Histol Histopathol (1999) 14: 1295-1308

http://www.ehu.es/histol-histopathol

Histology and Histopathology

Invited Review

Novel insight into current models of NADPH oxidase regulation, assembly and localization in human polymorphonuclear leukocytes

T. Kobayashi and H. Seguchi

Department of Anatomy and Cell Biology, Kochi Medical School, Kochi, Japan

Summary. We review herein the definition of the NADPH oxidase-activating site in human neutrophils and eosinophils, together with the new biochemical findings of the assembly of NADPH oxidase components and the signal transduction for the activation of NADPH oxidase. The activation of this enzyme is associated with multiple interrelated signaling pathways. Upon cell stimulation, the second messengers act on the assembly of NADPH oxidase components. The cytosolic components are first phosphorylated, and then associated with the membrane components. Small GTP-binding proteins and cytoskeletal components also participate in the activation of the NADPH oxidase. The cytochemical findings demonstrate that the superoxide generated by NADPH oxidase activity is initially localized in distinct types of intracellular granules, and not at the plasma membrane as previously believed. Thus, the assembly of NADPH oxidase components possibly occurs at the limiting membrane of the intracellular compartments. The oxidant-producing compartments mobilize and become associated with the plasma membrane upon cell stimulation with soluble stimulants, or fuse to phagosomes upon stimulation with particulate stimulants. Accordingly, superoxide is released to the extracellular space and into phagosomes in proportion to the oxidant-producing intracellular granule association with the plasma membrane and with the phagosomal membrane, respectively.

Key words: Cytochemistry, Degranulation, Eosinophils, NADPH oxidase, Neutrophils, Secretory granules, Superoxide

Introduction

Neutrophils and eosinophils play an essential role in the host defense against invading pathogens. They possess an enzymatic complex, the NADPH oxidase, which is able to catalyze the one-electron reduction of molecular oxygen to superoxide (O_2^-). Several reviews regarding NADPH oxidase activity based on the biochemical findings have been published in the last decade, discussing the mechanism of NADPH oxidase activation, and the definition of structure, function and assembly of NADPH oxidase components (Rossi, 1986; Cross and Jones, 1991; Morel et al., 1991; Bokoch, 1994, 1995a; Umeki, 1994; Edwards and Watson, 1995; Henderson and Chappel, 1996; Wojtaszek, 1997). While these biochemical studies undoubtedly represent major achievements, it is clear from cytochemical investigations that additional levels of complexity exist in the modulation of the NADPH oxidase complex in vivo (Robinson and Badway, 1995). Recently, the *in vivo* superoxide-producing site has been elucidated in human polymorphonuclear leukocytes using enzyme cytochemical methods. The aim of this review is to provide a novel insight into current models of NADPH oxidase regulation, assembly and localization in human polymorphonuclear leukocytes by re-evaluating the cytochemical and the biochemical data available.

Offprint requests to: H. Seguchi, Department of Anatomy and Cell Biology, Kochi Medical School, Kohasu, Okoh-cho, Nankoku, Kochi 783-8505, Japan. Fax: (0888)80-2304. e-mail: seguchih@kochi-ms.ac.jp

Signaling pathways for NADPH oxidase activation

Both soluble and particulate stimuli elicit a complex array of signal transduction upon the activation of NADPH oxidase in human phagocytes. This activation requires continuous contact between the cell and the stimulus; removal of the stimulus deactivates the oxidase, which is reactivated by a second exposure to the stimulus (Chanock et al., 1994). The cell surface receptors for platelet-activating factor (PAF), complement 5a (C5a), interleukin-8 (IL-8), leukotriene B_4 (LTB_4) and, N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) are linked to guanosine 5'-triphosphate (GTP)-binding proteins (Birnbaumer et al., 1990; Boulay et al., 1990; Bourne et al., 1990; Gerard and Gerard, 1991; Honda et al., 1991; Strosberg, 1991; Thomas et al., 1991; Helmreich and Hofmann, 1996), which participate in the activation of phospholipase A_2 (PLA_2), phosphoinositide-specific phospholipase C (PLC) and phospholipase D (PLD) as well as

phosphatidylinositol 3-kinase (PI3 kinase) and tyrosine protein kinase (Cockcroft, 1992; Thelen and Wirthmueller, 1994; Bokoch, 1995b; Divecha and Irvine, 1995). The phospholipases, in turn, generate bioactive phospholipids as second messengers for signal transduction. The signaling pathways leading the activation of NADPH oxidase in neutrophils involve activation of PLC and PLD, which is triggered by the binding of agonists to specific receptors, mediated by a heterotrimeric GTP-binding protein, at the plasma membrane. Diacylglycerol (DG) and inositol 1,4,5-triphosphate ($InsP_3$) are generated from the breakdown of phosphatidylinositol 4,5-bis phosphate ($PtdInsP_2$) by the activated PLC. DG is also produced from phosphatidic acid (PA) which is generated from phosphatidylcholine by the activated PLD which participates particularly in the regulation of the respiratory burst (McPhail et al., 1993). PLD has been demonstrated to be localized in secretory vesicles and to mobilize to the plasma membrane in human neutrophils upon cell stimulation (Morgan et al., 1997). Binding of $InsP_3$ to Ca^{2+} stores induces the release of Ca^{2+} to the cytosol (Clapham, 1995). The Ca^{2+} stores have been identified two regions of high Ca^{2+} concentration in human neutrophils, one towards the center of the cell, the other under the plasma membrane, on the cell periphery (Pettit et al., 1997). Ca^{2+} and DG activate protein kinase C which phosphorylates the cytosolic components of NADPH oxidase (Morel et al., 1991; Cockcroft, 1992; Rhee and Bae, 1997). Protein kinase C changes the subcellular distribution (Deli et al., 1987; Dekker and Parker, 1994). Protein kinase C represents a group of isoforms (Hug and Sarre, 1993). Human neutrophils express five protein kinase C isoforms (α, β_I, β_{II}, δ and ζ). Stimulation with opsonized zymosan is considered to result in the translocation of protein kinase C isoforms to the plasma membrane (β_{II}, δ and ζ) and granule fractions (δ and ζ). This redistribution promotes the participation of the protein kinase C isoforms in regulatory mechanisms involved in NADPH oxidase assembly/activation through phosphorylation of $p47^{phox}$ (Sergeant and McPhail, 1997). PA is also considered to participate in the activation of NADPH oxidase through phosphorylation of $p47^{phox}$ by PA-activated protein kinase which is different from protein kinase N, protein kinase C, p21(Cdc42/Rac)-activated protein kinase and mitogen-activated protein (MAP) kinase (Waite et al., 1997).

The activation of PI3-kinase located upstream of protein kinase C in the signaling cascade arising from fMLP receptors is considered to be necessary for the fMLP-induced respiratory burst (Ding and Badwey, 1994; Ahmed et al., 1995; Vlahos et al., 1995). Phosphatidylinositol 3,4,5-triphosphate (PIP_3) produced PI3-kinase is involved in the signaling cascade stimulated by fMLP in neutrophils (Stoyanov et al., 1995; Kular et al., 1997). Tyrosine protein kinase also participates in the activation of NADPH oxidase (Gomez-Cambronero et al., 1989; Nasmith et al., 1989; Berkow and Dodson, 1990; Grinstein et al., 1990; Naccache et al., 1990; Yamaguchi et al., 1995a; Ptasznik et al., 1996), and is considered to be required for the recruitment of PI3-kinase to the plasma membrane (Vossebeld et al., 1997), since the PI3-kinase has been demonstrated to contain a 110 kDa catalytic subunit and an 85 kDa regulatory subunit which contains an SH2 domain that can bind to tyrosine-phosphorylated proteins (Carpenter et al., 1990; Cantley et al., 1991). Furthermore, coupling of the src-related tyrosine protein kinase Lyn to the PI3-kinase is possibly involved in the signaling pathway for NADPH oxidase activation (Ptasznik et al., 1996).

The arachidonic acid (AA) generated by activated PLA_2 serves as a second messenger in the stimulation of the NADPH oxidase in neutrophils (Bromberg and Pick, 1983; Maridonneau-Parini and Tauber, 1986; Lu and Grinstein, 1990; Aebischer et al., 1993; Forehand et al., 1993; Henderson et al., 1993; White et al., 1993; Ely et al., 1995) and in eosinophils (Aebischer et al., 1993). The 85 kDa cytosolic PLA2 (cPLA2) possesses a high specificity for phospholipids that contain *sn*-2-arachidonate (Suga et al., 1990) and is considered to be translocated under elevated intracellular calcium levels into membranes where the substrate phospholipids are available (Channon and Leslie, 1990; Clark et al., 1991; Kramer et al., 1991; Lin et al., 1993; Nalefski et al., 1994). Sequence data have shown that the calcium-binding site of $cPLA_2$ is homologous to that of protein kinase C, which also translocates to the membrane upon elevation in intracellular calcium level (Channon and Leslie, 1990; Clark et al., 1991; Kramer et al., 1991; Wijkander and Sundler, 1992). This calcium elevation is an essential step in the signaling between the receptors such as the fMLP receptor and NADPH oxidase (Foyouzi-Youssefi et al., 1997). The microtubule associating protein (MAP) kinase cascade is a signaling pathway common to many polymorphonuclear leukocyte stimulants (Thompson et al., 1994). It has been recently shown that the $cPLA_2$ is activated by several protein kinases. That is, Ras, Raf (Avruch et al., 1994) and MEK kinase (MAP kinase kinase kinase) activated by the stimulation with opsonized zymosan through tyrosine kinase, activate ERK kinase (MAP kinase kinase) which then stimulates a MAP kinase family; ERKs. The activated ERKs finally stimulate $cPLA_2$ (Hazan et al., 1997). The involvement of tyrosine kinase in the activation of the MAP kinase cascade has been demonstrated in human neutrophils stimulated with various agonists such as concanavalin A, lipopolysaccharide, PAF, colony-stimulating factor and tumor necrosis factor α (Duci et al., 1994; Nahas et al., 1996). In addition, both PA and DG generated by PLD are known to induce $cPLA_2$ activity through the activation of protein kinase C linked to MAP kinase (Bauldry and Wooten, 1997; Nixon et al., 1997). $cPLA_2$ has, however, been reported to be phosphorylated also through a MAP kinase-independent pathway (Waterman and Sha'afi, 1995). The generated AA containing free COO^- groups

is considered to facilitate insertion into the membrane or may be a requirement for the interaction with the protein components to affect NADPH oxidase activity (Henderson and Chappell, 1996). It should be noted that protein phosphatases participate in the activation of NADPH oxidase (Gay et al., 1997). For example, the hyperphosphorylated p47^{phox} by protein serine/threonine phosphatase loses the ability to activate NADPH oxidase (Yamaguchi et al., 1995b), and tyrosine protein phosphatase (Walton and Dixon, 1993) also inhibits the activation (Le Cabec and Maridonneau-Parini, 1995). Furthermore, it is proposed that an increase in intracellular cAMP concentration activates protein kinase A which in turn negatively regulates the signaling pathway of NADPH oxidase at the downstream of protein kinase C (Mitsuyama et al., 1993).

