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Forum Review

Assembly and Activation of the Neutrophil NADPH Oxidase in Granule Membranes

ANNA KARLSSON and CLAES DAHLGREN

ABSTRACT

Phagocyte activation is accompanied by assembly of an NADPH oxidase that reduces oxygen to form a number of reactive species. These oxygen radicals can eradicate invading microorganisms, regulate the function of other immune reactive cells, and cause damage to "innocent bystander" cells. It is generally assumed that the NADPH oxidase is activated exclusively in the plasma membrane. In neutrophils, this assumption does not fit with the subcellular localization of the membrane component of the oxidase, which is stored in granule compartments. It has now become increasingly evident that oxidants are also produced in an intracellular compartment that we identify as the specific granules. Myeloperoxidase is stored in another granule subset, the azurophil granules, and participates in the processing of the oxidative metabolites. We suggest that neutrophil activation is accompanied by fusion between azurophil and specific granules, allowing these peroxidase-dependent reactions to take place. The presented data suggest a requisite role for neutrophil oxidants complementing their function as microbial killing agents. Signaling capabilities of the oxidants, affecting for example, the state of protein phosphorylation, regulation of transcription factors, and induction of apoptosis, are discussed. Antioxid. Redox Signal. 4, 49–60.

BACKGROUND

DURING PHAGOCYTOSIS OF MICROBIAL INTRUDERS, professional phagocytes of our innate immune system increase their consumption of molecular oxygen (6, 56). The importance of this so-called "respiratory burst" became obvious when the syndrome chronic granulomatous disease (CGD), characterized by predisposition to bacterial and fungal infections, was shown to be associated with decreased oxygen consumption, as well as defective microbial killing (36). The structure of the oxidase responsible for the respiratory burst has in major parts been resolved by studying phagocytes from CGD patients using a cell-free assay system developed in the 1980s (12). To date, the dormant oxidase has been shown to consist of a membrane-bound b-type cytochrome and a number of cytosolic components. Upon cell activation, the cytosolic proteins translocate to the b cytochrome. This forms a functional multicomponent electron-transfer system that catalyzes the reduction of molecular oxygen at the expense of NADPH. The electrons are ferried by the b cytochrome from NADPH in the cytosol, over the membrane to oxygen present in an intracellular compartment (e.g., a phagosome) or in the extracellular milieu (Fig. 1) (5). The b-type cytochrome is a membrane-spanning protein-heterodimer that originally was believed to be localized exclusively to the plasma membrane. However, subcellular frac-

The Phagocyte Research Laboratory, Department of Rheumatology, University of Göteborg, S-413 46 Göteborg, Sweden.

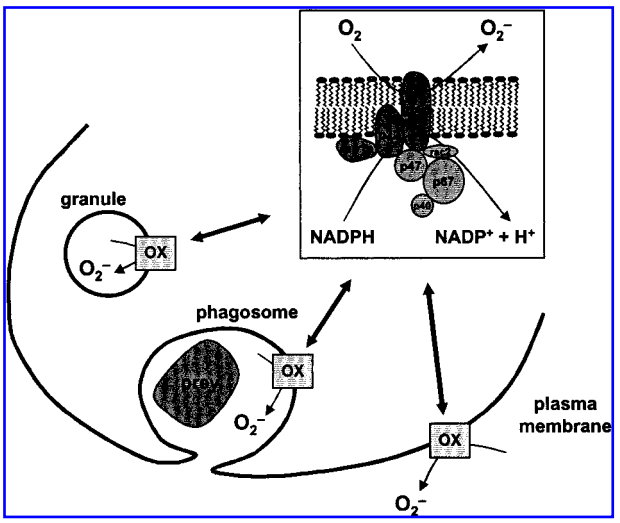

FIG. 1. Activation of the neutrophil NADPH oxidase is achieved through stimulus-induced translocation of cytosolic factors (p47, p67, p40, and rac2) to the membrane-bound b cytochrome. There are at least three possible sites for assembly and activation: the plasma membrane, phagosomal membranes, or granule membranes. Superoxide anion (O_2^-) can be measured at these different sites separately, and details about proper techniques for this purpose have been reviewed (19).

tionation studies revealed that only a minor fraction (~5%) resides in the plasma membrane, whereas the remaining part is present in membranes of mobilizable intracellular granules (11, 59).

