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Forum Review

Neutrophil Priming in Host Defense: Role of Oxidants as Priming Agents

STEVE D. SWAIN,[1] TROY T. ROHN,[2] and MARK T. QUINN[1]

ABSTRACT

Neutrophils play an essential role in the body's innate immune response to infection. To protect the host, these phagocytic cells possess an impressive array of microbicidal weapons that can be brought to bear on an invading pathogen, including a variety of toxic oxygen radical species and proteolytic enzymes. Although the neutrophil response is designed to restrict the damage to the smallest possible region where the pathogen is located, some of the damaging agents inevitably leak into the surrounding areas where they have the capacity to inflict tissue damage at sites of inflammation. Thus, it is essential that the host defense response of these cells is finely tuned to result in the appropriate level of response to any given situation. One of the regulatory mechanisms implicated in controlling neutrophil responses is priming. Through the action of priming agents, the level of activation and subsequent responses of the cell can be regulated so that a continuum of activation states is achieved. In this review, we describe key features of the priming response in host defense and disease pathogenesis and focus on the unique role of reactive oxygen species as priming agents. Antioxid. Redox Signal. 4, 69–83.

INTRODUCTION

THE INNATE HOST DEFENSE RESPONSE of humans is complex and multileveled, involving many cell types with distinct but overlapping roles. During this response, polymorphonuclear leukocytes (neutrophils) and other phagocytic cells are mobilized to sites of injury or infection where they serve to destroy invading pathogens. To accomplish this deed, neutrophils possess an impressive array of microbicidal weapons that can be activated. Although normally found traveling in the circulatory system, these cells rapidly sense the signs of microbial assault and respond by moving through the endothelial layer and out to the site of infection. As their initial mode of attack is to phagocytose the pathogen, neutrophils are known as professional phagocytes. Once ingested, the foreign particles can be destroyed by proteolytic enzymes stored in special granules, and by the production of reactive oxygen species (ROS). In certain instances, neutrophil enzymes and ROS can also be released into the extracellular environment, where they attack adjacent pathogens, as well as (inadvertently) host cells and tissues. Because of the potency of their microbicidal armaments, neutrophils could be considered effective but indiscriminate attackers, with little or no potential for selective inhibition of specific microbicidal responses. Recently, however, it has become clear that the neutrophil host defense response is actually very finely modulated to provide an appropriate response. Factors im-

[1]Department of Veterinary Molecular Biology, Montana State University, Bozeman, MT 59717.
[2]Department of Biology, Boise State University, Boise, ID 83725.

plicated in this modulatory role are known as priming agents. Thus, the ability of priming agents to modulate a graded response would allow the cell to respond proportionally to the severity of a stimulus. We present here an updated review of the concept of neutrophil priming, the role of ROS as priming agents, and the relevance of priming to host defense.

A DEFINITION OF PRIMING

The understanding of how a neutrophil undergoes functional transformation to facilitate an adequate host defense response has evolved over time. The initial standard for a neutrophil response was full activation. Presented with appropriate stimuli, a neutrophil that had been quietly circulating through the bloodstream would become attracted to a site of infection and fully activated. This process involved morphological polarization of the cell and subsequent chemotaxis, phagocytosis, production of ROS, and perhaps the release of granule enzymes into the extracellular environment. An enormous amount of research has focused on understanding this process, including not only the type of stimuli causing activation, and the consequences thereof, but also the molecular mechanisms involved in the activation process. It soon became evident, however, that activation was actually not an all-or-none process. Not only were neutrophils from infected patients sometimes found to be hyperresponsive to stimuli (46), but *in vitro* treatment of neutrophils with substimulatory doses of activating agents could cause heightened responses of those cells to subsequent stimuli (*e.g.*, 29). These types of studies led to the concept of a two-stage activation process. Neutrophils would first encounter a stimulus that would not activate the cells directly, but would leave the cells in a "primed" state. When primed neutrophils encountered a second appropriate stimulus, a fully activated state would result. Therefore, a consensus definition of priming has been generally accepted: priming is when the neutrophil's functional response (*e.g.*, ROS production, chemotaxis) to

