Exuberant Ca\(^{2+}\) Signaling in Neutrophils: A Cause for Concern

Mohammed M. Sayeed

Under inflammatory conditions after burn/trauma injuries, circulating neutrophils are frequently hyperactive, contributing to excessive superoxide production and related tissue damage. Although normal neutrophil activation is cooperatively controlled by Ca\(^{2+}\)-independent and Ca\(^{2+}\)-linked signaling pathways, exuberant Ca\(^{2+}\)-linked signaling appears to cause neutrophil hyperactivation in the injury conditions.

Leukocyte responses in mammalian organisms occur episodically not only after infectious challenges (bacterial, fungal, viral, or parasitic), but also after seemingly noninfectious (nonseptic) injury conditions, such as trauma and burn. Although early inflammatory phases after nonseptic challenges are probably independent of an invading pathogen, later phases are often complicated by pathogen invasion of the host organisms. The inflammatory response in the absence of a pathogen may be initiated by traumatized/burned tissue products serving as non-self “antigens.” An advanced state of burn/trauma ensuing in mammalian hosts after nonseptic or septic injuries has been referred to as the systemic inflammatory response syndrome (SIRS) (4). A hallmark of SIRS is the release of inflammatory mediators primarily, but not exclusively, from the leukocytes. At present, there is an exhaustive list of mediators identifiable in SIRS; inclusive in this list are cytokines tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, and IL-8; lipids platelet-activating factor (PAF) and prostaglandin E\(_2\); and reactive molecular species superoxide anion (O\(_2^-\)) and nitric oxide (8). If appropriately regulated in terms of their temporal release pattern and magnitude of release, the mediators have the potential to play adaptive roles in the host’s defense against the antigens/pathogens (9). Such adaptive roles are played through the actions of mediators on leukocytes, resulting in leukocyte responses that provide for neutralization and/or inactivation of the antigen/pathogen and protection of host cells/tissues. However, the outcome of leukocyte responses resulting from actions of mediators in SIRS is often damage and, ultimately, death of host cells/tissues. The host cell/tissue damage could occur not only from the actions of mediators leading to attenuated or disturbed leukocyte responses but also from actions of mediators causing an excessive or exaggerated response. Such maladaptive up- and downregulations of leukocyte responses can arise from inappropriate mediator release, mediator-receptor interactions, and/or receptor-mediated leukocyte signaling. An excessive neutrophil O\(_2^-\)generating response after trauma/burn conditions appears to be inappropriate in the early phases of injury; it can potentially cause O\(_2^-\)-mediated host tissue damage (10). Such an inappropriate neutrophil response could understandably be related to inappropriate activation of the signaling pathways linked to O\(_2^-\) generation. Recent studies have supported a role of altered Ca\(^{2+}\) signaling-related intracellular pathways in the exaggerated neutrophil O\(_2^-\)-generating response (13). Figure 1 illustrates the role of burn/trauma-induced inflammatory mediators in neutrophil activation and its consequences.

A broad understanding of the neutrophil signaling pathways involving Ca\(^{2+}\) and related signaling components, and of mechanisms by which these pathways control O\(_2^-\) production, has the potential to elucidate the excessive O\(_2^-\)-generative response. Such an understanding can also contribute to development of therapeutic strategies to abrogate O\(_2^-\)-related tissue damage in injury conditions. This article focuses on the present state of knowledge of Ca\(^{2+}\) and related signaling pathways and their role in O\(_2^-\) production in neutrophils and the impact of alterations in the signaling mechanisms on the oxidant response in trauma/burn injury conditions.