The respiratory burst products are not only able to eradicate invading microorganisms, but may also damage "innocent bystanders" and by that be responsible for the tissue destruction associated with many inflammatory reactions (for a review, see 29). In addition, reactive oxygen species generated by phagocytes function also as intracellular signaling molecules (64), *e.g.,* in apoptotic processes (48), and as regulators of other immune reactive cells (35, 50). Thus, it is of importance to increase our knowledge of the regulatory systems that determine the generation and release of phagocyte respiratory burst products. In this review, we focus on the role of granules in neutrophil production of reactive oxygen species. We hope to convince the reader that neutrophils produce large amounts of reactive oxygen species in intracellular compartments when no classical phagocytic process is taking place. At present, we can only speculate upon the precise role of these intracellular oxidants in neutrophil function and in the inflammatory process.

RESPIRATORY BURST IN THE PLASMA MEMBRANE AND THE GRANULES

Release of reactive oxygen species

Human neutrophils contain at least four types of granules that are mobilized (induced to fuse with the plasma membrane) (42, 58) hierarchically during *in vivo* extravasation of the cells from the blood stream to the tissue (10, 60). The two most easily mobilized organelles, *i.e.,* the secretory vesicles and the gelatinase granules, function as easily accessible reservoirs of various receptors that are exposed in increasing amounts on the surface as the vesicles/granules fuse with the plasma membrane. An increase in the number of surface-exposed receptors turns the neutrophil into a primed state or even alters the cells from being nonresponding into being responding with regard to certain stimuli (2, 38, 39). The classical granules, the specific granules and the azurophil peroxidase-positive granules, fuse primarily with the phagosome, and relatively small amounts of the matrix proteins from these granules are secreted to the surroundings during phagocytosis.

An assembly of an active NADPH oxidase in the plasma membrane will directly (or with a small delay) result in reduction of oxygen molecules in close vicinity to the plasma membrane, at the side exposed to the environment. Even though only a minor fraction (~5%) of the total cellular content of b cytochrome is present in the plasma membrane, it has been generally assumed that the NADPH oxidase is assembled and activated exclusively in this membrane or in an internal (phagosome) membrane derived hereof (32, 44). However, the presence of b cytochrome in mobilizable internal membranes introduces the possibility that this pool of b cytochrome could generate oxidants. The easily mobilized secretory vesicles harbor 10% of the b cytochrome (59), and although it has not been shown, an active NADPH oxidase could

possibly be assembled in the membrane of the secretory vesicles prior to their fusion with the plasma membrane. Most of the cellular content (85%) of cytochrome b is stored in the specific/gelatinase granules (11), but it is not clear whether this pool of b cytochrome retains the ability to ferry electrons after translocation from the granules to the plasma membrane (or the phagosome).

Intracellular production of reactive oxygen species

Reactive oxygen species generated by the plasma membrane-localized NADPH oxidase will be released from activated cells. If activation occurs in the phagosomal membrane, the oxygen radicals will be retained inside the phagocyte. The reactive oxygen species generated in an intracellular compartment, *e.g.*, the phagosome, are not reached by membrane-impermeable scavenger molecules such as superoxide dismutase (SOD) or catalase added extracellularly. These scavengers remove superoxide anions and hydrogen peroxide released from the cells, but have no access to intracellular sites. Consequently, intracellular oxidants produced during phagocytosis are unaffected by such scavengers and can be measured, provided that the experimental protocol used allows for detection of intracellular oxygen radicals (19, 31).