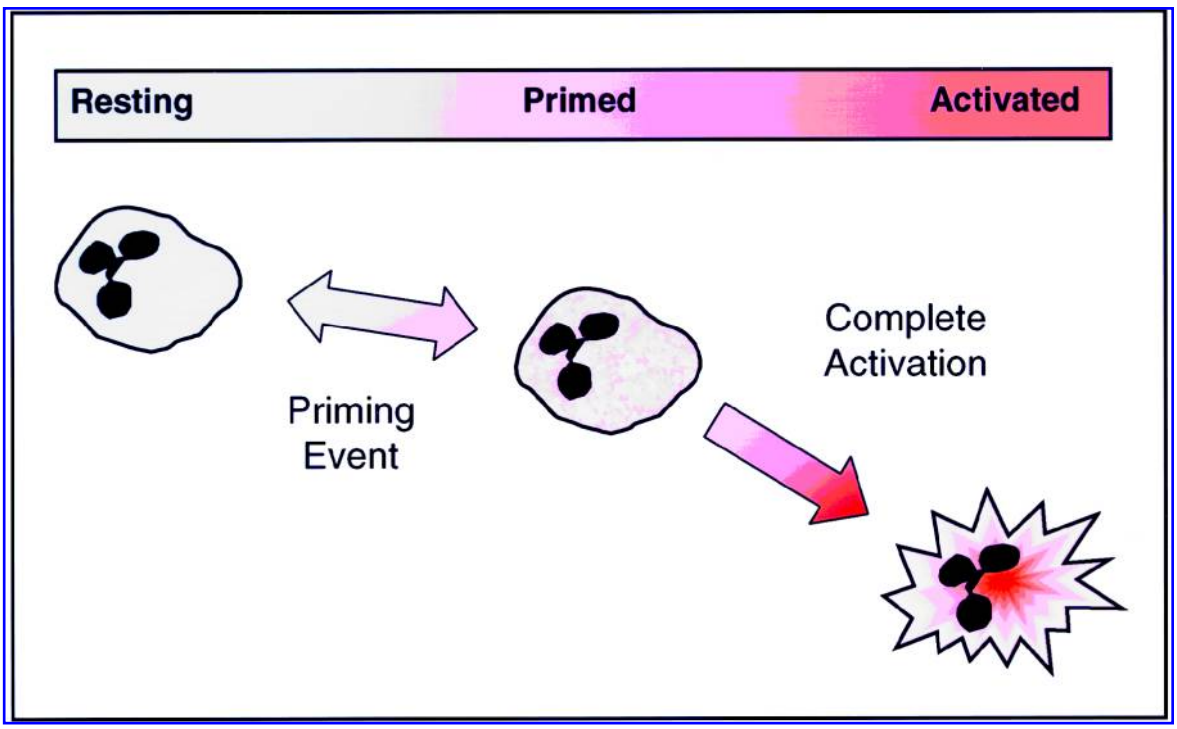

FIG. 1. A continuum model of neutrophil activation. Neutrophil activation can be modeled as a continuum from a resting to a completely activated state, whereas priming can modulate the location of the cell within this continuum and appears to be a reversible event. However, subsequent exposure of a primed cell to an activating stimulus pushes the cell irreversibly to a fully activated state.

a stimulus is amplified by previous exposure of the cell to a priming agent. The priming agent itself, however, does not normally cause a noticeable functional response, except possibly at very high concentrations of the priming agent.

In the last 10 years, there has been tremendous interest in the concept of neutrophil priming, and the number of agents that have been shown to have priming effects continues to grow (see below). Investigation into the biochemical mechanisms involved in neutrophil priming has demonstrated that multiple interacting pathways are commonplace in the signal transduction of priming events. It is also becoming evident that the phenotypes presented by "primed" neutrophils are equally diverse (see below). Furthermore, there is also evidence that primed neutrophils can return to a resting state (38). Clearly, it is an oversimplification to think of neutrophils as occurring in only three distinct states: resting, primed, and activated. Rather, the activation state of neutrophils seems to fall within a continuum, from nonresponsive to fully activated, in relation both to the functional response of the cells and to their morphological appearance (Fig. 1). Although this perspective of a neutrophil's responses might seem overly complex, it may allow for the development of new experimental approaches to investigate neutrophil function. This in turn may result in the elucidation of the mechanisms by which specific neutrophil functions are turned on and off, and the development of specific therapies against the detrimental effects of an excessive neutrophil response. We present here an overview of neutrophil priming, with an emphasis on the role of various ROS as inducers of the priming response. This is by no means an exhaustive review of all the pertinent literature, but rather an update to a fascinating and diverse field. The reader may refer to other recent priming-related reviews (*e.g.,* 12) for additional information and viewpoints.

PRIMING IN HOST DEFENSE

Neutrophil priming is thought to play a key role in the host-defense process, and the regulation of priming may be essential for host survival. For example, the level of neutrophil priming has been linked to the severity of disease and disease outcome, and a number of studies have suggested that priming may be a good indicator of clinical disease activity (59, 81). Indeed, neutrophils with a primed phenotype have been observed in the blood of subjects where accelerated host defense would be appropriate. Enhanced neutrophil ROS production has been reported in patients with infections (4, 68), although some of these studies have shown that only certain neutrophil subpopulations display a primed response (4). Primed neutrophils have also been found in the blood of trauma patients (9, 90), as well as patients with chronic inflammatory diseases such as rheumatoid arthritis (20).

The studies described above provide circumstantial evidence that neutrophil priming is important in host defense *in vivo.* However, the high variability of the clinical data has led investigators to develop *ex vivo* models to study the role of neutrophil priming in host tissue defense. Typically, these models involve the collection of exudate neutrophils from a skin lesion into an aseptic container that is placed over the lesion. These studies show that exudate neutrophils have many similarities to *in vitro* primed cells, including an enhanced respiratory burst in response to the chemotactic peptide *N*-formyl-methionine-leucine-phenylalanine (fMLF) (91) and increased cell surface expression of CD11b/CD18 (39, 66, 91), complement receptor 1 (66), alkaline phosphatase, CD45 (39), and fMLF receptors (91). At the same time, exudate neutrophils exhibit a substantial shedding of surface L-selectin, as do *in vitro* primed cells (39, 66). It has been proposed that the changes in surface antigen expression seen in exudate neutrophils are due to regulated degranulation, wherein most of the secretory granules and smaller amounts of the gelatinase and specific granules are mobilized to cause the observed changes in the cell-surface molecules (66).