Assembly of NADPH oxidase components

NADPH oxidase is activated by the translocation of the cytosolic components to the membrane components upon cell stimulation. The schematic diagram for the assembly of these components in current models of NADPH oxidase is shown in Figure 1.

Cytochrome b_{558}, a membrane factor of the NADPH oxidase (Segal et al., 1978), is a heterodimer consisting of a gp91^{phox} and a p22^{phox} (Huang et al., 1995) which are transmembrane proteins (Nakamura et al., 1988; Imajoh-Ohmi et al., 1992). These subunits are non-covalently associated with each other (Parkos et al., 1987), and are present in membranes in a molar ratio of 1:1 (Wallach and Segal, 1996). The gp91^{phox} has been reported to possess the binding sites for FAD and NADPH (Kleinberg et al., 1989; Segal et al., 1992) and to function as the NADPH oxidase-associated H^+ channel (Demaurex et al., 1993) which is activated by AA (Henderson et al., 1995, 1997). The heme in cytochrome b_{558} is thought to be bound to either two p22^{phox} molecules or to one p22^{phox} molecule and a histidine from gp91^{phox} (Chanock et al., 1994). This p22^{phox} is thought to be considered as the terminal component of the respiratory-burst electron transport system (Umeki, 1994).

Five types of proteins (p40^{phox}, p47^{phox}, p67^{phox}, p21-rac and Rap1A) have been so far known to be classified as the cytosolic factors. p47^{phox} and p67^{phox} have been reported to be associated to each other (Nauseef, 1993; Finan et al., 1994) and exist as a complex that very likely involves at least one additional cytosolic protein (Iyer et al., 1994; McPhail, 1994; Uhlinger et al., 1994). The three cytosolic phox proteins (p40^{phox}, p47^{phox} and p67^{phox}) are presently considered

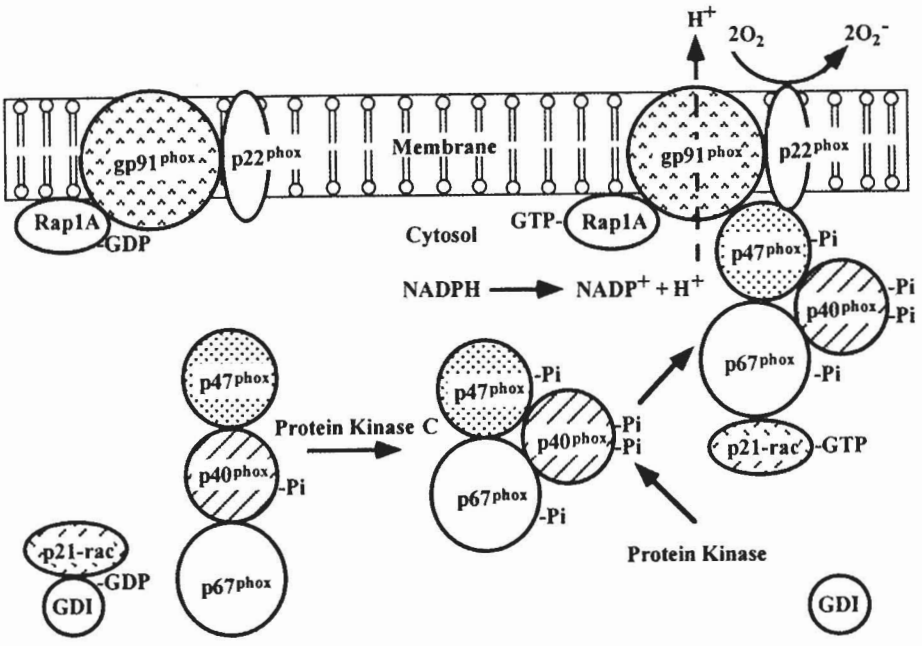

Fig. 1. A schematic diagram showing the assembly of NADPH oxidase components. A cytosolic triphox consisting of p40^{phox}, p47^{phox} and p67^{phox} is phosphorylated by protein kinases, followed by the conformational change upon cell stimulation. This triphox is then associated with p22^{phox} and gp91^{phox} which are located at the membrane, resulting in the production of superoxide. The two small GTP-binding proteins (Rap1A and p21-rac) regulate the oxidant generation. p21-rac located in a cytosolic complex with Rho GDP dissociation inhibitor (GDI) in resting cells, is released from RhoGDI upon cell stimulation, and is then associated with the p67^{phox}. Rap1A associated tightly with cytochrome b_{558} is a regulator of O_2^- formation and functions as the final activation switch involving direct physical interaction with the cytochrome b_{558}.

1

to translocate *en bloc* and to become associated with cytochrome b_{558} upon cell stimulation. First this trimeric phox complex alters its own conformation: in resting cells $p40^{phox}$ is inserted between $p67^{phox}$ and $p47^{phox}$, and upon cell stimulation a conformational change occurs, resulting in the exclusion of $p40^{phox}$ and the direct contact between $p47^{phox}$ and $p67^{phox}$ (Wientjes et al., 1993; Ito et al., 1996; Tsunawaki et al., 1996; Sathyamoorthy et al., 1997; Fuchs et al., 1995, 1996, 1997). The $p47^{phox}$ of the trimeric phox complex is then translocated to the membrane where it is associated with cytochrome b_{558} via multiple binding regions, including the sites on $gp91^{phox}$ and $p22^{phox}$ (DeLeo et al., 1995; Adams et al., 1997). Concerning the interaction between $p47^{phox}$ and $p22^{phox}$, the SH3 domain of $p47^{phox}$ is thought to bind to proline-rich sequences in $p22^{phox}$ (Leto et al., 1994). The $p67^{phox}$ regulates the transfer of electrons from NADPH for reduction of flavin, and the $p47^{phox}$ controls the electron flow between flavin and the heme groups (Cross and Curnutte, 1995), or serves merely as an adaptor protein to enhance the binding of other cytosolic components to the oxidase complex (Freeman and Lambeth, 1996).

Low molecular weight GTP-binding proteins (Rap1A and p21-rac) are known to participate in the activation of NADPH oxidase. In resting cells the p21-rac is located in a cytosolic complex with an inhibitory protein, Rho GDP dissociation inhibitor (GDI), which prevents nucleotide exchange on the p21-rac. Upon cell activation, the p21-rac is released from RhoGDI possibly by biologically active lipids including AA, PA and phosphatidylinositol. The p21-rac is then associated with the $p67^{phox}$. The interaction between $p67^{phox}$ and p21-rac is essential for translocation of the cytosolic trimeric phox proteins and activation of the NADPH oxidase (Bokoch, 1994, 1995a; Prigmore et al., 1995; Kreck et al., 1996; Leusen et al., 1996; Nishimoto et al., 1997). This p21-rac is classified into two types of proteins: Rac1 and Rac2. In human cells, Rac2 is the main $p67^{phox}$-interacting GTPase (Dorseuil et al., 1996). Rap1A which is tightly associated with cytochrome b_{558} is a regulator of O_2^- formation and functions as the final activation switch involving direct physical interaction with cytochrome b_{558} (Quinn et al., 1989; Maly et al., 1994; Gabig et al., 1995). Recently, p125ras GTP-activating protein has been reported to be related to signaling pathway resulting in the activation of NADPH oxidase (Duci et al., 1996).

The phosphorylation of the cytosolic factors is essential for the activation of NADPH oxidase (Heyworth et al., 1989). Both $p47^{phox}$ and $p67^{phox}$ are phosphorylated by protein kinase C during cell stimulation (El Benna et al., 1994, 1996, 1997; Park et al., 1997; Sergeant and McPhail, 1997). On the other hand, the phosphorylation of $p40^{phox}$ is under the control of a different kinase pathway from that of the phosphorylation of $p47^{phox}$ (Fuchs et al., 1997). In addition, several protein kinases, including PA-activated protein kinase, have been suggested to phosphorylate $p47^{phox}$ and to be involved in the activation of NADPH oxidase (McPhail et al., 1995; Yamaguchi et al., 1996; Waite et al., 1997). The phosphorylation of the cytosolic factors is thus necessary to change their conformation to facilitate the assembly of NADPH oxidase complex (Jesaitis et al., 1986; Segal et al., 1996; Fuchs et al., 1997). The dynamics of the cytoskeletal components are essential for the activation of NADPH oxidase. The p21-rac has been reported to bind tubulin and to be required for actin assembly (Xu et al., 1994; Best et al., 1996), indicating that the cytoskeleton may participate in the activation of NADPH oxidase (Woodman et al., 1991; Wiles et al., 1995). Cofilin, a 21-kDa phosphoprotein, is also considered to participate in the continual polymerization and depolymerization of F-actin, and to give rise to the oscillatory pattern of H_2O_2 production (Heyworth et al., 1997).

Eosinophils show a similar, although in some ways more potent, oxidative burst and hence generate more O_2^- than neutrophils (DeChatelet et al., 1977; Yamashita et al., 1985; Petreccia et al., 1987). The eosinophils possess membrane-bound cytochrome b_{558}, cytosolic $p47^{phox}$, $p67^{phox}$, $p40^{phox}$ and p21-rac. The amounts of these components are greater in eosinophils than in neutrophils. Upon activation, $p47^{phox}$, $p67^{phox}$ and $p40^{phox}$ are translocated to the membrane, but larger amounts of these components are translocated in eosinophils compared to those in neutrophils. *K*m values of activated oxidase for NADPH are almost the same in both leukocytes, indicating that oxidase components are likely to be very similar in both eosinophils and neutrophils (Miyamoto et al., 1994; Someya et al., 1997).

Biochemical localization of NADPH oxidase components

In an early study using an advantageous method for subcellular fractionation of human neutrophils, the b-cytochrome component of NADPH oxidase was reported to be in the membrane of the specific granules of unstimulated neutrophils and that stimulus-induced fusion of these granules with the plasma membrane results in translocation of the cytochrome (Borregaard et al., 1983).