It has become increasingly evident that, in the absence of a phagocytosable prey, a major part (or sometimes a minor part, depending on the stimulus; see below) of the stimulus-induced oxidants are generated in an intracellular compartment, *i.e.*, they are not affected by SOD and catalase. No such activity is detected in neutrophils isolated from individuals suffering from CGD (Table 1), and we thus conclude that phagocytosis-independent intracellular oxidant production truly reflects NADPH oxidase activity.

As mentioned above, the neutrophil specific granule membrane is the main storage pool for the b cytochrome, suggesting that this is the membrane at which the intracellular NADPH oxidase is assembled and activated (44, 46). This suggestion gains support from experiments performed with neutrophil cytoplasts. Organelle-free cytoplasts are unique membrane-enclosed cellular structures that lack subcellular granules (as well as all other subcellular organelles), but contain cytosol and have an intact receptor–response coupling mechanism and are capable of oxygen consumption and oxygen radical production (55). Cytoplasts thus provide means for studying the participation of granules in the intracellular phase of the respiratory burst. It turns out that the NADPH oxidase in cytoplasts can be activated to release superoxide anion and hydrogen peroxide at a rate and magnitude similar to that seen in intact neutrophils. However, no production originating from an intracellular compartment can be detected (Table 1) (21), implicating that the intracellular oxidant production is due to assembly and activation of the oxidase in the granules of intact cells. This suggestion gains further support from results obtained in many different experimental systems using specific granule-deficient HL-60 cells (17, 28). In many respects, differentiated HL-60 cells resemble normal neutrophils; they expose different chemoattractant and opsonin recep-

TABLE 1. SUBCELLULAR LOCALIZATION OF SUPEROXIDE PRODUCTION IN DIFFERENT CELL TYPES

	Intracellular production of O_2^-	*Extracellular production of O_2^-*	*Subcellular localization of the active NADPH oxidase*
PMNL	+	+	Plasma membrane, specific granules
HL-60	—	+	Plasma membrane
Cytoplasts	—	+	Plasma membrane
CGD PMNL	—	—	—
MPO-deficient PMNL	+*	+	Plasma membrane, specific granules

O_2^-, superoxide anion; PMNL, polymorphonuclear leukocyte.
*Not measurable by chemiluminescence.

tors, and they are equipped with an intact receptor–response coupling mechanism, as well as all the components required for assembly of a functional NADPH oxidase. However, HL-60 cells lack specific granules (8). In agreement with the results obtained with cytoplasts, the NADPH oxidase in differentiated HL-60 cells can be activated to release superoxide anion and hydrogen peroxide, but the intracellular phase is missing, correlating to the lack of specific granules (Table 1).

Additional data in support of our suggestion that the specific granules represent the most likely candidate as the source of intracellular NADPH oxidase activity are provided by subcellular fractionation studies (22, 67). Specific granule membranes can be isolated from cells that have been activated with a Ca^{2+} ionophore (*e.g.,* ionomycin, inducing production of intracellular oxidants). In addition to the dormant NADPH oxidase, which requires addition of cytosolic factors and sodium dodecyl sulfate or arachidonic acid to become active, such activated granule membranes contain an assembled and superoxide-producing NADPH oxidase, as well as an assembled but nonactive NADPH oxidase that can be activated by sodium dodecyl sulfate (without addition of cytosolic components; 22). Taken together, data from experiments with cytoplasts, HL-60 cells, and isolated neutrophil granules show that the oxidase can be assembled and activated at the b cytochrome-containing specific granule membrane (Table 1).