The clinical use of cytokines with potential priming actions has also shed light on the role of priming in host defense. For example, administration of granulocyte-macrophage colony-stimulating factor (GM-CSF) induced

effects consistent with priming: the fMLF-induced respiratory burst of neutrophils obtained from these patients was enhanced compared with controls (13), and up-regulation of CD11b/CD18 was observed on neutrophil surfaces (13). Tumor necrosis factor-α (TNF-α) has been shown to induce priming responses, and neutrophils removed from TNF-α-treated subjects exhibited an enhanced respiratory burst (62). In addition, plasma indicators of neutrophil degranulation were also elevated in these individuals (74).

It is likely that a complex interplay occurs between the neutrophils and all pertinent priming and modulatory agents that are present. Given the evidence that neutrophil priming occurs *in vitro* and *in vivo* to various degrees, one has to consider the usefulness of such a mechanism. Because of the possible damage to host tissue that could occur if neutrophils were inappropriately activated, priming allows for a more carefully regulated activation process. In an ideal system, circulating neutrophils would be quiescent, and priming (at the appropriate level) and activation would occur at localized sites of infection and over an appropriate time course. In most of the studies described above, neutrophils and priming agents are in suspension together. However, in a biological system, some mechanisms are in place to localize these types of priming interactions. For example, platelet activating factor (PAF) has been proposed to have several modes of interaction with target cells. One of these is juxatcrine, where PAF remains bound to the cell of origin, thus helping to regulate its actions in a localized fashion (92). The action of interleukin-8 (IL-8) on neutrophils is also enhanced at local sites by binding to extracellular matrix proteins such as heparan sulfate (84). Another priming cytokine, TNF-α, can be modulated by circulating, soluble forms of its receptor, which may allow for dampening of TNF-α effects away from the site of production (76). Together, the sequential nature of priming and activation, the gradation of priming responses, and the localization mechanisms discussed above would all allow for precise control over the magnitude and scope of neutrophil response to infection.

PRIMING IN DISEASE PATHOGENESIS

Given the potent armament that neutrophils possess, they also have great potential for damaging host tissue, should these weapons become uncontrolled and/or misdirected. Indeed, there are a large number of pathological conditions in which host tissue damage by neutrophils has been demonstrated. For example, neutrophil-derived ROS have been implicated as the damaging agent in various inflammatory diseases, including adult respiratory distress syndrome (7) and rheumatoid arthritis (18).

The mechanisms of host tissue damage by neutrophils are, in essence, the same used to destroy pathogenic microorganisms (58). For example, oxygen radicals can damage cellular proteins and lipids. Degradative enzymes that normally serve in the destruction of phagocytosed bacteria and tissue repair can be released from dying neutrophils or by cells that have been activated extensively enough to result in azurophil granule release. Also, neutrophil cationic proteins that are directly bactericidal can damage host cells when released in sufficient concentrations (17). Although there is little doubt that neutrophils contribute to host tissue damage during inflammation, much less is known about why the neutrophil's response becomes unregulated in inflammation, leading to disease pathology. As priming seems to play a role in determining the magnitude of the host defense response, one possible factor contributing to the excessive response of neutrophils in these conditions may be inappropriate levels of priming (44). As an example, Robinson *et al.* (60) reported that synovial fluid from patients with rheumatoid arthritis contained not only insoluble antibody aggregates, but also soluble antibody aggregates, and that this material could activate synovial neutrophils, but not peripheral neutrophils from healthy donors, unless they are primed with GM-CSF. Thus, these studies implicate neutrophil priming in the pathogenesis of rheumatoid arthritis. Indeed, synovial fluid in rheumatoid arthritis joints has been shown to contain many priming agents, including interleukin-1β, IL-8, TNF-α, and GM-CSF (18). In addi-

tion, the production of peroxynitrite in the joint (36) suggests the possibility that it may contribute to neutrophil priming in this area.