Human neutrophils contain a heterogenous population of intracellular granules. The identification and the characterization of the granules and their contents have been investigated over the past decades using biochemical and cytochemical methods (Bainton, 1973, 1993; Bainton et al., 1987; Borregaard et al., 1990, 1993a,b; Kjeldsen et al., 1993; Kjeldsen, 1995; Sengeløv, 1996; Sengeløv et al., 1995; Borregaard and Cowland, 1997; Gullberg et al., 1997). These granules are now classified into five distinct types: (i) defensin-positive azurophil granules, (ii) defensin-negative azurophil granules, (iii) specific granules, (vi) gelatinase granules, and (v) secretory vesicles (Borregaard and Cowland, 1997). It has been so far reported that the

cytochrome b_{558} is localized at the membrane of gelatinase granules and secretory vesicles in addition to specific granules (Bjerrum and Borregaard, 1989; Jesaitis et al., 1990; Calafat et al., 1993; Kjeldsen et al., 1992, 1994). In quantitative studies using cytochrome b_{558} as the marker for membrane-bound components of NADPH oxidase, it appeared that the major part of this cytochrome b_{558} co-sediments with markers for specific granules, gelatinase granules and secretory vesicles, and only a small amount of the cytochrome b_{558} co-localizes with the plasma membrane (Bjerrum and Borregaard, 1989). In addition, the specific granule fraction containing cytochrome b_{558} has been reported to possess the greater part of total NADPH oxidase activity (Clark et al., 1987). These findings indicate that NADPH oxidase complex is not fundamentally localized in the plasma membrane but in the intracellular granules. In human neutrophils, 85% of cytochrome b_{558} is localized in specific granules and gelatinase granules, while the remaining 15% is localized in secretory vesicles (Bjerrum and Borregaard, 1989). However, the secretory vesicles, in comparison with specific granules and gelatinase granules, may play an important role as stores of membrane proteins that are easily mobilized to the cell surface during stimulation by inflammatory mediators in early neutrophil activation (Borregaard et al., 1990; Sengeløv et al., 1993), indicating that the secretory vesicles have a principal role in the production of O_2^- by NADPH oxidase.

Immunocytochemical localization of NADPH oxidase components

In situ localization of NADPH oxidase components has been identified using the immunocytochemical method. Electron microscopy revealed that cytochrome b_{558} in human neutrophils is localized in intracellular granules which also contain lactoferrin, being, thus, specific granules (Ginsel et al., 1990; Jesaitis et al., 1990). It has also been reported that phagocytosing human neutrophils exhibit cytochrome b_{558} localized in the phagosomal membrane adjoining the bacterial cell wall (Jesaitis et al., 1990). However, the $p22^{phox}$ of NADPH oxidase is not localized at the plasma membrane of the resting neutrophils in cryosections (Ginsel et al., 1990). In human eosinophils, the $p22^{phox}$ is localized over a large population of small cytoplasmic granules, most of which are situated between the specific granules which are not labeled by the antibody against $p22^{phox}$. This antibody does not yet label the plasma membrane of the resting eosinophils (Ginsel et al., 1990). The cytochrome b_{558} was also shown to be localized in secretory vesicles in human neutrophils and in albumin-containing vesicles in eosinophils under electron microscopic observation (Calafat et al., 1993). Indirect immunofluorescence study also showed that a distinct granular staining of the cytoplasm is seen for cytochrome b_{558} in human neutrophils (Johansson et al., 1995). These studies did not eliminate the existence of the cytochrome b_{558} on the plasma membrane. The mobilization of intracellular granules is considered to differ according to the granular type (Wright et al., 1977). It should be noted that, as compared to other intracellular granules, an intracellular store of NADPH oxidase can be easily mobilized even under the simple stress condition of density gradient centrifugation, and that such mobilization may result in the expression of cytochrome b_{558} on the plasma membrane (Calafat et al., 1993).

Enzyme cytochemical localization of NADPH oxidase activity

In situ localization of enzyme activity in cells and tissues can be achieved by enzyme cytochemical analyses. Several cytochemical methods have been reviewed to detect NADPH oxidase activity (Karnovsky, 1994; Robinson and Badwey, 1995). Two distinct types of the methods, the cerium method and the DAB/Mn^{2+} method, have been mainly employed to detect the localization of NADPH oxidase activity in human phagocytes under the electron microscope. The cerium method, developed by Briggs et al. (1975), has contributed towards the visualization of the NADPH oxidase-activating site in cells. The enzymatic reaction is as follows: O_2^- generated by NADPH oxidase activity is dismutated to form hydrogen peroxide (H_2O_2). Cerium ion (Ce^{3+}) reacts with H_2O_2 to form cerium perhydroxide ($Ce(OH)_2OOH$) which is the electron-dense precipitate observed under the electron microscope. The usefulness of the cerium-based cytochemical method has been evaluated elsewhere (Robinson and Karnovsky, 1983a,b; Hardonk et al., 1985; Van Noorden and Frederiks, 1993; Kobayashi et al., 1997, 1998a). Using this method it appears that the NADPH oxidase-activating sites are restricted to the plasma membrane and phagosomes in the phagocytizing human neutrophils attached onto coverslips (Briggs et al., 1975). In addition, it has been demonstrated that the oxidant-producing sites are localized on the cell surface and within intracellular vesicles in coverslip-attached cells (Robinson and Batten, 1990) stimulated with phorbol 12-myristate 13-acetate (PMA), a protein kinase C activator (Nishizuka, 1986) and NADPH oxidase activator (Robinson et al., 1985), under scanning laser reflectance confocal microscope (Robinson and Batten, 1989a-c; Halbhuber et al., 1996). In the case of phagocytizing neutrophils suspended in appropriate buffers, not attached onto coverslips, the enzyme reaction sites are detected on the part of the plasma membrane engulfing the particles and on the phagosomal membrane, but not on the free surface of the plasma membrane (Ohno et al., 1982a). The oxidant-reaction product is localized at the contact surface between adjoining neutrophils in suspension stimulated with PMA or lectin, and on the whole surface membrane in these cells stimulated with digitonin or A23187 (Ohno et al., 1982a,b; Hirai et al., 1991).

The DAB/Mn^{2+} method was originally developed to detect O_2^--producing sites in neutrophils (Briggs et al., 1986; Steinbeck et al., 1992, 1993). The enzymatic reaction is as follows: diaminobenzidine (DAB) is oxidized by Mn^{3+} which is formed from Mn^{2+} by the oxidization with O_2^-. The oxidized DAB then reacts with osmium ions to form electron-dense insoluble polymers. Using this DAB/Mn^{2+} method it has been demonstrated that O_2^- generation is associated with the plasma membrane and the endocytic vacuoles formed by the invagination of the plasma membrane in coverslip-attached neutrophils stimulated with zymosan or PMA (Briggs et al., 1986). While it has been reported that O_2^- generation is restricted to the vesicular membrane in coverslip-attached cells stimulated with PMA (Steinbeck et al., 1993), these previous studies indicate that the activating sites of NADPH oxidase in cells are altered by different conditions of cell incubation and stimulants.

As described above, the secretory vesicles among the heterogeneous population of intracellular granules relate to early neutrophil activation. Borregaard et al. (1987) were the first to report biochemically that this compartment contains alkaline phosphatase (ALPase) activity and is mobilized to the plasma membrane much more readily than any identified granule subset and has kinetics of up-regulation to the membrane similar to those reported for a variety of receptor proteins. The ALPase-containing compartments have been enzyme-cytochemically demonstrated to be short rod-shaped organelles distributed dispersely throughout the cytoplasm (Kobayashi and Robinson, 1991; Takizawa and Robinson, 1993), that rapidly undergo a dramatic reorganization upon cell stimulation with either a chemoattractant fMLP or PMA, and exhibit an unusual exocytotic pathway in that these small organelles fuse to form elongated tubular structures before their association with the plasma membrane (Kobayashi and Robinson, 1991; Cain et al., 1993; Fernández-Segura et al., 1995). ALPase activity has been reported to be localized in the Golgi complex, and the secretory granules, referred to as the secretory vesicles, to be located near the Golgi complex (Kobayashi and Robinson, 1991), indicating that the secretory granules are produced through the Golgi complex and are hence essentially intracellular in origin, in spite of the fact that it has been reported that albumin is contained in this compartment indicating that it is endocytic in origin (Borregaard and Cowland, 1997). Recently, it appears that the sites of active NADPH oxidase are in an intracellular compartment (Fig. 2) possessing ALPase activity, and not at the plasma membrane in PMA-stimulated human neutrophils suspended in appropriate buffer using the DAB/Mn^{2+} method, and that these organelles are intracellular in origin, as can be demonstrated using exogenously added ferritin particles as a marker for endocytosis (Fig. 3). These oxidant-producing granules have been further demonstrated to fuse directly with the plasma membrane or to fuse to form larger intracellular vesicles which then become associated with the plasma membrane (Seguchi et al., 1997; Kobayashi et al., 1998b). It has also been shown that oxidant-producing sites are restricted to the tubular compartment similar to

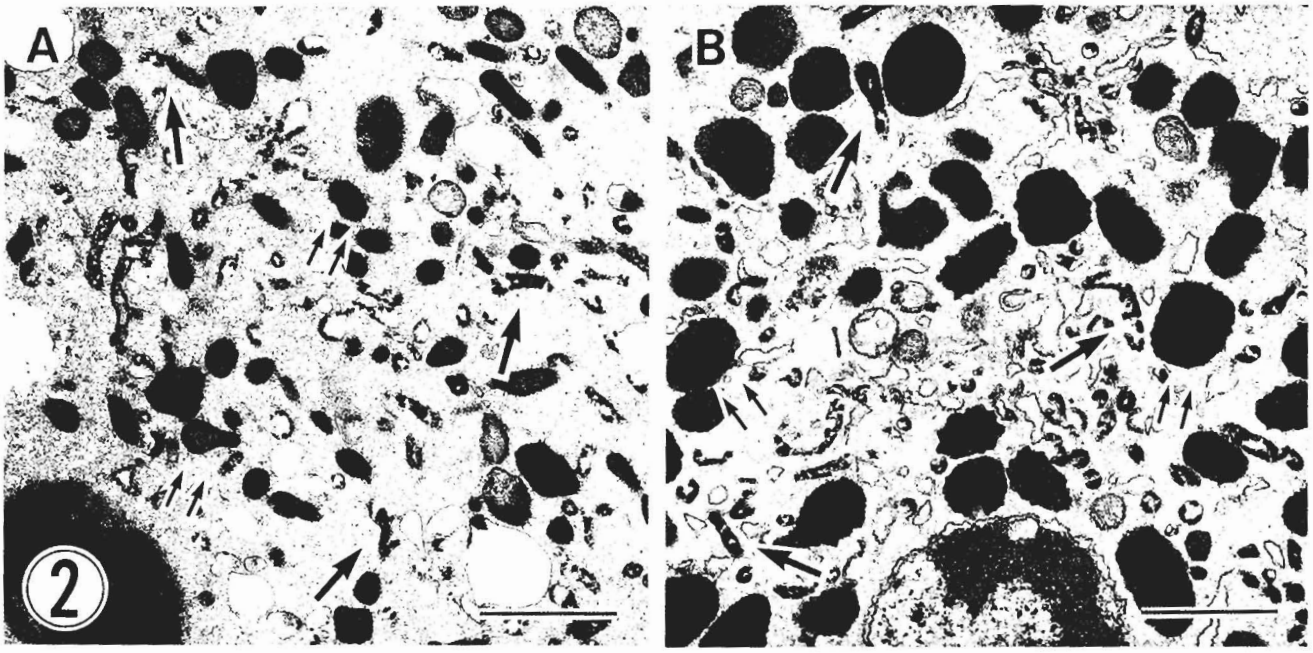

Fig. 2. Electron micrographs showing the intracellular compartments possessing NADPH oxidase activity in human neutrophils **(A)** and eosinophils **(B)** stimulated with PMA. The cytochemical reaction was done using the cerium-based method. The oxidant is localized in smaller intracellular compartments (arrows) compared to specific granules (double arrows) in both cells. Bars :1 μm.

tubular structures of the secretory granules in prefixed cells stimulated with PMA (Kobayashi and Seguchi, 1994; Kobayashi et al., 1999). In human neutrophils phagocytizing human IgG-coated latex beads, intracellular compartment showing NADPH oxidase activity has been reported to gather around the phagosomes and fuse to the phagosomal membranes, but not to the plasma membrane (Seguchi et al., 1997; Kobayashi et al., 1998b). This finding indicates that exocytosis of O_2^--producing granules is essential to carry reactive oxygen species to the extracellular space or into the lumen of phagosome, as it has been suggested that oxidase activity may be influenced by degranulation (Wymann et al., 1987; Suchard and Boxer, 1994). These investigations demonstrate clearly that the sites of activation of NADPH oxidase are not at the plasma membrane, but at the intracellular compartment, and that O_2^- is released from stimulated neutrophils through exocytosis of an oxidant-producing intracellular granule, indicating that the intracellular granule is not merely a site where cytochrome b_{558} is reserved and delivered to the plasma membrane.