O\(_2^-\) production: role of inflammatory mediators

Although intracellular O\(_2^-\) release after phagocytic uptake of pathogens by neutrophils leads to oxidant-mediated pathogen killing and thus to an efficient host defense, excessive O\(_2^-\) release in the environment in close proximity outside the neutrophil can cause oxidative host cell/tissue damage at sites traversed by neutrophils during their migration from blood to tissue regions of inflammation/infection (Fig. 1). Such neutrophil-caused oxidant injury is known to occur in pathological conditions of rheumatoid arthritis, myocardial infarction, stroke, acute respiratory distress syndrome (ARDS), and tissue ischemia (10). Inflammatory mediators, which originate from cellular elements (e.g., tissue macrophages, dendritic cells) at sites of inflammation and diffuse into blood, evidently stimulate neutrophils’ chemotactic response, which is responsible for their migration to the inflamed tissue sites. The chemotactic mediators are also effective stimuli for neutrophil O\(_2^-\) production. Although neutrophil chemotaxis results from membrane skeletal and cytoskeletal changes contributing to adhesion of neutrophils to other cells and the extracellular matrix, the O\(_2^-\)-generative effector response is dependent on the ultimate activation of the neutrophil plasma membrane NADPH oxidase (O\(_2^-\) oxidoreductase).

Some of the chemotactic mediators that activate the O\(_2^-\)-generating NADPH oxidase system are activated serum...
complement fragment C5a, bacteria-derived N-formylated methionyl peptides [e.g., formyl-methionyl-leucyl-phenylalanine (f-MLP)], bioactive lipids PAF and leukotriene B4 (LTB4), and the chemokine IL-8 (1). Inflammatory cytokines TNF-α, granulocyte colony-stimulating factor (GCSF), and granulocyte-macrophage colony-stimulating factor (GMCSF) also contribute, but indirectly, to O_2^- production. The chemotactic mediators f-MLP, C5a, PAF, LTB4, and IL-8 bind to neutrophil plasma membrane receptors (the so-called serpentine receptors), which have seven intramembrane peptides in series, with an NH_2-terminal peptide as the extracellular and a COOH-terminal peptide as the cytosolic domain. On the other hand, TNF-α, GCSF, and GMCSF bind to a single transmembrane peptide receptor. The activation of NADPH oxidase is also effected by Fc and complement receptors (FcR and CR, respectively), which are involved in the neutrophil's phagocytic and adhesive responses. Neutrophils express two types of FcRs (which bind to the Fc component of immune complexes), FcRII and FcRIII, and two types of CRs (which bind to complement-pathogen complexes), CR1 and CR3. CR3, known as CD11b/CD18 or β_2-integrin, also binds to its cognate ligand intracellular adhesion molecule 1 (ICAM-1), leading to neutrophil adhesion to a target cell such as the endothelial cell. Neutrophil adhesive molecule L-selectin, which binds to the endothelial cell sialyl-Lewis ligand, also participates in firm neutrophil adhesion to endothelium. Ligation of these phagocytosis- and adhesion-promoting molecules contributes to potentiation of neutrophil O_2^- production (4). In addition, CD11b/CD18 and FcRIII may directly interact to enhance O_2^- production. Recent studies have shown that there is a high degree of coordination between the phagocytic/adhesive effector responses and NADPH oxidase activation in neutrophils (2). The neutrophil’s receptor systems involved in O_2^- production are illustrated in Fig. 2.

The actions of various inflammatory mediators and the roles of their receptors in neutrophil O_2^- production have mostly been studied in vitro using neutrophils from healthy humans and/or animals. Whereas the earlier studies obtained information pertaining to effects and roles of individual mediators, more recent studies have focused on effects of multiple mediators sequentially impinging on neutrophils. The studies focusing on the mediators’ cumulative effects have given us a better understanding of both the potentially adaptive as well as a maladaptive neutrophil respiratory burst responses that may be elicited in pathophysiological inflammatory states, including those associated with the trauma/burn injury conditions. The in vitro studies have clearly shown that stimulation of resting neutrophils by a given mediator either results in O_2^- generation or simply leads to “priming” of the O_2^- production mechanism without actual O_2^- production (12, 15). O_2^- is
produced by the primed neutrophils only after a subsequent stimulation by either the same mediator, at a high concentration, or a different mediator; the response in the preprimed cells is of a greater magnitude than that produced in unprimed resting cells (Fig. 1). The chemotactic mediators acting on the serpentine receptors are known to be capable of eliciting $O_2^-$ generation in both resting and primed neutrophils (7); the response, however, is much greater in primed than in resting cells. On the other hand, the cytokines TNF-$\alpha$, GMCSF, and GCSF are only capable of priming (7). Neutrophils adhered to other cells (such as endothelial cells), extracellular matrix substances, or to immune complexes deposited into tissues in pathological conditions are also known to exhibit enhanced $O_2^-$ release (15). Such enhanced oxidant responses in the adhered state are also ascribed to an initial priming of neutrophils followed by the potentiated $O_2^-$ response. Both the serpentine and single transmembrane peptide receptor agonists can cause the enhanced $O_2^-$ response in adhered-primed neutrophils (Figs. 1 and 4).