EFFECTS OF PRIMING ON THE NEUTROPHIL

The overall effect of neutrophil priming is to prepare the cell for the appropriate level of response to a given stimulus. In the classical example of priming, exposure of neutrophils to an agent [*e.g.,* lipopolysaccharide (LPS)] was found to potentiate the oxidative burst of these cells to a subsequent stimulation by fMLF (29). In fact, most of the published reports on neutrophil priming focus on enhancement of the respiratory burst, and this has come to be the most accepted criteria to decide whether an agent has priming abilities. However, many other neutrophil functions can be enhanced by prior exposure to priming agents (Table 1). The release of neutrophil granule contents is an example, and several agents have been reported to enhance the fMLF-induced release of granule enzymes (2, 24). The understanding of priming-potentiated degranulation is complicated, however, as most investigators only examine a subset of the neutrophil granules (azurophil, specific, or secretory granules). Additionally, the use of the cytoskeletal destabilizing agents (*e.g.,* cytochalasin B) in many studies makes direct comparison of degranulation experiments difficult. Another important neutrophil function that is related to the priming response is the modulation of receptor and adhesion molecule expression on the cell surface. Finally, priming agents can modulate neutrophil production of inflammatory mediators, such as leukotrienes and cytokines, which can have localized paracrine action on neighboring cells. Thus, it is becoming clear that priming agents can produce many effects in neutrophils, and that one priming effect may influence another. In addition, it is now evident that different priming agents can operate via distinctly different cellular mechanisms and may induce different combinations of priming responses. A current list of agents that have been reported to elicit priming responses in neutrophils, based on the classical definition of enhancing the oxidative burst, is shown in Table 2. Below, five of the most common priming agents are examined in more detail.

TABLE 1. FUNCTIONAL CHANGES INDUCED BY PRIMING AGENTS

- Enhancement of the respiratory burst
- Changes in the level of cell-surface adhesion molecule expression
- Up-regulation of cell-surface receptors
- Production and release of bioactive lipids
- Degranulation and release of granule contents
- Actin polymerization
- Phosphorylation events
- Increased phagocytosis
- Activation of signal transduction enzymes
- Change in rate of neutrophil apoptosis

TNF-α

TNF-α is a 17-kDa protein produced by monocytes, macrophages, and, to a lesser extent, endothelial cells and neutrophils (78). This potent cytokine plays a major role in the inflammatory response and is important in host defense against infectious agents and malignant tumors (78). TNF-α has long been recognized as a potent priming agent, and neutrophils treated with TNF-α followed by stimulation with fMLF will produce an oxidative burst greater than that of unprimed cells (2, 11, 21, 70). The priming response to TNF-α also occurs in cells stimulated with C5a (2) or opsonized zymosan (73), but not when phorbol myristate acetate (PMA) is used as the stimulus (2, 70).

Besides effects on the respiratory burst, priming by TNF-α also has been shown to stimulate release of certain neutrophil granules. TNF-α can cause a dose-dependent degranulation of specific granules; however, the effect is very small for cells in suspension, as compared with cells adherent to fibronectin (42). Like specific granules, gelatinase granules can also be mobilized by TNF-α treatment (30). As a result of degranulation, upregulation of certain cell-surface molecules can occur during TNF-α priming. For example, CD11b/CD18 and CD11c/CD18 are

TABLE 2. PRIMING AGENTS FOR HUMAN NEUTROPHILS

Cytokines	Interleukin-1 (IL-1)
	Interleukin-3 (IL-3)
	Interleukin-6 (IL-6)
	Interleukin-8 (IL-8)
	Interferon-γ (IFN-γ)
	Granulocyte colony-stimulating factor (G-CSF)
	Granulocyte-macrophage colony-stimulating factor (GM-CSF)
	Tumor necrosis factor-α (TNF-α)
Lipid mediators	Platelet-activating factor (PAF)
	Leukotriene B_4 (LTB_4)
	Ceramide (C2 and C6)
	Phorbol myristate acetate (PMA)
	Alkylacylglycerols
Hormones/ growth factors	Insulin-like growth factor I (IGF-I)
	Hepatocyte growth factor (HGF)
	Recombinant human growth hormone (rHGH)
	Vasoactive intestinal peptide (VIP)
	Brain natriuretic hormone
	Atrial natriuretic peptide (ANP)
	β-Endorphin
	Met-enkephalin
	Melatonin
	Substance P
	Neurokinin A and B
	Vitamin D binding protein
	Thrombopoietin
Other agents	Adenosine triphosphate (ATP)
	Diadenosine polyphosphates
	Inositol hexakisphosphate ($InsP_6$)
	Urokinase-type plasminogen activator
	Kallikrein
	Fibronectin
	Peroxynitrite
	Calmodulin antagonist W-7
	Antibodies to neutrophil cell-surface antigens
	Cigarette smoke condensate
	Proteases
	Glycoxidized collagen

rapidly up-regulated upon TNF-α exposure, whereas CD11a/CD18 expression remains unchanged (11). The number, but not the affinity, of cell-surface fMLF receptors is also up-regulated during TNF-α priming (21, 50), explaining the mechanism behind TNF-α enhancement of the fMLF-induced oxidative burst. Because surface molecule expression can be altered rapidly by TNF-α treatment, it is likely that these proteins are drawn from intracellular pools, such as secretory granules (65) and/or specific and gelatinase granules (30). Although TNF-α has been reported to cause release of azurophil granule proteins in whole blood (16), degranulation of azurophil granules is not normally observed when neutrophils are treated *in vitro* with TNF-α in the absence of cytochalasin B (2, 42). One explanation for this discrepancy is the possibility that other unknown factors or soluble mediators present in whole blood may have contributed to the observed effects.