THE SUBCELLULAR LOCALIZATION OF THE RESPIRATORY BURST IS DETERMINED BY THE NATURE OF THE ACTIVATING AGONIST

Activation through pertussis toxin-sensitive heterotrimeric G protein-linked receptors

Different chemoattractants [*e.g., N*-formylated peptides, C5a, interleukin-8 (IL-8), Hp(2–20), WKYMVM/m] induce leukocyte infiltration and activation through binding to G protein-coupled seven-transmembrane cell-surface receptors (1, 72). The chemoattractant-mediated dissociation of $G_{\alpha i2}$ and the $G_{\beta\gamma}$ subunit complex results in activation of several downstream signaling effector enzymes that promote intracellular Ca^{2+} mobilization, modifications in the metabolism of phosphoinositides, and activation of mitogen-activated protein kinases (MAPK) (9). Integration of the different chemoattractant-activated signaling pathways results not only in directed cell migration, recruitment of new receptors from the granules to the cell surface, and release of proteolytic enzymes. Large amounts of superoxide are also produced by the NADPH oxidase (13, 15, 23). With respect to the subcellular localization of the activated oxidase, the receptors investigated so far [*i.e.,* FPR for formylated peptides, FPRL1 for WKYMVM/m and Hp(2–20), C5aR for C5a, and CCR for IL-8] all generate signals that induce assembly of the NADPH oxidase primarily in the plasma membrane. Consequently, the produced oxidants are released from the cells (Table 2).

Activation through the galectin-3 receptor(s)

We have recently described a new inflammatory mediator, galectin-3 (39), that interacts with and activates only "primed" neutrophils. Galectin-3 is a mammalian, lactose-binding lectin that is secreted from different inflammatory cells upon activation, *e.g.,* macrophages, mast cells, and epithelial cells. This lectin activates neutrophils that have encountered an extravasation process *in vivo* or been exposed to bacteria-derived lipopolysaccharides *in vitro.* The molecular mechanism behind the primed state with respect to galectin-3 responsiveness is the up-regulation to the cell surface of galectin-3 receptors accompanying the mobilization of the gelatinase granules (2, 39). The receptors are tentatively identified as CD66a and/or CD66b (26).

The cellular responses induced by occupation of the galectin-3 receptor(s) differ from those induced by occupation of the seven-transmembrane G protein-linked receptors. In addition to inducing release of reactive oxygen species, the lectin also activates the specific granule-localized pool of the oxidase, and the reactive oxygen species generated are thus retained intracellularly (Table 2). The

TABLE 2. LOCALIZATION OF THE RESPIRATORY BURST IN NEUTROPHILS AFTER STIMULATION BY DIFFERENT AGONISTS

	Localization of activated NADPH oxidase		
	Plasma membrane	*Specific granule*	*Activation mechanisms/receptors*
7-TM-R agonists*	+	—	7-TM-R ligation activates heterotrimeric G-protein giving hydrolysis of PIP_2, Ca^{2+} mobilization, etc.
Galectin-3	+	+	Ligation of β-galactoside-containing receptors, *e.g.*, CD66a/CD66b mobilized from gelatinase and specific granules
Phagocytic prey	—	+	Uptake through CRs (CR1 and CR3) or FcRs
α-CR3 pansorbins	—	+	Ligation of CR3 but without particle uptake
PMA	+	+	Stimulation of PKC; differs in dependency for PI 3-kinase
Ionomycin	—	+	Elevation of cytosolic free Ca^{2+} through ionophore activity

*Seven-transmembrane spanning receptor agonists, *e.g., N*-formylated peptides, C5a, IL-8, WKYMVM/m, Hp(2–20).

same type of activation pattern is observed with wheat germ agglutinin, a lectin that activates neutrophils through yet unidentified glycosylated receptors exposing sialic acid and/or *N*-acetylglucosamine (37).