Although mediators that can cause priming and those that can lead to potentiated $O_2^-$ responses have been identified, the neutrophil intracellular mechanisms responsible for priming have not been adequately elucidated, particularly in pathophysiological or pathological states in vivo (7).

**Signaling to $O_2^-$ production: role of $Ca^{2+}$-dependent and $Ca^{2+}$-independent pathways**

The activation of serpentine receptors in neutrophils leads to their coupling within the membrane to the heterotrimeric pertussis toxin-sensitive G protein, which complexes with GTP in an amplified manner. The activated G proteins in turn stimulate an array of intracellular phospholipases and kinases.

An early event following G protein activation is stimulation of phosphatidylinositol 4,5-bisphosphate (PIP$_2$)-specific phospholipase C-$\beta$ (PLC-$\beta$) which hydrolyzes PIP$_2$ into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP$_3$) (5). The resulting DAG serves as the second messenger to stimulate protein kinase C (PKC), and IP$_3$ induces a rapid release of $Ca^{2+}$ from intracellular nonmitochondrial stores (calciosome or endoplasmic reticulum), thereby elevating the concentration of intracellular $Ca^{2+}$ ([Ca$^{2+}$]). An initial transient increase in...
[Ca\(^{2+}\)]\(_i\) is attributable to emptying of intracellular Ca\(^{2+}\) stores; this transient increase is followed by a somewhat sustained influx of Ca\(^{2+}\) from the extracellular space. The influx of Ca\(^{2+}\) appears to be regulated by the state of filling/depletion of intracellular Ca\(^{2+}\) stores. Although the downstream molecular targets of elevated [Ca\(^{2+}\)]\(_i\), in neutrophils are not well understood, Ca\(^{2+}\) signal is known to synergize the stimulation of PKC by DAG. In neutrophils, the most abundant PKC isozyme is PKC-\(\beta\), whose activation through translocation to the plasma membrane is dependent on Ca\(^{2+}\). A direct activation of PKC, bypassing signaling through PLC, via neutrophil stimulation with phorbol 12-myristate 13-acetate (PMA) is known to stimulate NADPH oxidase. PKC activation can also occur in neutrophils independently of the upstream Ca\(^{2+}\) signal, since chemotactic mediators are known to stimulate phosphatidylcholine-specific phospholipase D (PLD) to lead to generation of the PKC activator DAG (Fig. 2A). Whereas earlier studies had shown involvement of PLC/Ca\(^{2+}\)/PKC signaling in neutrophil O\(_2\)\(_{\text{ production}}\) (6), later studies provided evidence that signaling to O\(_2\)\(_{\text{ production}}\) could also occur independently of the activation of this pathway. Ca\(^{2+}\)/PKC-independent pathways, demonstrated under conditions that abrogate phosphorylation of the cytosolic protein p47phox is...correlated with O\(_2\)\(_{\text{ generation}}\...\)