In addition to up-regulation of cell-surface molecules, neutrophil priming can also result in down-regulation of certain antigens, and TNF-α priming has been found to induce shedding of L-selectin from the neutrophil surface (6, 11).

PAF

Many cell types, including neutrophils, produce the lipid mediator PAF by enzymatic cleavage of membrane phospholipids (77). PAF has potent direct effects on neutrophils and can induce chemotaxis, actin polymerization, and aggregation, but it also has many priming-type actions (27, 92). Like TNF-α, PAF priming has been shown to enhance fMLF-stimulated oxidant production over that observed in unprimed cells (27, 28, 32, 38). This enhancement is strongly concentration-dependent over PAF concentrations of 10^{-9} *M* to 10^{-5} *M* (27, 28, 38). It is noteworthy, however, that high concentrations of PAF can actually activate a weak oxidative burst (28, 38). In contrast to TNF-α, PAF has little priming effect on the respiratory burst induced by opsonized zymosan, although it strongly enhances the oxidative burst in response to PMA (27, 28). The potentiation of the PMA-activated oxidative burst by PAF both enhances the maximal rate of superoxide production (as with fMLF) and reduces the time required to reach the maximal rate (27). Up-regulation of cell-surface molecules (such as β_2 integrins) also occurs in neutrophils treated with PAF (6, 11, 52). The PAF-induced priming effects also occur very rapidly (11, 38, 52), implicating mobilization of granule protein stores in the priming response. The rapid up-regulation of CR1 receptors (11), alkaline phosphatase, and CD11b/CD18 (6) suggests that secretory granule mobilization is also a part of the PAF

priming response. Although several groups have reported that PAF induces degranulation of azurophil granules in the presence of cytochalasin B (52, 71), little or no azurophil degranulation is reported in the absence of cytochalasin B (32).

Recently, we characterized the dose response of bovine neutrophils to PAF with respect to a number of functional parameters, including Ca^{2+} flux, membrane potential changes, actin polymerization, degranulation, and the production and/or priming of the oxidative burst (69). Based on the overall response pattern to PAF, we found that lower concentrations of PAF promote neutrophil sensitivity to subsequent stimuli by selective degranulation, up-regulation of adhesion molecules, and increased actin polymerization. In contrast, higher concentrations seem to promote more direct bactericidal responses, such as the release of ROS and granule enzymes. The ability of priming PAF to modulate a graded response in neutrophils, depending on the concentration of PAF encountered, would allow the cell to respond proportionally to the severity of a stimulus. This model for PAF action also fits within the responses previously characterized for human neutrophils and is suggested to represent a general paradigm for the action of many priming agents.

LPS

LPS, a component of the cell wall of gram-negative bacteria, is a potent inflammatory mediator, which induces production of proinflammatory cytokines and functional alterations in many cells during sepsis (72). Because LPS can be produced from many sources and can be a contaminant in biological buffers, there are conflicting reports in the literature regarding the effective concentration of LPS needed for priming. The interaction of LPS with its receptor, CD14, is enhanced by a plasma/serum protein called LPS binding protein (for review, see 72). Whether or not plasma or serum is included in experimental protocols is most likely the major reason for the variable results reported in LPS priming investigations. In most cases, LPS has potent priming actions on the fMLF-stimulated oxidative burst (1, 24, 49, 80). The stimulation is concentration-dependent (usually in the range of 10–1,000 ng/ml) (1), although this varies depending on whether LPS was immobilized (49) or in the presence of LPS binding protein and/or lipoproteins (80). In addition to fMLF, LPS can also enhance the oxidative burst in response to IgG or C3b-opsinized zymosan (25) and to PMA (26).

LPS priming appears to result in mobilization of various neutrophil granules; however, the results on some granule types have been highly variable. Recruitment of secretory granules, as evaluated by an increase in cell-surface alkaline phosphatase, can occur by treatment with relatively low doses of LPS (1, 49). Gelatinase granule release also appears to occur after LPS treatment (54). LPS has been reported to induce azurophil granule release (16); however, this response seems to vary widely (24). Recently, it has been suggested that LPS has a true priming effect on azurophil granule release in that it can potentiate fMLF-stimulated elastase release, but has no direct effect in mobilizing granules (51). Thus, LPS treatment may facilitate subsequent activation-induced azurophil granule release. Some degree of specific granule degranulation does seem to occur in response to LPS, even in the absence of cytochalasin B (24). For example, up-regulation of adhesion molecules (β_2 integrins) and fMLF receptors on the cell surface is observed with priming doses of LPS (11, 49, 80).

GM-CSF

Endothelial cells and mononuclear leukocytes produce proteins called colony-stimulating factors, named for their ability to promote hematopoiesis (48). Additionally, some of these compounds have also been shown to have priming effects on neutrophils. One such factor that has been heavily studied is GM-CSF, a 23-kDa glycoprotein (56). GM-CSF has some similarities in its priming abilities to TNF-α and enhances the fMLF-induced oxidative burst (3, 19, 22, 37). As with most priming agents, oxidative burst priming occurs in a concentration-dependent fashion (3,

37), with priming apparent at as low as 10 pg/ml, and reaching a plateau at 1 ng/ml (3). GM-CSF has no effect, however, on stimulation of the oxidative burst by PMA (85) or opsonized zymosan (41). In the absence of cytochalasin B, GM-CSF does not cause degranulation of either the specific or azurophil granules (3). However, treatment of neutrophils with GM-CSF does cause up-regulation of CD11b/CD18 (6, 19, 41), complement receptor 1 (41), as well as the fMLF receptor (19). Increases in cell-surface alkaline phosphatase supports the hypothesis that the up-regulation of these surface antigens is due to mobilization of secretory granules (6).

