertson, D. B. Lowrie, A. R. Cross and O. T. G. Jones, Induction of synthesis of components of the hydrogen peroxide generating oxidase during activation of the human monocytic cell line U937 by interferon-gamma, Biochem. J. 248: 281 (1987).

132. P. E. Newburger, R. A. B. Ezekowitz, C. Whitney, J. Wright and S. H. Orkin, Induction of phagocyte cytochrome b heavy chain gene expression by interferon gamma, Proc. Natl. Acad. Sci. U.S.A.: 85, 5215 (1988).

133. G. Berton, M. Cassatella, G. Cabrini and F. Rossi, Activation of mouse macrophages causes no change in expression and function of phorbol diester receptors, but is accompanied by alterations in the activity and kinetic parameters of NADPH oxidase, Immunology 54: 371 (1985).

134. C. F. Nathan, C. R. Horowitz, J. DeLaHarpe, S. Vadhan-Raj, S. A. Sherwin, H. F. Oettgen and S. Krown, Administration of recombinant interferon gamma to cancer patients enhances monocyte secretion of hydrogen peroxide, Proc. Natl. Acad. Sci. U.S.A. 82: 8686 (1985).

135. R. A. B. Ezekowitz, S. H. Orkin and P. E. Newburger, Recombinant interferon gamma augments superoxide production and X-chronic granulomatous disease gene expression in X-linked variant chronic granulomatous disease, J. Clin. Invest. 80: 1009 (1987).

136. J. M. G. Sechler, H. R. Malech, C. J. White and J. I. Gallin, Recombinant human interferon-gamma reconstitutes defective phagocyte function in patients with chronic granulomatous disease of childhood, Proc. Natl. Acad. Sci. U.S.A. 85: 4874 (1988).