Activation through CR3, the receptor for complement factor C3bi

It has been claimed that occupation of the complement receptor 3 (CR3) in neutrophils does not lead to an activation of the NADPH oxidase (7, 71). Nevertheless, a number of studies using serum opsonized microorganisms have shown that binding of a particle to CR3 results in activation of the oxidase (31, 34). This discrepancy is due in part to the choice of technique to determine oxidase activity, where the latter studies, in addition to measuring released oxidants, also investigated intracellular production (see below; Table 2). However, the studies showing oxidase activity during CR3 occupation have the drawback that structures other than C3bi on the surface of the particles are potentially capable of binding to the neutrophil. To overcome this problem, specific antibodies were used, directed either against the α subunit of CR3 or the β subunit common to the β2-integrins. When such antibodies *per se* are allowed to bind to CR3 on the neutrophil surface, no NADPH oxidase activity is triggered. To mimic the pattern of interaction between phagocyte and prey, neutrophils were instead challenged with antibodies bound to pansorbins (heat-killed protein A-positive *Staphylococcus aureus*). The *S. aureus* protein A binds the Fc moiety of the antibodies, leaving the antigen-binding site free to interact with CR3. Interestingly, an interaction between such particles and neutrophils results in activation of the oxidase (62). It would fit into the general idea about the function of CR3 as an opsonin receptor, if the difference in induction of oxidase activity between introducing the antibodies *per se* or the antibodies bound to a particle was due to induction of a phagocytic process by the latter. It is, however, intriguing that particles coated with antibodies to CR3 bind and generate signals that activate the oxidase, but do not trigger phagocytosis (62). Taken together, these results suggest that a local clustering of CR3, such as that induced by the antibody-exposing particles, is a prerequisite for oxidase activation.

The oxygen metabolites formed in response to CR3 ligation are not released from the cells, but formed at an intracellular site, and as stated above this occurs without formation of a classical phagosome/phagolyso-

some (Table 2). Despite the fact that activation is achieved without any uptake of the prey, the activation process is inhibited by cytochalasin B (an inhibitor of the dynamic reorganization of cytoskeletal actin), suggesting that the signaling pathway initiated by CR3 and leading to oxidase activation is highly dependent on a functional cytoskeleton (31, 62). This contrasts the oxidase activation and radical release induced by agonists operating through heterotrimeric G protein-coupled receptors, whose signaling capacity increases in the absence of an intact cytoskeleton.

LOCALIZATION OF THE NADPH OXIDASE ACTIVATION IS LINKED TO THE INTRACELLULAR SIGNALING PATHWAY INVOLVED IN THE ACTIVATION PROCESS

The protein kinase C (PKC) route of activation

As discussed above, different stimuli induce different patterns of extra- versus intracellular production of oxygen radicals, indicating a diversity in the regulating pathways (16, 40, 47, 70). Several of the neutrophil signaling pathways have a common starting point in the cleavage of membrane phosphoinositides, a process that generates membrane-localized activators of protein kinases, as well as soluble inositol metabolites of importance for mobilization of Ca^{2+} from intracellular stores. The direct stimulation of PKC by phorbol esters, *e.g.*, phorbol myristate acetate (PMA), mimics the effects of the natural PKC activator, diacylglycerol. PMA has been suggested to activate the NADPH oxidase through redistribution of PKC and phosphorylation of several proteins, including the cytosolic NADPH oxidase component p47phox (24, 25). Accordingly, PMA has been reported by many authors to induce extracellular release of oxygen metabolites. However, during PMA stimulation, an assembly of the oxidase occurs not only in the plasma membrane (resulting in a release of oxidants), but also in the specific granule membrane, giving rise to production of intracellular oxygen metabolites in amounts similar to those released (Table 2) (47). Hence, PMA comprises a good tool to investigate the signal transduction pathways leading to activation of the NADPH oxidase both in the plasma membrane and in the granule membrane.

Phosphatidylinositol 3-kinase (PI 3-Kinase) phosphorylates phosphatidylinositol (PtdIns), PtdIns 4-monophosphate (PtdIns4P), and PtdIns 4,5-bisphosphate [PtdIns(4,5)P_2], to form PtdIns3P, PtdIns(3,4)P_2, and PtdIns 3,4,5-trisphosphate [PtdIns(3,4,5)P_3], lipids that have been suggested to act as second messengers (14). Activation of PI 3-kinase is an early event in neutrophil responses to chemoattractants and has been shown to participate in intracellular signaling leading to activation of different neutrophil functions (43, 63, 65, 68). With respect to PI 3-kinase, neither wortmannin nor another PI 3-kinase inhibitor, LY294002, affects the release of superoxide anion induced by PMA (69), suggesting that this effect is independent of PI 3-kinase activity. The activation of the intracellular pool of NADPH oxidase, however, shows a dependency for PI 3-kinase, because wortmannin alters the intracellular oxygen radical production (40). Additional inhibitor studies show that activation of both pools of oxidase are dependent on extracellular signal-regulated kinase/MAPK kinase activity and protein phosphatase 1 and/or 2A.