IL-8

Another molecule that has distinct priming abilities is the proinflammatory cytokine IL-8. IL-8 is produced by a large number of cell types, including monocytes, granulocytes, endothelial cells, and epithelial cells (87). As with the other priming agents discussed here, IL-8 treatment causes enhancement of the fMLF-stimulated oxidative burst (14, 22, 75, 86), and its actions are concentration-dependent, beginning at 1–5 ng/ml, with a maximal effect at 10 ng/ml (86). In contrast to TNF-α and GM-CSF, IL-8 does appear to prime the PMA-stimulated oxidative burst (75, 86), although the priming enhancement is less than what is seen with the other priming agents described above. Limited degranulation also occurs during the IL-8-induced priming response. For example, IL-8 treatment induces secretory granule degranulation, based on increase in cell-surface alkaline phosphatase (6) and results in up-regulation of fMLF receptors (22), and CD11b/CD18 (6). Although azurophil degranulation does not seem to occur in the absence of cytochalasin B, a small amount of specific granule degranulation may occur in response to priming doses of IL-8 (8).

ROS AS PRIMING AGENTS

The possibility that the very cells generating reactive oxidants might also be impacted by the ROS they generate (autocrine effect) or by ROS generated by neighboring cells (paracrine effect) is a very intriguing idea and has received significant attention in the last 10 years. The interaction of ROS with neutrophils probably involves multiple mechanisms of action, and some responses are more distinct than others (Fig. 2). Below, we describe several of the primary mechanisms involved in the ROS-induced priming response.

Membrane perturbation

Plasma membrane organization plays an important role in regulating neutrophil responsiveness (15). For example, Jesaitis and coworkers (34) found that neutrophil chemoattractant receptors were laterally segregated into membrane microdomains, resulting in loss of access to G proteins. Similarly, the NADPH oxidase system also appears to be laterally organized within the plasma membrane (55). Additionally, Kusner *et al.* (40) demonstrated that one mechanism behind protease priming of the neutrophil respiratory burst was protease-induced alterations in membrane structure, resulting in increased lateral mobility. ROS can have potent effects on neutrophil membrane lipids, resulting in changes in membrane fluidity. However, whereas some investigators have reported that ROS cause decreased neutrophil membrane fluidity (31, 43), others have reported that oxidant exposure results in increased mobility of proteins within the neutrophil plasma membrane (35, 57).

Modulation of kinase activity

Analyses of the effects of ROS on specific neutrophil activation pathways have demonstrated specific and intriguing effects of ROS. For example, select signal transduction pathways that utilize mitogen-activated protein kinases, a group of serine/threonine kinases that are strongly implicated in neutrophil priming and activation (88), show redox-dependent increases in tyrosine phosphorylation and activation (23). The same type of redox-sensitive activation has also been demonstrated for several tyrosine kinases involved in adhesion-dependent neutrophil oxidative burst and phagocytosis, including $p56/59^{hck}$ and $p72^{syk}$ (10) and $p58^{c\text{-}fgr}$ and