[Ca\(^{2+}\)]\(_i\) elevation and PKC activation, involve activation of various protein kinases besides PKC (3, 5). The stimulation of neutrophils through either the serpentine or single-transmembrane domain receptor leads to Ca\(^{2+}\)-independent activation of protein tyrosine kinases (PTKs). Diverse neutrophil effector responses, including NADPH oxidase activation, are linked to protein tyrosine phosphorylations. In case of stimulation of neutrophils with the cytokine TNF-\(\alpha\), GMCSF, or GCSF, the receptor’s cytoplasmic domain itself, which possesses the PTK activity, catalyzes phosphorylation of tyrosine on some selected target protein(s). The serpentine receptors activate the src family nonreceptor tyrosine kinase, Lyn; the activation process consists of Lyn autophosphorylation followed by phosphorylation of target protein Shc (Fig. 2B). Lyn-Shc activation leads to stimulation of phosphatidylinositol 3-kinase (PI3K), which in turn could activate NADPH oxidase (2). A somewhat specific blockade of PI3K by wortmannin or LY-294002 is known to inhibit the neutrophil oxidative burst. PI3K may be located upstream to Ca\(^{2+}\)-signal-independent PKC signaling. The target protein Shc can also be linked to activation of the “small GTP-binding protein/GTPase,” Ras (2). The events downstream to Ras activation in neutrophils are not adequately elucidated. The f-MLP and C5a activation of neutrophils leads to activation of p21 Ras, which in turn may play a role in the activation of a group of serine/threonine kinases, mitogen-activated protein kinases (MAPKs) (11). Three subfamilies of MAPKs have been identified in mammalian cells: 1) the extracellular signal-related kinases (ERKs/p42–44 MAPKs); 2) p38 MAPK; and 3) c-Jun NH\(_2\)-terminal kinases (JNKs) (11). All three MAPK subfamilies are characterized by tripeptide sequences that can be phosphorylated both at threonine and tyrosine sites; such phosphorylations are catalyzed by a family of dual-purpose tyrosine/threonine kinases, known as MAPK-ERK kinases (MEKs). MEKs in turn are activated via serine/threonine phosphorylation by MEK kinases (MEKs) (Fig. 2C). To date, studies have shown that stimulation of neutrophils by C5a, IL-8, and f-MLP can activate ERKs and that f-MLP can stimulate both ERKs and p38 MAPK. The role of MAPKs in neutrophil effector response generation is not adequately understood. Whereas some studies have dissociated ERK and p38 MAPK activation from neutrophil O\(_2\)\(_{\text{ production}}\), other studies have supported p38 MAPK involvement with f-MLP stimulation of the respiratory burst (11). A secondary ERK activation may also be required for the respiratory burst oxidase activity.

The activation of neutrophil Fc receptors and \(\beta\)-integrin/L-selectin adhesive molecules involves PTK activation as well as [Ca\(^{2+}\)]\(_i\) elevation due primarily to Ca\(^{2+}\) release from the intracellular stores (Fig. 2D) (5). In this case, src family PTK may play a role in tyrosine phosphorylation of PLC-\(\alpha\) isozyme which, like PLC-\(\beta\), triggers Ca\(^{2+}\)/PKC signaling. Thus adhesion of neutrophils to immune complexes (via FcR), and to other cells or matrix (via \(\beta\)-integrin/L-selectin) can also lead to NADPH oxidase activation through Ca\(^{2+}\)-dependent as well as Ca\(^{2+}\)-independent pathways.

The O\(_2\)\(_{\text{ generation}}\) NADPH oxidase is a multicomponent enzyme comprised of a membrane-bound cytochrome b\(_{558}\) unit and several cytosolic factors that translocate to the membrane and form the active enzyme complex upon neutrophil activation (10, 14). The phosphorylation of the cytosolic protein p47phox is one of the early and key events in NADPH oxidase assemblage and is correlated with O\(_2\)\(_{\text{ generation}}\) (10). Other cytosolic factors that appear to be important are p67phox and Rac-related low-molecular weight GTP-binding protein, Rac2. Both p67phox and Rac2 requirements for O\(_2\)\(_{\text{ generation}}\) have been shown in human neutrophils. The cytosolic protein p47phox can be phosphorylated at multiple sites. All three cytosolic factors translocate to the membrane and participate in the activation of NADPH oxidase. The upstream signaling contributing to activation of the cytosolic factors (p47phox, p67phox, and Rac2) is not definitively known. PKC would appear to be involved in the activation of p47phox. Both PKC activation and an inhibitory modulation of protein phosphatases play a role in the p47phox phosphorylation and related O\(_2\)\(_{\text{ production}}\). Rac activation appears to require src family or other PTK. Thus the signaling components immediately proximal to NADPH oxidase activation are phosphoprotein products of both Ca\(^{2+}\)-dependent and Ca\(^{2+}\)-independent pathways.