The PI 3-kinase has been suggested to be localized upstream of PKC (68), and at least one PKC isozyme, PKCζ, is directly activated by PtdIns(3,4,5)P_3 (53). However, the fact that activation of the granule-localized oxidase is inhibited by wortmannin suggests that PI 3-kinase localizes downstream of PKC. The positioning of PI 3-kinase both upstream and downstream of PKC is not necessarily a contradiction. The phosphorylation of pleckstrin in platelets shows a two-mode stimulation pattern, characterized by an initial rapid activation of a cPKC followed by a slower, PtdIns(3,4)P_2-dependent activation of nPKCs (66). Also ras has been suggested to be both upstream and downstream of PI 3-kinase (14).

At present, five PKC isozymes, α, βI, βII, δ, and ζ, are known to be present in human neutrophils. The IC_{50} values for inhibition of the

oxidase activation with the PKC inhibitor Gö-6850 differed significantly between the plasma membrane-localized and the intracellular NADPH oxidase (40), indicating that different PKC isozymes could mediate the activation of the two NADPH oxidase pools. This is in line with recent data showing that heterogeneous translocation of PKC isozymes to neutrophil membranes occurs during phagocytosis (61), as well as in response to intracellular Ca^{2+} elevation (49).

The PI 3-kinase is also involved in the signaling leading to NADPH oxidase activation in response to formyl-methionyl-leucyl-phenylalanine (fMLF). However, it should be noted that although both fMLF and PMA induce assembly and activation of the NADPH oxidase in the plasma membrane, two different signaling pathways are engaged for this purpose. This is illustrated by the fact that phosphatase inhibitors such as okadaic acid and calyculin A have opposite effects on the activity induced by the two stimuli. The PMA-induced release of superoxide anion is inhibited by the phosphatase inhibitors, whereas the fMLF-induced oxidant production and release is markedly enhanced, in both magnitude and duration (33).

The Ca^{2+} route of activation

A common feature in activation of secretory systems in many cell types is an elevation of the free cytosolic concentration of Ca^{2+} ($[Ca^{2+}]_c$), and the role of Ca^{2+} as a regulator of neutrophil function has been recognized for several years (4). The increase in $[Ca^{2+}]_c$ associated with neutrophil activation is derived from two sources; release into the cytoplasm of Ca^{2+} present in intracellular storing organelles, and influx of Ca^{2+} from the extracellular milieu through an opening of Ca^{2+} channels in the plasma membrane (3). The two sources are interlinked, as an emptying of intracellular Ca^{2+} stores is believed to induce opening of so-called store-operated Ca^{2+} channels in the plasma membrane (54). So far, neither the identity of the store-operated Ca^{2+} channels nor the molecular mechanism for communication between the intracellular stores and the plasma membrane is known. Specific ionophores that allow for an influx of Ca^{2+} from the extracellular environment and release Ca^{2+} from intracellular stores can be used to mimic the rise in intracellular Ca^{2+} induced by physiological stimuli. Using this approach, we have shown that a rise in $[Ca^{2+}]_c$ is a signal sufficient to activate the granule-localized NADPH oxidase, but not that in the plasma membrane (16, 21) (Table 2). Mobilization to the plasma membrane of b cytochrome-containing granules is associated with an increased secretion of superoxide in response to, *e.g.*, fMLF, concomitant to a loss of the Ca^{2+} sensitivity of the mobilized oxidase component (18). An increase in $[Ca^{2+}]_c$ is thus not sufficient to initiate activation of the NADPH oxidase in the plasma membrane, but such an increase can prime the cells for an increased response to other stimuli (17). Furthermore, it is clear that translocation of the cytosolic oxidase components to a b cytochrome-containing membrane (a prerequisite for activation of the oxidase) is not induced by Ca^{2+} *per se* (52). The responsible signaling molecules generated by an elevation of $[Ca^{2+}]_c$ have thus yet to be identified.