In human eosinophils little is known about the electron microscopical localization of the NADPH oxidase-activating site. We have found that the oxidant-producing site is restricted to the intracellular compartment in PMA-stimulated cells in suspension (Fig. 2). This structure, smaller than the specific granules, excludes exogenously-added ferritin particles, indicating that this is intracellular in origin (Fig. 3). The oxidant-producing compartment in eosinophils, like that in neutrophils, is associated with the plasma membrane upon stimulation with PMA, while this structure accumulates around phagosomes and is associated with the limiting membrane of the phagosomes resulting in the release of $O2^-$ into the lumen of the phagosomes (Seguchi et al., 1997).

The intracellular dynamics of the NADPH oxidase-conveying granules are illustrated as a schematic diagram in Figure 4.

Conclusion and consideration

In the present review new insights have been discussed into current models of NADPH oxidase assembly and regulation and localization in human polymorphonuclear leukocytes. Multiple signaling pathways which may interrelate with each other exist in the activation of NADPH oxidase. The assembly of the NADPH oxidase components induced by these signals is also a complex issue. The simplest view of O_2^- release is that the various components of NADPH oxidase complex are brought into juxtaposition at the plasma membrane. However, we emphasize herein that the situation is more complex as the intracellular compartments in human neutrophils and eosinophils are considered to be the site where cytosolic components are associated with the membrane components of NADPH oxidase. Accordingly, O_2^- accumulates initially in the intracellular compartments. O_2^- is further released into

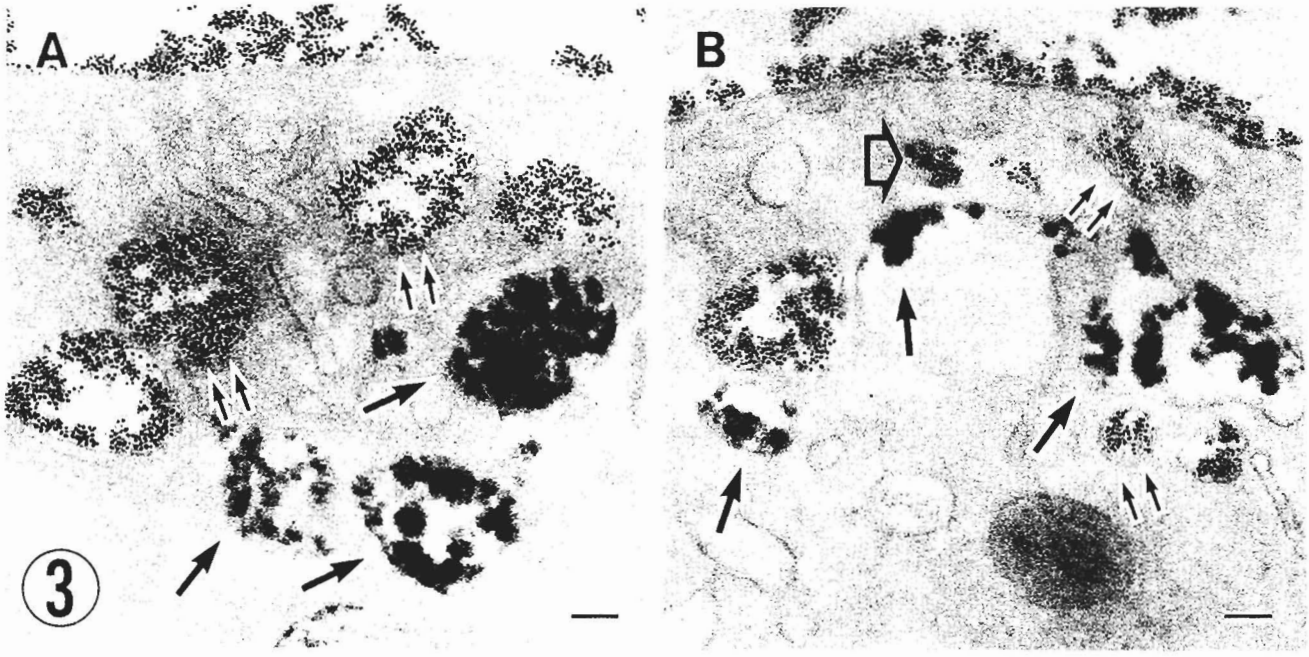

Fig. 3. Electron micrographs showing localization of oxidant and cationized ferritin particles added exogenously in human neutrophils **(A)** and eosinophils **(B)** stimulated with PMA. There is no mixing of the oxidant-positive vesicles (arrows) and the cationized ferritin-loaded endocytic structures (double arrows). Intracellular vesicles containing both oxidant reaction and ferritin particles (open arrow) indicating that the oxidant-producing compartments fuse with the endocytic vesicles. Note that free cell surface shows no oxidant reaction. Bars: 0.1 μm.

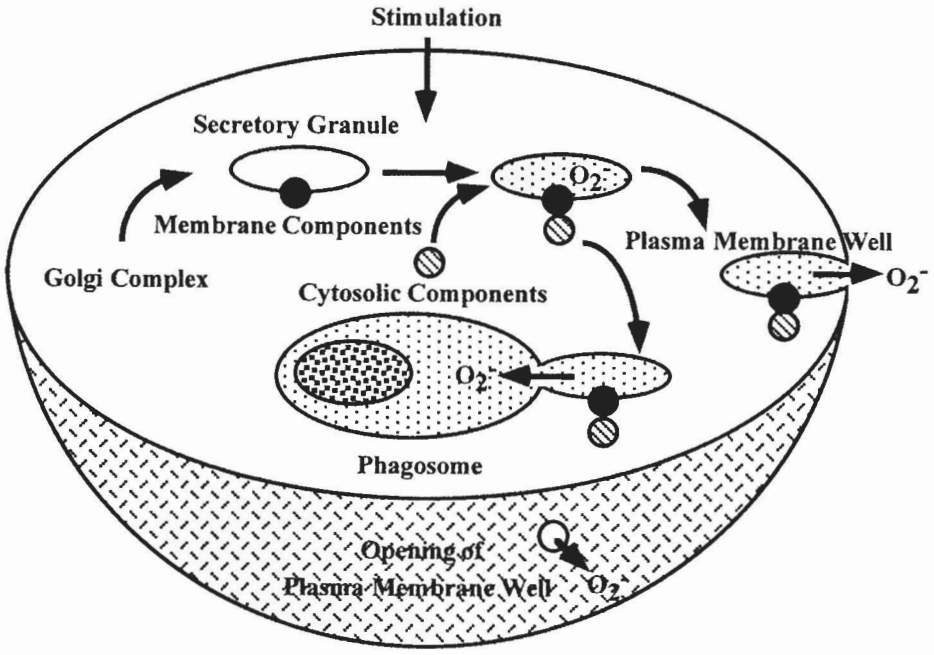

Fig. 4. A schematic diagram showing intracellular dynamics of secretory granules possessing NADPH oxidase activity in human polymorphonuclear leukocytes. Upon cell stimulation, cytosolic components of NADPH oxidase are associated with the membrane components localized at the limiting membrane of the secretory granules. Accordingly, superoxide is released into this lumen. The oxidant-producing granules are associated with the plasma membrane to form *plasma membrane well*. Superoxide is then released extracellularly from the opening of this structure. In another case, the secretory granules are associated with phagosomal membrane, releasing superoxide into the phagosomal lumen.

4

the extracellular space and phagosome in proportion to how the oxidant-producing intracellular granule is associated with the plasma membrane and the phagosomal membrane, respectively. The present view coordinates with the previous investigations: degranulation is essential to NADPH oxidase activity (Wymann et al., 1987; Suchard and Boxer, 1994), the production of O_2^- occurs in phagosome (Johansson et al., 1995) where the oxidase substrate, NADPH, is transported to this vicinity prior to oxidation of targets (Liang and Petty, 1992), the lag period before the onset of the respiratory burst represents the time necessary for degranulation (Suchard and Boxer, 1994), and the priming could, in part, be a result of a translocation of intracellular membranes containing constituents of NADPH oxidase to the plasma membrane (Sengeløv et al., 1995).

Concerning the signal transduction, one of the possible explanations of the signaling pathways for the activation of NADPH oxidase is that $cPLA_2$, PLD, protein kinase C or other effectors may translocate, under the elevation of intracellular calcium level, to the limiting membrane of the oxidant-producing intracellular compartment followed by the assembly of NADPH oxidase components, although there are still many questions that need to be elucidated before the intracellular machinery that triggers the activation of the NADPH oxidase is fully understood. Analyses of the intracellular dynamics of NADPH oxidase components and the investigations of the signaling pathways from the cell surface receptor to the oxidant-producing intracellular compartments will contribute to elucidate the mechanisms of NADPH oxidase activation.

Acknowledgments. This study was in part supported by Grants-in-Aid for Scientific Research (C) (Nos. 07807101 and 07670021) from the Ministry of Education, Science, Sports, and Culture of Japan.

References

Adams E.R., Dratz E.A., Gizachew D., DeLeo F.R., Yu L., Volpp B.D., Vlases M., Jesaitis A.J. and Quinn M.T. (1997). Interaction of human neutrophil flavocytochrome b with cytosolic proteins: transfered-NOESY NMR studies of a gp91^{phox} C-terminal peptide bound to p47^{phox}. Biochem. J. 325, 249-257.

Aebischer C-P., Pasche I. and Jörg A. (1993). Nanomolar arachidonic acid influences the respiratory burst in eosinophils and neutrophils induced by GTP-binding protein. A comparative study of the respiratory burst in bovine eosinophils and neutrophils. Eur. J. Biochem. 218, 669-677.