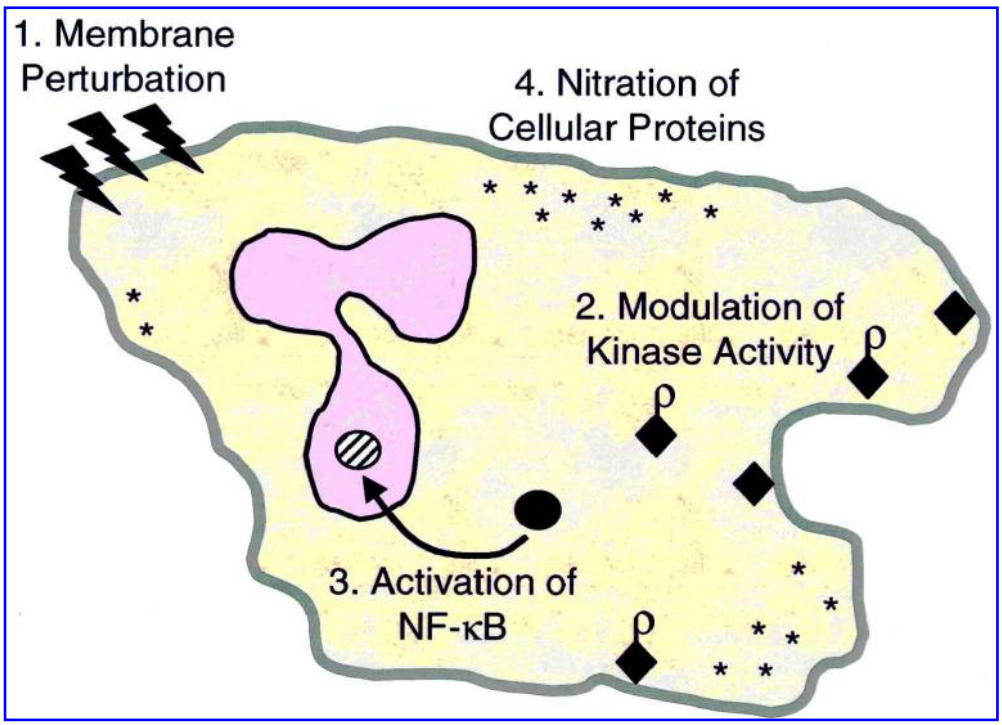

FIG. 2. Modulation of neutrophil function by ROS priming. The various priming events induced by ROS are indicated with numbers. **1. Membrane perturbation** by ROS can also have potent effects on neutrophil membrane lipids, resulting in changes in membrane fluidity. **2. Modulation of kinase activity** via redox-dependent increases in tyrosine phosphorylation (kinases indicated by solid diamonds; phosphorylation indicated by ρ). **3. Activation of NF-κB** results in translocation to the nucleus and activation of gene transcription. **4. Nitration of cellular proteins** (indicated by the asterisks) can modulate functional responses and signaling pathways. See text for further details.

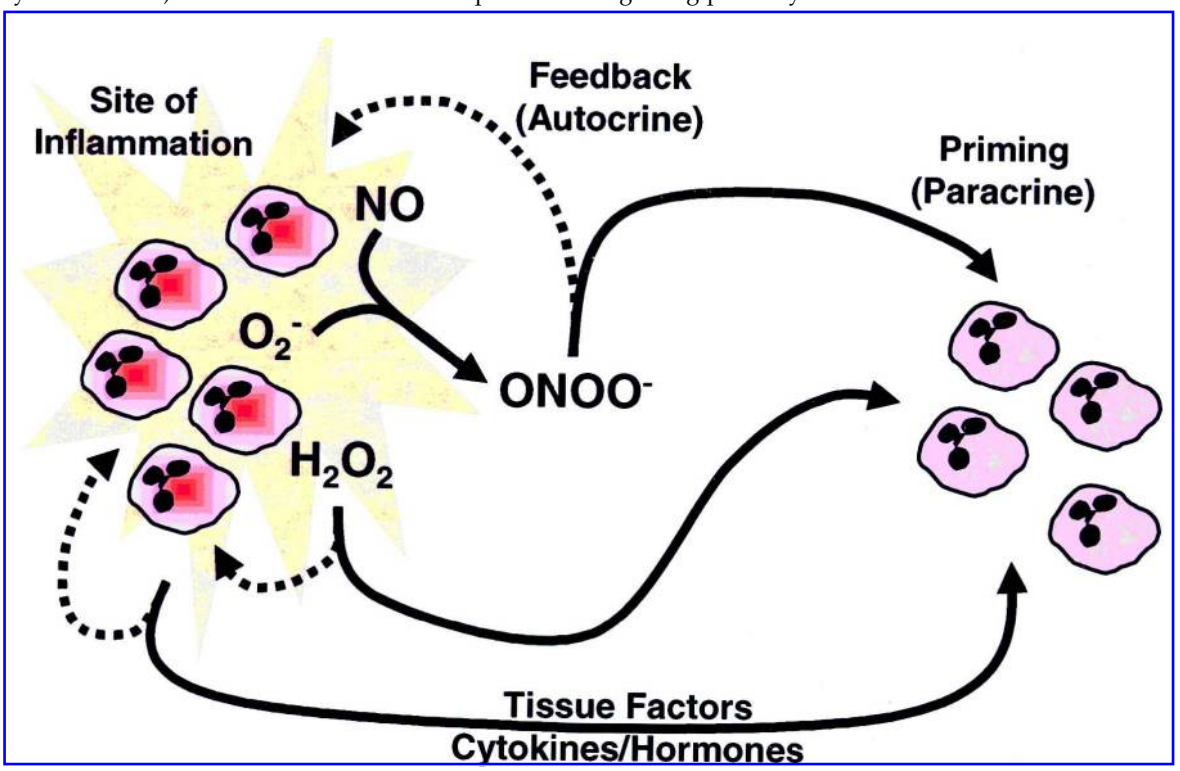

FIG. 3. Model of neutrophil priming by ROS at sites of inflammation. The formation of ROS, such as hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$), can serve to prime neutrophils arriving at sites of inflammation (solid lines) and, thereby, increase the efficiency by which neutrophils kill microorganisms (paracrine effect). Priming by ROS could also be used as feedback regulatory input (dashed lines) to cells already at the site (autocrine effect). Additionally, ROS-induced effects may act synergistically with other tissue factors and priming agents to provide an integrated priming effect.

p53/56^{lyn} (89). Additionally, ROS-related tyrosine kinase activation has been shown to be involved in priming of neutrophil phagocytosis (53). In all of these cases, the exact mechanisms responsible for ROS-induced effects on signaling kinases are uncertain. There have been suggestions, however, that inhibition of a phosphatase (*e.g.*, CD45) is responsible for the increased phosphorylation observed (23). What is most interesting about these studies is that there appears to be some degree of specificity involved, and only selected kinases examined showed increased ROS-related phosphorylation. Furthermore, the effect seems to involve some intermediate reaction, as oxidants applied directly to kinases in solution did not result in the phosphorylation changes seen when those agents were applied to intact neutrophils.