THE AZUROPHIL GRANULES AND THE IMPLICATIONS FOR GRANULE–GRANULE FUSION

Myeloperoxidase (MPO), stored in the azurophil granules, participates in the processing of oxidants generated by the NADPH oxidase

One of the techniques that allow for a determination of intracellular respiratory burst activity is the luminol-amplified chemiluminescence (for details about the technique and how it can be used to follow intracellular as well as extracellular generation of respiratory burst products, see 19). In this technique, reactive oxygen species generated by the neutrophil NADPH oxidase excite the luminol molecule that, when it returns to its ground state, releases energy in the form of light (Fig. 2A). The luminol reaction in relation to neutrophils is highly dependent on the participation of MPO. This is illustrated by the fact that neutrophils isolated from persons with a

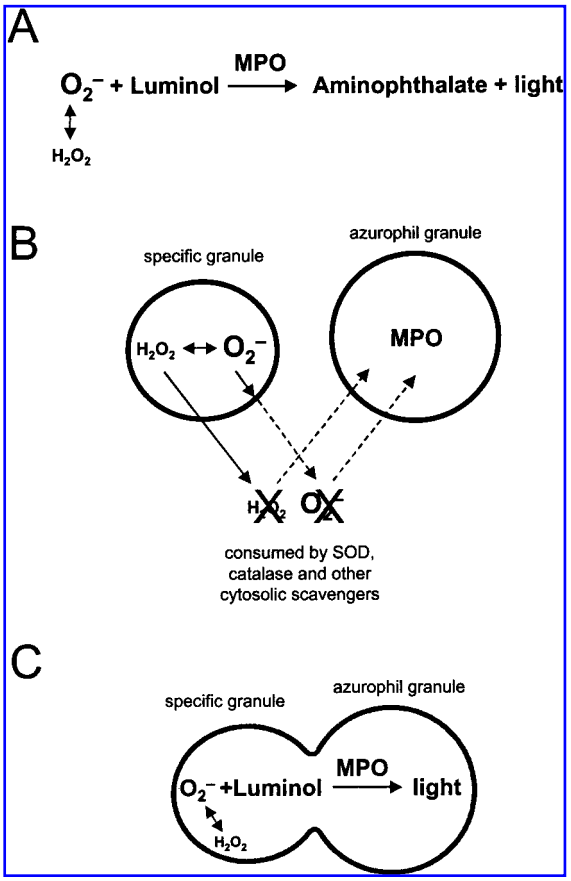

FIG. 2. (**A**) In the presence of MPO, superoxide anions (O_2^-) generated by the NADPH oxidase can excite luminol that, when it returns to its ground state, releases energy in the form of light. (**B**) One possible mechanism for the superoxide to reach MPO is that it diffuses from the specific granules, through the cytoplasm, and into the azurophil granules. This is contradicted, however, by the facts that (a) superoxide cannot traverse biological membranes and (b) oxidants are consumed by scavengers in the cytoplasm. (**C**) Fusion between the specific and azurophil granules comprise a plausible hypothesis for how superoxide comes in contact with MPO to give rise to chemiluminescence. H_2O_2, hydrogen peroxide.

selective deficiency in MPO, one of the more frequent granulocyte defects (45), produce very low levels of chemiluminescence despite a pronounced (in fact higher than that of normal cells) production of superoxide anion and hydrogen peroxide.