Ahmed M.U., Hazeki K., Hazeki O., Katada T. and Ui M. (1995). Cyclic AMP-increasing agents interfere with chemoattractant-induced respiratory burst in neutrophils as a result of the inhibition of

phosphatidylinositol 3-kinase rather than receptor-operated Ca^{2+} influx. J. Biol. Chem. 270, 23816-23822.

Avruch J., Zhang X-F. and Kyriakis J.M. (1994). Raf meets Ras: completing the framework of a signal transduction pathway. TIBS 19, 279-283.

Bainton D.F. (1973). Sequential degranulation of the two types of polymorphonuclear leukocyte granules during phagocytosis of microorganisms. J. Cell Biol. 58, 249-264.

Bainton D.F. (1993). Neutrophilic leukocyte granules: from structure to function. ANCA-associated vasculitides: immunological and clinical aspects. Gross W.L. (ed). Plenum Press. New York. pp 17-33.

Bainton D.F., Miller L.J., Kishimoto T.K. and Springer T.A. (1987). Leukocyte adhesion receptors are stored in peroxidase-negative granules of human neutrophils. J. Exp. Med. 166, 1641-1653.

Bauldry S.A. and Wooten R.E. (1997). Induction of cytosolic phospholipase A_2 activity by phosphatidic acid and diglycerides in permeabilized human neutrophils: interrelationship between phospholipases D and A_2. Biochem. J. 322, 353-363.

Best A., Ahmed S., Kozma R. and Lim L. (1996). The Ras-related GTPase Rac1 binds tubulin. J. Biol. Chem. 271, 3756-3762.

Berkow R.L. and Dodson R.W. (1990). Tyrosine-specific protein phosphorylation during activation of human neutrophils. Blood 75, 2445-2452.

Birnbaumer L., Abramowitz J. and Brown A.M. (1990). Receptor-effector coupling by G proteins. Biochim. Biophys. Acta 1031, 163-224.

Bjerrum O.W. and Borregaard N. (1989). Dual granule localization of the dormant NADPH oxidase and cytochrome b_{559} in human neutrophils. Eur. J. Haematol. 43, 67-77.

Bokoch G.M. (1994). Regulation of the human neutrophil NADPH oxidase by the Rac GTP-binding proteins. Curr. Op. Cell Biol. 6, 212-218.

Bokoch G.M. (1995a). Regulation of the phagocyte respiratory burst by small GTP-binding proteins. Trends in Cell Biol. 5, 109-113.

Bokoch G.M. (1995b). Chemoattractant signaling and leukocyte activation. Blood 86, 1649-1660.

Borregaard N., Heiple J.M., Simons E.R. and Clark R.A. (1983). Subcellular localization of the b-cytochrome component of the human neutrophil microbicidal oxidase: translocation during activation. J. Cell Biol. 97, 52-61.

Borregaard N. and Cowland J.B. (1997). Granules of the human neutrophilic polymorphonuclear leukocyte. Blood 89, 3503-3521.

Borregaard N., Miller L.J. and Springer T.A. (1987). Chemoattractant-regulated mobilization of a novel intracellular compartment in human neutrophils. Science 237, 1204-1206.

Borregaard N., Christensen L., Bjerrum O.W., Birgens H.S. and Clemmensen I. (1990). Identification of a highly mobilizable subset of human neutrophil intracellular vesicles that contains tetranectin and latent alkaline phosphatase. J. Clin. Invest. 85, 408-416.

Borregaard N., Lollike K., Kjeldsen L., Sengeløv H., Bastholm L., Nielsen M.H. and Bainton D.F. (1993a). Human neutrophil granules and secretory vesicles. Eur. J. Haematol. 51, 187-198.

Borregaard N., Kjeldsen L., Lollike K. and Sengeløv H. (1993b). Granules and vesicles of human neutrophils. The role of endomembranes as source of plasma membrane proteins. Eur. J. Haematol. 51, 318-322.

Boulay F., Tardif M., Brouchon L. and Vignais P. (1990). The human N-formylpeptide receptor. Characterization of two cDNA isolates and evidence for a new subfamily of G-coupled receptors. Biochemistry 29, 11123-11133.

Bourne H.R., Sanders D.A. and McCormick F. (1991). The GTPase superfamily: conserved structure and molecular mechanism. Nature 349, 117-127.

Briggs R.T., Drath D.B., Karnovsky M.L. and Karnovsky M.J. (1975). Localization of NADH oxidase on the surface of human polymorphonuclear leukocytes by a new cytochemical method. J. Cell Biol. 67, 566-586.

Briggs R.T., Robinson J.M., Karnovsky M.L. and Karnovsky M.J. (1986). Superoxide production by polymorphonuclear leukocytes. A cytochemical approach. Histochemistry 84, 371-378.

Bromberg Y. and Pick E. (1983). Unsaturated fatty acids as second messengers of superoxide generation by macrophages. Cell. Immunol. 79, 240-252.

Cain T.J., Liu Y., Kobayashi T. and Robinson J.M. (1993). Rapid purification of glycosylphosphatidylinositol-anchored alkaline phosphatase from human neutrophils after up-regulation to the cell surface. J. Histochem. Cytochem. 41, 1367-1372.

Calafat J., Kuijpers T.W., Janssen H., Borregaard N., Verhoeven A.J. and Roos D. (1993). Evidence for small intracellular vesicles in human blood phagocytes containing cytochrome b_{558} and the adhesion molecule CD11b/CD18. Blood 81, 3122-3129.

Cantley L.C., Auger K.R., Carpenter C., Duckworth B., Graziani A., Kapeller R. and Soltoff S. (1991). Oncogenes and signal transduction. Cell 64, 281-302.

Carpenter C.L., Duckworth B.C., Auger K.R., Cohen B., Schaffhausen B.S. and Cantley L.C. (1990). Purification and characterization of phosphoinositide 3-kinase from rat liver. J. Biol. Chem. 265, 19704-19711.

Channon J.Y. and Leslie C.C. (1990). A calcium-dependent mechanism for associating a soluble arachidonoyl-hydrolyzing phospholipase A_2 with membrane in the macrophage cell line RAW264.7. J. Biol. Chem. 265, 5409-5413.

Chanock S.J., El Benna J., Smith R.M. and Babior B.M. (1994). The respiratory burst oxidase. J. Biol. Chem. 269, 24519-24522.

Clapham D.E. (1995). Calcium signaling. Cell 80, 259-268.

Clark R.A., Leidal K.G., Pearson D.W. and Nauseef W.M. (1987). NADPH oxidase of human neutrophils. Subcellular localization and characterization of an arachidonate-activatable superoxide-generating system. J. Biol. Chem. 262, 4065-4074.

Clark J.D., Lin L.L., Kriz R.W., Ramesha C.S., Sultzman L.A., Lin A.Y., Milona N. and Knopf J.L. (1991). A novel arachidonic acid-selective cytosolic PLA_2 contains a Ca^{2+}-dependent translocation domain with homology to PKC and GAP. Cell 65, 1043-1051.

Cockcroft S. (1992). G-protein-regulated phospholipase C, D and A_2-mediated signaling in neutrophils. Biochim. Biophys. Acta 1113, 135-160.

Cross A.R. and Curnutte J. T. (1995). The cytosolic activating factors $p47^{phox}$ and $p67^{phox}$ have distinct roles in the regulation of electron flow in NADPH oxidase. J. Biol. Chem. 270, 6543-6548.

Cross A.R. and Jones O.T.G. (1991). Enzymatic mechanisms of superoxide production. Biochim. Biophys. Acta 1057, 281-298.

DeChatelet L.R., Shirley P.S., McPhail L.C., Huntley C.C., Muss H.B. and Bass D.A. (1977). Oxidative metabolism of the human eosinophil. Blood 50, 525-535.

Dekker L.V. and Parker P.J. (1994). Protein kinase C - a question of specificity. TIBS 19, 73-77.

DeLeo F.R., Nauseef W.M., Jesaitis A.J., Burritt J.B., Clark R.A. and Quinn M. T. (1995). A domain of $p47^{phox}$ that interacts with human neutrophil flavocytochrome b_{558}. J. Biol. Chem. 270, 26246-26251.

Deli E., Kiss Z., Wilson E., Lambeth J.D. and Kuo J.F. (1987). Immunocytochemical localization of protein kinase C in resting and activated human neutrophils. FEBS Lett. 221, 365-369.

Demaurex N., Schrenzel J., Jaconi M.E., Lew D.P. and Krause K-H. (1993). Proton channels, plasma membrane potential, and respiratory burst in human neutrophils. Eur. J. Haematol. 51, 309-312.

Ding J. and Badwey J.A. (1994). Wortmannin and 1-butanol block activation of a novel family of protein kinases in neutrophils. FEBS Lett. 348, 149-152.

Divecha N. and Irvine R.F. (1995). Phospholipid signaling. Cell 80, 269-278.

Dorseuil O., Reibel L., Bokoch G.M., Camonis J. and Gacon G. (1996). The Rac target NADPH oxidase p67^{phox} interacts preferentially with Rac2 rather than Rac1. J. Biol. Chem. 271, 83-88.

Duci S., Donini M. and Rossi F. (1994). Tyrosine phosphorylation and activation of NADPH oxidase in human neutrophils: a possible role for MAP kinases and for a 75 kDa protein. Biochem. J. 304, 243-250.

Duci S., Donini M., Wientjes F. and Rossi F. (1996). Tyrosine phosphorylation and subcellular redistribution of p125ras guanosine triphosphate-activating protein in human neutrophils stimulated with FMLP. FEBS Lett. 383, 181-184.

Edwards S.W. and Watson F. (1995). The cell biology of phagocytes. Immunol. Today 16, 508-510.

El Benna J., Faust L.R.P. and Babior B.M. (1994). The phosphorylation of the respiratory burst oxidase component p47^{phox} during neutrophil activation. Phosphorylation of sites recognized by protein kinase C and by proline-directed kinases. J. Biol. Chem. 269, 23431-23436.

El Benna J., Faust L.R.P., Johnson J.L. and Babior B.M. (1996). Phosphorylation of the respiratory burst oxidase subunit p47^{phox} as determined by two-dimensional phosphopeptide mapping. Phosphorylation by protein kinase C, protein kinase A, and a mitogen-activated protein kinase. J. Biol. Chem. 271, 6374-6378.

El Benna J., Dang P.M-C., Gaudry M., Fay M., Morel F., Hakim J. and Gougerot-Pocidalo M-A. (1997). Phosphorylation of the respiratory burst oxidase subunit p67^{phox} during human neutrophil activation. Regulation by protein kinase C-dependent and independent pathways. J. Biol. Chem. 272, 17204-17208.

Ely E.W., Seeds M.C., Chilton F.H. and Bass D.A. (1995). Neutrophil release of arachidonic acid, oxidants, and proteinases: causally related or independent. Biochim. Biophys. Acta 1258, 135-144.