Regulation of nuclear factor-κB (NF-κB)

Redox regulation of NF-κB activation also appears to play a role in neutrophil priming (64). This intensely studied transcription factor mediates the expression of many proteins in response to agents of infection and inflammation, such as LPS or TNF-α (45). In many cell types, activation of NF-κB appears to be dependent on the presence of ROS, including H_2O_2 (63), O_2^- (83), and peroxynitrite (33). Furthermore, ROS production by activated neutrophils has been implicated in paracrine activation of NF-κB in nearby cells under certain inflammatory conditions (67). However, although inflammatory mediators (LPS, TNF-α, fMLF) can stimulate activation of NF-κB in neutrophils (47), there is still no evidence that ROS are essential for this response (79). Thus, whereas some effects of ROS on neutrophils appear to have a positive feedback effect on neutrophil priming, other responses are quite removed from this process.

Protein nitration

Another widespread action of ROS on cells is nitration of protein tyrosines via peroxynitrite, which is generated by the reaction of O_2^- with nitric oxide (5). Interestingly, we found that peroxynitrite was also an effective priming agent for human neutrophils, and that neutrophil priming was mediated primarily by nitration of tyrosine residues on neutrophil proteins (61). Peroxynitrite priming resulted in enhanced respiratory burst activity upon subsequent activation with low doses of PMA or fMLF, changes in the expression of neutrophil surface markers (L-selectin, Mac-1, flavocytochrome b, and fMLF receptor), and increased intracellular Ca^{2+} levels (61). Analysis of the mechanism of neutrophil priming by peroxynitrite demonstrated that peroxynitrite treatment resulted in little or no neutrophil membrane lipid peroxidation or protein sulfhydryl group oxidation, but did result in significant nitration of tyrosine residues on neutrophil proteins. In addition, inhibition of tyrosine nitration with a pyrrolopyrimidine antioxidant (U-101033E) blocked the majority of peroxynitrite-induced priming effects, further demonstrating that neutrophil priming was mediated primarily by nitration of tyrosine residues on neutrophil proteins (61). Thus, the ability of peroxynitrite and possibly other ROS to serve as effective neutrophil priming agents suggests that oxidant-mediated modification of neutrophil proteins may play a role in modulating the host-defense process at sites of inflammation (Fig. 3).

It is obvious from this survey of only a very few of the known priming agents that these agents act through many mechanistic pathways and, increasingly, demonstrate a diversity of functional responses and phenotypes. Thus, we must consider here what generalizations, if any, we can make from this survey of priming agents. Although enhancement of the fMLF-induced oxidative burst may have been the original definition of priming, the term is now being used to describe a wider variety of phenomena. However, the common theme of these observations may be thought of as appropriately preparing the cell for a given response, *i.e.*, sensitizing them to respond appropriately, based on the severity of the stimulus. Because priming normally exhibits a concentration-dependent behavior with regard to the priming agent, variations in the levels of priming agents encountered can have significant effects on the subsequent response of the cell.

The cellular and biochemical mechanisms behind priming may be even more diverse than the functional responses, and it has been suggested that there may be multiple pathways leading to the same priming effect (82). The up-regulation of cell-surface receptors and adhesion molecules is a common, but not universal, event in priming. Thus, selective granule mobilization may be part of a common priming response at the cellular level. Fusion of specific, gelatinase, and secretory granules can all result in changes in the complement of neutrophil cell-surface molecules and, thereby, modulate the way in which a cell interacts with its environment. At a biochemical level, many mechanisms (Ca^{2+} mobilization, serine and/or tyrosine phosphorylation of various target molecules, lipase activation, protein synthesis, etc.) may be involved in priming responses. Thus, it is clear that priming is more than a simple on/off switch. Mechanistically and functionally, priming may be more of a spectrum, where different subsets of responses and the level of those responses are regulated by the interaction with various priming, activating, and inhibitory agents. Ultimately, the result would be a finely tuned attack on the pathogen, resulting in the appropriate level of host defense response. In addition, as the localization of priming agents is often near the site of inflammation, priming may help to focus the responding neutrophils' attack on the target area, while neutrophils in other areas of the body remain at rest.

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ABBREVIATIONS

fMLF, *N*-formyl-methionine-leucine-phenylalanine; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-8, interleukin-8; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; PAF, platelet activating factor; PMA, phorbol myristate acetate; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α.

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Address reprint requests to:
Mark T. Quinn, Ph.D.
Veterinary Molecular Biology
Montana State University
Bozeman, MT 59717

E-mail: mquinn@montana.edu

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