Addition of a peroxidase (MPO or horseradish peroxidase) to MPO-deficient cells regenerates the extracellular chemiluminescence activity induced, *e.g.*, by fMLF (20), but is without effect on intracellular activity induced by activation through CR3 or through a rise in $[Ca^{2+}]_c$ (unpublished observation). The fact that addition of a peroxidase to the extracellular fluid is not sufficient to regenerate the intracellular activity in peroxidase-deficient cells suggests that MPO, stored in the azurophil granules of normal neutrophils, participates in the processing of the intracellularly produced oxidative metabolites. There are several potential mechanisms for this effect. Theoretically, reactive oxygen species released to the extracellular milieu during neutrophil activation could reach the azurophil granules by diffusing back into the cell and react with the granule-localized MPO to generate light. However, the fact that an intracellular chemiluminescence reaction can occur without any release of superoxide anion or hydrogen peroxide suggests that the reactive oxygen species reach the MPO-containing compartment by a more direct route. An alternative would be that superoxide (the oxygen metabolite that reacts with MPO and excites luminol; for details, see 19) produced intracellularly first diffuses through one membrane (that of the specific granules), then through the cytosol, and then through yet another membrane (that of the azurophil granule), to reach the MPO-containing compartment (Fig. 2B). This mechanism is unlikely, because superoxide cannot pass biological membrane in the absence of anion channels (no such channels have been described in neutrophils). If the superoxide anions by "chance" would pass the specific granule membrane, they will face a cytoplasm loaded with oxygen radical scavengers that would consume the oxygen metabolites before reaching the azurophil granules. Superoxide spontaneously dismutates to hydrogen peroxide, which can diffuse over a membrane. However, also this metabolite will be consumed by scavengers in the cytosol (Fig. 2B). Another scenario is that the oxygen radical-producing organelle (the specific granule) is induced to fuse with the peroxidase-storing azurophil granule, a fusion process that would allow the superoxide anion generating NADPH oxidase and MPO to cooperatively excite luminol and produce chemiluminescence with an intracellular origin (Fig. 2C). The fusion hypothesis gains support from the facts that such a process has been shown to occur during activation of eosinophils (57), and that the intracellular chemiluminescence

reaction can be inhibited without any change in the intracellular NADPH oxidase activity (Karlsson, unpublished observations).

CONCLUDING REMARKS

CGD is the inherited disorder that is characterized by a defect in the phagocyte respiratory burst oxidase and impaired microbial killing. Basic studies of CGD patients and their phagocytes have clearly revealed that superoxide anion and hydrogen peroxide generated through activation of the electron-transporting NADPH oxidase give rise to yet other reactive oxygen species that are of ultimate importance for the ability of the innate immune system to combat invading microbes. It is important to note, however, that a defect in the respiratory burst oxidase is associated also with defect apoptosis (programmed cell death) (41) and development of chronic inflammatory lesions unrelated to the presence of a viable microbial target (51). This suggests a role for the oxidants beside their function as microbial killing agents. In line with this, it has been shown that oxygen radicals have direct or indirect signaling capabilities, affecting, for example, the state of protein phosphorylation of second or third messengers (30, 64). The fact that some transcription factors appear to be regulated by their redox state (27) strongly implies that respiratory burst products may participate also in regulating expression of individual genes or whole regulons. Nevertheless, the precise role of the reactive oxygen metabolites produced by the phagocyte respiratory burst oxidase in cellular compartments devoid of invading microbes has yet to be defined.

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ABBREVIATIONS

$[Ca^{2+}]_c$, free cytosolic concentration of Ca^{2+}; CGD, chronic granulomatous disease; CR3, complement receptor 3; fMLF, formyl-methionyl-leucyl-phenylalanine; IL-8, interleukin-8; MAPK, mitogen-activated protein kinase; MPO, myeloperoxidase; PI 3-kinase, phosphatidylinositol 3-kinase; PKC, protein kinase C; PMA, phorbol myristate acetate; PtdIns, phosphatidylinositol; SOD, superoxide dismutase.

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Address reprint requests to:
Anna Karlsson
The Phagocyte Research Laboratory
Department of Rheumatology
Guldhedsgatan 10
S-41346
S-430 05 Göteborg, Sweden

E-mail: anna.karlsson@microbio.gu.se

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