Fernández-Segura E., García J.M. and Campos A. (1995). Dynamic reorganization of the alkaline phosphatase-containing compartment during chemotactic peptide stimulation of human neutrophils imaged by backscattered electrons. Histochem. Cell Biol. 104, 175-181.

Finan P., Shimizu Y., Gout I., Hsuan J., Truong O., Butcher C., Bennett P., Waterfield M.D. and Kellie S. (1994). An SH3 domain and proline-rich sequence mediate an interaction between two components of the phagocyte NADPH oxidase complex. J. Biol. Chem. 269, 13752-13755.

Forehand J.R., Johnston Jr. R.B. and Bomalaski J.S. (1993). Phospholipase A_2 activity in human neutrophils. Stimulation by lipopolysaccharide and possible involvement in priming for an enhanced respiratory burst. J. Immunol. 151, 4918-4925.

Foyouzi-Youssefi R., Petersson F., Lew D.P., Krause K-H. and Nüsse O. (1997). Chemoattractant-induced respiratory burst: increases in cytosolic Ca^{2+} concentrations are essential and synergize with a kinetically distinct second signal. Biochem. J. 322, 709-718.

Freeman J.L. and Lambeth J.D. (1996). NADPH oxidase activity is independent of p47^{phox} in vitro. J. Biol. Chem. 271, 22578-22582.

Fuchs A., Dagher M-C. and Vignais P.V. (1995). Mapping the domains of interaction of p40^{phox} with both p47^{phox} and p67^{phox} of the neutrophil oxidase complex using the two-hybrid system. J. Biol. Chem. 270, 5695-5697.

Fuchs A., Dagher M-C., Fauré J. and Vignais P.V. (1996). Topological organization of the cytosolic activating complex of the superoxide-generating NADPH-oxidase. Pinpointing the sites of interaction between p47^{phox}, p67^{phox} and p40^{phox} using the two-hybrid system. Biochim. Biophys. Acta 1312, 39-47.

Fuchs A., Bouin A-P., Rabilloud T. and Vignais P.V. (1997). The 40-kDa component of the phagocyte NADPH oxidase (p40^{phox}) is phosphorylated during activation in differentiated HL60 cells. Eur. J. Biochem. 249, 531-539.

Gabig T.G., Crean C.D., Mantel P.L. and Rosli R. (1995). Function of wild-type or mutant Rac2 and Rap1a GTPases in differentiated HL60 cell NADPH oxidase activation. Blood 85, 804-811.

Gay J.C., Raddassi K., Truett III A.P. and Murray J.J. (1997). Phosphatase activity regulates superoxide anion generation and intracellular signaling in human neutrophils. Biochim. Biophys. Acta 1336, 243-253.

Gerard N.P. and Gerard C. (1991). The chemotactic receptor for human C5a anaphylatoxin. Nature 349, 614-617.

Ginsel L.A., Onderwater J.J.M., Fransen J.A.M., Verhoeven A.J. and Roos D. (1990). Localization of the low-M_r subunit of cytochrome b_{558} in human blood phagocytes by immunoelectron microscopy. Blood 76, 2105-2116.

Gómez-Cambronero J., Huang C.K., Bonak V.A., Wang E., Casnellie J.E., Shiraishi T. and Sha'afi R.I. (1989). Tyrosine phosphorylation in human neutrophils. Biochem. Biophys. Res. Commun. 162, 1478-1485.

Grinstein S., Furuya W., Lu D.J. and Mills G.B. (1990). Vanadate stimulates oxygen consumption and tyrosine phosphorylation in electropermeabilized human neutrophils. J. Biol. Chem. 265, 318-327.

Gullberg U., Andersson E., Garwicz D., Lindmark A. and Olsson I. (1997). Biosynthesis, processing and sorting of neutrophil proteins: insight into neutrophil granule development. Eur. J. Haematol. 58, 137-153.

Halbhuber K-J., Scheven C., Jirikowski G., Feuerstein H. and Ott U. (1996). Reflectance enzyme histochemistry (REH): visualization of cerium-based and DAB primary reaction products of phosphatases and oxidases in cryostat sections by confocal laser scanning microscopy. Histochem Cell. Biol. 105, 239-249.

Hardonk M.J., Kallicharan D. and Hulstaert C.E. (1985). Cytochemical demonstration of ATPase in the rat kidney basement membrane using the cerium-based method. Acta Histochem. 31, 253-261.

Hazan I., Dana R., Grano Y. and Levy R. (1997). Cytosolic phospholipase A_2 and its mode of activation in human neutrophils by opsonized zymosan. Correlation between 42/44 kDa mitogen-activated protein kinase, cytosolic phospholipase A_2 and NADPH oxidase. Biochem. J. 326, 867-876.

Helmreich E.J.M. and Hofmann K-P. (1996). Structure and function of proteins in G-protein-coupled signal transfer. Biochim. Biophys. Acta 1286, 285-322.

Henderson L.M., Moule S.K. and Chappell J.B. (1993). The immediate activator of the NADPH oxidase is arachidonate not phosphorylation. Eur. J. Biochem. 211, 157-162.

Henderson L.M., Bainting G. and Chappell J.B. (1995). The arachidonate-activable, NADPH oxidase-associated H^+ channel. Evidence that gp91-phox functions as an essential part of the channel. J. Biol. Chem. 270, 5909-5916.

Henderson L.M. and Chappell J.B. (1996). NADPH oxidase of neutrophils. Biochim Biophys. Acta 1273, 87-107.

Henderson L.M., Thomas S., Banting G. and Chappell J.B. (1997). The arachidonate-activatable, NADPH oxidase-associated H^+ channel is contained within the multi-membrane-spanning N-terminal region of gp91-*phox*. Biochem. J. 325, 701-705.

Heyworth P.G., Shrimpton C.F. and Segal A.W. (1989). Localization of the 47 kDa phosphoprotein involved in the respiratory-burst NADPH oxidase of phagocytic cells. Biochem. J. 260, 243-248.

Heyworth P.G., Robinson J.M., Ding J., Ellis B.A. and Badwey J.A. (1997). Cofilin undergoes rapid dephosphorylation in stimulated neutrophils and translocates to ruffled membranes enriched in products of the NADPH oxidase complex. Evidence for a novel cycle of phosphorylation and dephosphorylation. Histochem. Cell Biol. 108, 221-233.

Hirai K-I., Moriguchi K. and Wang G-Y. (1991). Human neutrophils produce free radical from the cell-zymosan interface during phagocytosis and from the whole plasma membrane when stimulated with calcium ionophore A23187. Exp. Cell Res. 194, 19-27.

Honda Z-I., Nakamura M., Miki I., Minami M., Watanabe T., Seyama Y., Okado H., Toh H., Ito K., Miyamoto T. and Shimazu T. (1991). Cloning by functional expression of platelet-activating factor receptor from guinea-pig lung. Nature 349, 342-346.

Huang J., Hitt N.D. and Kleinberg M.E. (1995). Stoichiometry of p22-*phox* and gp91-*phox* in phagocyte cytochrome b_{558}. Biochemistry 34, 16753-16757.

Hug H. and Sarre T.F. (1993). Protein kinase C isoenzymes: divergence in signal transduction? Biochem. J. 291, 329-343.

Imajoh-Ohmi S., Tokita K., Ochiai H., Nakamura M. and Kanegasaki S. (1992). Topology of cytochrome b_{558} in neutrophil membrane analyzed by anti-peptide antibodies and proteolysis. J. Biol. Chem. 267, 180-184.

Ito T., Nakamura R., Sumimoto H., Takeshige K. and Sakaki Y. (1996). An SH3 domain-mediated interaction between the phagocyte NADPH oxidase factors $p40^{phox}$ and $p47^{phox}$. FEBS Lett. 385, 229-232.

Iyer S.S., Pearson D.W., Nauseef W.M. and Clark R.A. (1994). Evidence for a readily dissociable complex of p47phox and p67phox in cytosol of unstimulated human neutrophils. J. Biol. Chem. 269, 22405-22411.

Jesaitis A.J., Tolley J.O. and Allen R.A. (1986). Receptor-cytoskeleton interactions and membrane traffic may regulate chemoattractant-induced superoxide production in human granulocytes. J. Biol. Chem. 261, 13662-13669.

Jesaitis A.J., Buescher E.S., Harrison D., Quinn M.T., Parkos C.A., Livesey S. and Linner J. (1990). Ultrastructural localization of cytochrome b in the membranes of resting and phagocytosing human granulocytes. J. Clin. Invest. 85, 821-835.

Johansson A., Jesaitis A.J., Lundqvist H., Magnusson K-E., Sjölin C., Karlsson A. and Dahlgren C. (1995). Different subcellular localization of cytochrome b and the dormant NADPH-oxidase in neutrophils and macrophages: effect on the production of reactive oxygen species during phagocytosis. Cell. Immunol. 161, 61-71.

Karnovsky M.J. (1994). Cytochemistry and reactive oxygen species: a retrospective. Histochemistry 102, 15-27.

Kjeldsen L. (1995). Gelatinase granules in human neutrophils. Eur. J. Haematol. 54 (suppl. 56), 9-30.

Kjeldsen L., Bjerrum O.W., Askaa J. and Borregaard N. (1992). Subcellular localization and release of human neutrophil gelatinase, confirming the existence of separate gelatinase-containing granules. Biochem. J. 287, 603-610.

Kjeldsen L., Bainton D.F., Sengeløv H. and Borregaard N. (1993). Structural and functional heterogeneity among peroxidase-negative granules in human neutrophils: identification of a distinct gelatinase-containing granule subset by combined immmunocytochemistry and subcellular fractionation. Blood 82, 3183-3191.

Kjeldsen L., Sengeløv H., Lollike K., Nielsen M.H. and Borregaard N. (1994). Isolation and characterization of gelatinase granules from human neutrophils. Blood 83, 1640-1649.

Kleinberg M.E., Rotrosen D. and Malech H.L. (1989). Asparagine-linked glycosylation of cytochrome b_{558} large subunit varies in different human phagocytic cells. J. Immunol. 143, 4152-4157.

Kobayashi T. and Robinson J.M. (1991). A novel intracellular compartment with unusual secretory properties in human neutrophils. J. Cell Biol. 113, 743-756.

Kobayashi T. and Seguchi H. (1994). Localization of NADPH oxidase in human polymorphonuclear leukocytes. J. Histochem. Cytochem. 42, 983.

Kobayashi T., Okada T., Garcia del Saz E. and Seguchi H. (1997). Internalization of ecto-ATPase activity in human neutrophils upon stimulation with phorbol ester or formyl peptide. Histochem. Cell Biol. 107, 353-363.

Kobayashi T., Zinchuk V.S., Okada T., Garcia del Saz E. and Seguchi H. (1998a). Intracellular dynamics of alkaline phosphatase-containing granules in electropermeabilized human neutrophils. Histochem. Cell Biol. 110, 395-406.

Kobayashi T., Robinson J.M. and Seguchi H. (1998b). Identification of intracellular sites of superoxide production in stimulated neutrophils. J. Cell Sci. 111, 81-91.

Kobayashi T., Garcia del Saz E., Hendry E. and Seguchi H. (1999). Detection of oxidant producing-sites in glutaraldehyde-fixed human neutrophils and eosinophils stimulated with phorbol myristate acetate. Histochem. J. (in press).

Kramer R.M., Roberts E.F., Manetta J. and Putnum J.E. (1991). The Ca^{2+}-sensitive cytosolic phospholipase A_2 is a 100-kDa protein in human monoblast U937 cells. J. Biol. Chem. 266, 5268-5272.

Kreck M.L., Freeman J.L., Abo A. and Lambeth J.D. (1996). Membrane association of Rac is required for high activity of the respiratory burst oxidase. Biochemistry 35, 15683-15692.

Kular G., Loubtchenkov M., Swigart P., Whatmore J., Ball A., Cockcroft S. and Wetzker R. (1997). Co-operation of phosphatidylinositol transfer protein with phosphoinositide 3-kinase γ in the formyl-methionyl-leucylphenylalanine-dependent production of phosphatidylinositol 3,4,5-triphosphate in human neutrophils. Biochem. J. 325, 299-301.

Le Cabec V. and Maridonneau-Parini I. (1995). Complete and reversible inhibition of NADPH oxidase in human neutrophils by phenylarsine oxide at a step distal to membrane translocation of the enzyme subunits. J. Biol. Chem. 270, 2067-2073.

Leto T.L., Adams A.G. and de Mendez I. (1994). Assembly of the phagocyte NADPH oxidase: binding of src homology 3 domains to proline-rich targets. Proc. Natl. Acad. Sci. USA 91, 10650-10654.

Leusen J.H.W., de Klein A., Hilarius P.M., Åhlin A., Palmblad J., Smith C.I.E., Diekmann D., Hall A., Verhoeven A.J. and Roos D. (1996).

Disturbed interaction of p21-rac with mutated p67-phox causes chronic granulomatous disease. J. Exp. Med. 184, 1243-1249.

Liang B. and Petty H.R. (1992). Imaging neutrophil activation: analysis of the translocation and utilization of NAD(P)H-associated autofluorescence during antibody-dependent target oxidation. J. Cell. Physiol. 152, 145-156.

Lin L.L., Wartmann M., Lin A.Y., Knopf J.L., Seth A. and Davis R.J. (1993). cPLA2 is phosphorylated and activated by MAP kinase. Cell 72, 269-278.

Lu D.J. and Grinstein S. (1990). ATP and guanine nucleotide dependence of neutrophil activation. Evidence for the involvement of two distinct GTP-binding proteins. J. Biol. Chem. 265, 13721-13729.

Maly F-E., Quilliam L.A., Dorseuil O., Der C.J. and Bokoch G.M. (1994). Activated or dominant inhibitory mutants of Rap1A decrease the oxidative burst of Epstein-Barr virus-transformed human B lymphocytes. J. Biol. Chem. 269, 18743-18746.

Maridonneau-Parini I. and Tauber A.I. (1986). Activation of NADPH-oxidase by arachidonic acid involves phospholipase A_2 in intact human neutrophils but not in the cell-free system. Biochem. Biophys. Res. Commun. 138, 1099-1105.

McPhail L.C. (1994). SH3-dependent assembly of the phagocyte NADPH oxidase. J. Exp. Med. 180, 2011-2015.

McPhail L.C., Qualliotine-Mann D., Agwu D.E. and McCall C.E. (1993). Phospholipases and activation of the NADPH oxidase. Eur. J. Haematol. 51, 294-300.

McPhail L.C., Qualliotine-Mann D. and Waite K.A. (1995). Cell-free activation of neutrophil NADPH oxidase by a phosphatidic acid-regulated protein kinase. Proc. Natl. Acad. Sci. USA 92, 7931-7935.

Mitsuyama T., Takeshige K. and Minakami S. (1993). Cyclic AMP inhibits the respiratory burst of electropermeabilized human neutrophils at a downstream site of protein kinase C. Biochim. Biophys. Acta 1177, 167-173.

Miyamoto D., Someya A., Nunoi H., Nagaoka I. and Yamashita T. (1994). Analysis of the NADPH oxidase components during differentiation of HL-60 cells to eosinophilic lineage. Biochim. Biophys. Acta 1224, 11-16.

Morel F., Doussiere J. and Vignais P.V. (1991). The superoxide-generating oxidase of phagocytic cells. Physiological, molecular and pathological aspects. Eur. J. Biochem. 201, 523-546.

Morgan C.P., Sengeløv H., Whatmore J., Borregaard N. and Cockcroft S. (1997). ADP-ribosylation-factor-regulated phospholipase D activity localizes to secretory vesicles and mobilizes to the plasma membrane following N-formylmethionyl-leucyl-phenylalanine stimulation of human neutrophils. Biochem. J. 325, 581-585.

Naccache P.H., Gilbert C., Caon A.C., Gaudry M., Huang C.K., Bonak V.A., Umezawa K. and McColl S.R. (1990). Selective inhibition of human neutrophil functional responsiveness by erbstatin, an inhibitor of tyrosine protein kinase. Blood 76, 2098-2104.

Nahas N., Molski T.F.P., Fernandez G.A. and Sha'afi R.I. (1996). Tyrosine phosphorylation and activation of a new mitogen-activated protein (MAP)-kinase cascade in human neutrophils stimulated with various agonists. Biochem. J. 318, 247-253.

Nakamura M., Sendo S., van Zwieten R., Koga T., Roos D. and Kanegasaki S. (1988). Immunocytochemical discovery of the 22- to 23-Kd subunit of cytochrome b_{558} at the surface of human peripheral phagocytes. Blood 72, 1550-1552.

Nalefski E.A., Sultzman L.A., Martin D.M., Kriz R.W., Towler P.S., Knopf J.L. and Clark J.D. (1994). Delineation of two functionally distinct domains of cytosolic phospholipase A_2, a regulatory Ca^{2+}-dependent lipid-binding domain and a Ca^{2+}-independent catalytic domain. J. Biol. Chem. 269, 18239-18249.

Nasmith P.E., Mills G.B. and Grinstein S. (1989). Guanine nucleotides induce tyrosine phosphorylation and activation of the respiratory burst in neutrophils. Biochem. J. 257, 893-897.

Nauseef W.M. (1993). Cytosolic oxidase factors in the NADPH-dependent oxidase of human neutrophils. Eur. J. Haematol. 51, 301-308.

Nishimoto Y., Freeman J.L.R., Motalebi S.A., Hirshberg M. and Lambeth J.D. (1997). Rac binding to $p67^{phox}$. Structural basis for interactions of the Rac1 effector region and insert region with components of the respiratory burst oxidase. J. Biol. Chem. 272, 18834-18841.

Nishizuka Y. (1986). Studies and perspectives of protein kinase C. Science 233, 305-312.

Nixon A.B., Seeds M.C., Bass D.A., Smitherman P.K., O'Flaherty J.T., Daniel L.W. and Wykle R.L. (1997). Comparison of alkylacylglycerol vs. diacylglycerol as activators of mitogen-activated protein kinase and cytosolic phospholipase A_2 in human neutrophil priming. Biochim. Biophys. Acta 1347, 219-230.

Ohno Y-I., Hirai K-I., Kanoh T., Uchino H. and Ogawa K. (1982a). Subcellular localization of H_2O_2 production in human neutrophils stimulated with particles and an effect of cytochalasin-B on the cells. Blood 60, 253-260.

Ohno Y-I., Hirai K-I., Kanoh T., Uchino H. and Ogawa K. (1982b). Subcellular localization of hydrogen peroxide production in human polymorphonuclear leukocytes stimulated with lectins, phorbol myristate acetate, and digitonin: an electron microscopic study using $CeCl_3$. Blood 60, 1195-1202.

Park J-W., Hoyal C.R., El Benna J. and Babior B.M. (1997). Kinase-dependent activation of the leukocyte NADPH oxidase in a cell-free system. Phosphorylation of membranes and $p47^{phox}$ during oxidase activation. J. Biol. Chem. 272, 11035-11043.

Parkos C.A., Allen R.A., Cochrane C.G. and Jesaitis A.J. (1987). Purified cytochrome b from human granulocyte plasma membrane is comprised of two polypeptides with relative molecular weights of 91,000 and 22,000. J. Clin. Invest. 80, 732-742.

Petreccia D.C., Nauseef W.M. and Clark R.A. (1987). Respiratory burst of normal human eosinophils. J. Leukoc. Biol. 41, 283-288.

Pettit E.J., Dacies E.V. and Hallett M.B. (1997). The microanatomy of calcium stores in human neutrophils: Relationship of structure to function. Histol. Histopathol. 12, 479-490.

Prigmore E., Ahmed S., Best A., Kozma R., Manser E., Segal A.W. and Lim L. (1995). A 68-kDa kinase and NADPH oxidase component $p67^{phox}$ are targets for Cdc42Hs and rac1 in neutrophils. J. Biol. Chem. 270, 10717-10722.

Ptasznik A., Prossnitz E.R., Yoshikawa D., Smrcka A., Traynor-Kaplan A.E. and Bokoch G.M. (1996). A tyrosine kinase signaling pathway accounts for the majority of phosphatidylinositol 3,4,5-triphosphate formation in chemoattractant-stimulated human neutrophils. J. Biol. Chem. 271, 25204-25207.

Quinn M.T., Parkos C.A., Walker L., Orkin S.H., Dinauer M.C. and Jesaitis A.J. (1989). Association of a Ras-related protein with cytochrome b of human neutrophils. Nature 342, 198-200.

Rhee S.G. and Bae Y.S. (1997). Regulation of phosphoinositide-specific phospholipase C isozymes. J. Biol. Chem. 272, 15045-15048.

Robinson J.M. and Karnovsky M.J. (1983a). Ultrastructural localization of 5'-nucleotidase in guinea pig neutrophils based upon the use of cerium as capturing agent. J. Histochem. Cytochem. 31, 1190-1196.

Robinson J.M. and Karnovsky M.J. (1983b). Ultrastructural localization

of several phosphatases with cerium. J. Histochem. Cytochem. 31, 1197-1208.

Robinson J.M., Badwey J.A., Karnovsky M.L. and Karnovsky M.J. (1985). 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, 1052-1058.

Robinson J.M. and Batten B.E. (1989a). Detection of diaminobenzidine reactions using scanning laser confocal reflectance microscopy. J. Histochem. Cytochem. 37, 1761-1765.

Robinson J.M. and Batten B.E. (1989b). Localization of cytochemical reactions by scanning-laser confocal microscopy with the reflectance mode. J. Histochem. Cytochem. 37, 940.

Robinson J.M. and Batten B.E. (1989c). Detection of cytochemical reactions using scanning laser confocal reflectance microscopy. J. Cell Biol. 109, 308a.

Robinson J.M. and Batten B.E. (1990). Localization of cerium-based reaction products by scanning laser reflectance confocal microscopy. J. Histochem. Cytochem. 38, 315-318.

Robinson J.M. and Badway J.A. (1995). The NADPH oxidase complex of phagocytic leukocytes: a biochemical and cytochemical view. Histochemistry 103, 163-180.

Rossi F. (1986). The O_2^--forming NADPH oxidase of the phagocytes: nature, mechanisms of activation and function. Biochim. Biophys. Acta 853, 65-89.

Sathyamoorthy M., de Mendez I., Adams A.G. and Leto T.L. (1997). $p40^{phox}$ down-regulates NADPH oxidase activity through interactions with its SH3 domain. J. Biol. Chem. 272, 9141-9146.

Segal A.W., Jones O.T., Webster D. and Allison A.C. (1978). Absence of a newly described cytochrome b from neutrophils of patients with chronic granulomatous disease. Lancet 2, 446-449.

Segal A.W., West I., Wientjes F., Nugent J.H.A., Chavan A.J., Haley B., Garcia R.C., Rosen H. and Scrace G. (1992). Cytochrome b-245 is a flavocytochrome containing FAD and the NADPH-binding site of the microbicidal oxidase of phagocytes. Biochem. J. 284, 781-788.

Segal A.W., Grogan A., Reeves E., Keep N., Wientjes F., Burlingame A.L., Totty N.F. and Hsuan J.J. (1996). $p47^{phox}$ regulates "amoebic" cytoskeleton in neutrophils. J. Leukoc. Biol. suppl. abstr. 110.

Seguchi H., Kobayashi T. and Garcia del Saz E. (1997). Intracellular granules are the site of superoxide production in human neutrophils. In: Recent advances in microscopy of cells, tissues and organs. Motta P.M. (ed). Antonio Delfino Editore. Rome. pp 129-134.

Sengeløv H. (1996). Secretory vesicles of human neutrophils. Eur. J. Haematol. 57 (suppl. 58), 6-24.

Sengeløv H., Kjeldsen L. and Borregaard N. (1993). Control of exocytosis in early neutrophil activation. J. Immunol. 150, 1535-1543.

Sengeløv H., Follin P., Kjeldsen L., Lollike K., Dahlgren C. and Borregaard N. (1995). Mobilization of granules and secretory vesicles during in vivo exudation of human neutrophils. J. Immunol. 154, 4157-4165.

Sergeant S. and McPhail L.C. (1997). Opsonized zymosan stimulates the redistribution of protein kinase C isoforms in human neutrophils. J. Immunol. 159, 2877-2885.

Someya A., Nishijima K., Nunoi H., Irie S. and Nagaoka I. (1997). Study on the superoxide-producing enzyme of eosinophils and neutrophils - Comparison of the NADPH oxidase components. Arch. Biochem. Biophys. 345, 207-213.

Steinbeck M.J., Khan A.U. and Karnovsky M.J. (1992). Intracellular singlet oxygen generation by phagocytosing neutrophils in response to particles coated with a chemical trap. J. Biol. Chem. 267, 13425-13433.

Steinbeck M.J., Khan A.U., Appel Jr. W.H. and Karnovsky M.J. (1993). The DAB-Mn^{++} cytochemical method revisited: validation of specificity for superoxide. J. Histochem. Cytochem. 41, 1659-1667.

Stoyanov B., Volinia S., Hanck T., Rubio I., Loubtchenkov M., Malek D., Stoyanova S., Vanhaesebroeck B., Dhand R., Nünberg B., Gierschik P., Seedorf K., Hsuan J.J., Waterfield M.D. and Wetzker R. (1995). Cloning and characterization of a G protein-activated human phosphoinositide-3 kinase. Science 269, 690-693.

Strosberg A.D. (1991). Structure/function relationship of proteins belonging to the family of receptors coupled to GTP-binding proteins. Eur. J. Biochem. 196, 1-10.

Suchard S.J. and Boxer L.A. (1994). Exocytosis of a subpopulation of specific granules coincides with H_2O_2 production in adherent human neutrophils. J. Immunol. 152, 290-300.

Suga K., Kawaski T., Blank M.L. and Snyder F. (1990). An arachidonyl (polyenoic)-specific phospholipase A_2 activity regulates the synthesis of platelet-activating factor in granulocytic HL-60 cells. J. Biol. Chem. 265, 12363-12371.

Takizawa T. and Robinson J.M. (1993). Combined immunocytochemistry and enzyme cytochemistry on ultra-thin cryosections: a new method. J. Histochem. Cytochem. 41, 1635-1639.

Thelen M. and Wirthmueller U. (1994). Phospholipases and protein kinases during phagocyte activation. Curr. Opin. Immunol. 6, 106-112.

Thomas K.M., Taylor M. and Navarro J. (1991). The interleukin-8 receptor is encoded by a neutrophil-specific cDNA clone, F3R. J. Biol. Chem. 266, 14839-14841.

Thompson H.L., Marshall C.J. and Saklatvala J. (1994). Characterization of two different forms of mitogen-activated protein kinase kinase induced in polymorphonuclear leukocytes following stimulation by N-formylmethionyl-leucyl-phenylalanine or granulocyte-macrophage colony-stimulating factor. J. Biol. Chem. 269, 9486-9492.

Tsunawaki S., Kagara S., Yoshikawa K., Yoshida L.S., Kuratsuji T. and Namiki H. (1996). Involvement of $p40^{phox}$ in activation of phagocyte NADPH oxidase through association of its carboxyl-terminal, but not its amino-terminal, with $p67^{phox}$. J. Exp. Med. 184, 893-902.

Uhlinger D.J., Taylor K.L. and Lambeth J.D. (1994). p67-phox enhances the binding of p47-phox to the human neutrophil respiratory burst oxidase complex. J. Biol. Chem. 269, 22095-22098.

Umeki S. (1994). Activation factors of neutrophil NADPH oxidase complex. Life Sci. 55, 1-13.

Van Noorden C.J.F. and Frederiks W.M. (1993). Cerium methods for light and electron microscopical histochemistry. J. Microsc. 171, 3-16.

Vlahos C.J., Matter W.F., Brown R.F., Traynor-Kaplan A.E., Heyworth P.G., Prossnitz E.R., Ye R.D., Marder P., Schelm J.A., Rothfuss K.J., Serlin B.S. and Simpson P.J. (1995). Investigation of neutrophil signal transduction using a specific inhibitor of phosphatidylinositol 3-kinase. J. Immunol. 154, 2413-2422.

Vossebeld P.J.M., Homburg C.H.E., Schweizer R.C., Ibarrola I., Kessler J., Koenderman L., Roos D. and Verhoeven A.J. (1997). Tyrosine phosphorylation-dependent activation of phosphatidylinositide 3-kinase occurs upstream of Ca^{2+}-signalling induced by Fcγ receptor cross-linking in human neutrophils. Biochem. J. 323, 87-94.

Waite K.A., Wallin R., Qualliotine-Mann D. and McPhail L.C. (1997). Phosphatidic acid-mediated phosphorylation of the NADPH oxidase

component p47-phox. Evidence that phosphatidic acid may activate a novel protein kinase. J. Biol. Chem. 272, 15569-15578.

Wallach T.M. and Segal A.W. (1996). Stoichiometry of the subunits of flavocytochrome b_{558} of the NADPH oxidase of phagocytes. Biochem. J. 320, 33-38.

Walton K.M. and Dixon J.E. (1993). Protein tyrosine phosphatases. Annu. Rev. Biochem. 62, 101-120.

Waterman W.H. and Sha'afi R.I. (1995). A mitogen-activated protein kinase independent pathway involved in phosphorylation and activation of cytosolic phospholilase A_2 in human neutrophils stimulated with tumor necrosis factor-α. Biochem. Biophys. Res. Commun. 209, 271-278.

White S.R., Strek M.E., Kulp G.V.P., Spaethe S.M., Burch R.A., Neeley S.P. and Leff A.R. (1993). Regulation of human eosinophil degranulation and activation by endogenous phospholipase A_2. J. Clin. Invest. 91, 2118-2125.

Wientjes F.B., Hsuan J.J., Totty N.F. and Segal A.W. (1993). $p40^{phox}$, a third cytosolic component of the activation complex of the NADPH oxidase to contain src homology 3 domains. Biochem. J. 296, 557-561.

Wijkander J. and Sundler R. (1992). Macrophage arachidonate-mobilizing phospholipase A2: role of Ca^{2+} for membrane binding but not for catalytic activity. Biochem. Biophys. Res. Commun. 184, 118-124.

Wiles M.E., Dykens J.A. and Wright C.D. (1995). Human neutrophil (PMN) oxygen radical production and the cytoskeleton. Life Sci. 57, 1533-1546.

Wojtaszek P. (1997). Oxidative burst: an early plant response to pathogen infection. Biochem. J. 322, 681-692.

Woodman R.C., Ruedi J.M., Jesaitis A.J., Okamura N., Quinn M.T., Smith R.M., Curnutte J.T. and Babior B.M. (1991). Respiratory burst oxidase and three of four oxidase-related polypeptides are associated with the cytoskeleton of human neutrophils. J. Clin. Invest. 87, 1345-1351.

Wright D.G., Bralove D.A. and Gallin J.I. (1977). The differential mobilization of human neutrophil granules. Am. J. Pathol. 87, 273-284.

Wymann M.P., von Tscharner V., Deranleau D.A. and Baggiolini M. (1987). The oneset of the respiratory burst in human neutrophils: real-time studies of H_2O_2 formation reveal a rapid agonist-induced transduction process. J. Biol. Chem. 262, 12048-12053.

Xu X., Barry D.C., Settleman J., Schwartz M.A. and Bokoch G.M. (1994). Differing structural requirements for GTPase-activating protein responsiveness and NADPH oxidase activation by Rac. J. Biol. Chem. 269, 23569-23574.

Yamaguchi M., Oishi H., Araki S., Saeki S., Yamane H., Okamura N. and Ishibashi S. (1995a). Respiratory burst and tyrosine phosphorylation by vanadate. Arch. Biochem. Biophys. 323, 382-386.

Yamaguchi M., Saeki S., Yamane H., Okamura N. and Ishibashi S. (1995b). Hyperphosphorylated p47-phox lost the ability to activate NADPH oxidase in guinea pig neutrophils. Biochem. Biophys. Res. Commun. 216, 203-208.

Yamaguchi M., Saeki S., Yamane H., Okamura N. and Ishibashi S. (1996). Involvement of several protein kinases in the phosphorylation of p47-phox. Biochem. Biophys. Res. Commun. 220, 891-895.

Yamashita T., Someya A. and Hara E. (1985). Response of superoxide anion production by guinea pig eosinophils to various soluble stimuli: comparison to neutrophils. Arch. Biochem. Biophys. 241, 447-452.

Accepted April 19, 1999