**Signaling modulations in burn inflammation: relationship to neutrophil priming**

Although there is little feasibility of directly measuring O\(_2\)\(_{\text{ production}}\) by tissue neutrophils under pathological conditions in vivo, there is evidence that circulating neutrophils under such conditions have an enhanced ability to produce O\(_2\)\(_{\text{ generation}}\) and to inflict oxidative damage to host tissues (10).
Whereas a number of studies of blood neutrophils harvested from human trauma and burn patients have ascertained the enhanced $O_2^-$ response and have evaluated the roles of various inflammatory mediators in its generation (1), fewer studies have investigated intracellular signaling mechanisms responsible for the enhanced response. Recent studies from the author’s laboratory assessed the signaling and $O_2^-$ generation was measured after stimulating neutrophils with the chemotactic mediator f-MLP. The f-MLP-stimulated $O_2^-$ production 1–3 days postburn was substantially greater than the marginal response observed in control, sham, or burn rats 7–10 days postinjury. Thus neutrophil respiratory burst occurred during the early but not late burn inflammatory phase. This is in keeping with the concept that hyperactivation of neutrophils in the early periods after severe burns is followed by decreased neutrophil responsiveness (1).

$[Ca^{2+}]_{i}$ in neutrophils was measured before and after f-MLP stimulation using fura 2 fluorescence spectroscopy and imaging techniques (13). A substantially greater elevation in $[Ca^{2+}]_{i}$ occurred on neutrophil stimulation with exogenous f-MLP in the 1–3 day postburn rat groups than in control, sham, or 7–10 day postburn groups. These results clearly indicated enhanced responsiveness of $Ca^{2+}$ signaling in neutrophils during early burn inflammatory phase. Assessments of PKC activation (translocation of the protein kinase activity from cytosol to membrane) in control, sham, and burn rat neutrophils, with and without f-MLP stimulation, yielded results indicating a close relationship between PKC and $Ca^{2+}$ signaling changes. The enhanced $Ca^{2+}$ and PKC signaling in the early phase after burn was correlated with the increased $O_2^-$ production response (Fig. 3). The observed enhanced signaling and effector response can be rationally attributed to priming of neutrophils, in vivo, during early periods of burn inflammation.

Previous studies have shown that neutrophils that were first primed by TNF-$\alpha$ or GMCSF and then stimulated with Fc ligand (immune complexes) exhibited a greater elevation in $[Ca^{2+}]_{i}$ compared with that observed in unprimed neutrophils. Furthermore, these studies showed that, whereas $[Ca^{2+}]_{i}$ elevation in unprimed neutrophils stimulated with the Fc ligand was due primarily to $Ca^{2+}$ release from the intracellular stores, an influx of $Ca^{2+}$ from the extracellular fluid contributed to the observed $Ca^{2+}$ signal in the primed neutrophils (15). The upregulation of neutrophil $Ca^{2+}$/PKC signaling, in our study, also could have resulted from $Ca^{2+}$ influx, because it was abolished when the rats were treated with the $Ca^{2+}$ entry blocker diltiazem (Fig. 3). The diltiazem treatment of rats also abrogated the enhanced $O_2^-$ response in the burn rat neutrophils (Fig. 3). Because $Ca^{2+}$ blocker prevented the upregulation of both $Ca^{2+}$/PKC signaling and $O_2^-$ production in neutrophils that would be presumably primed in vivo after burn injury, $Ca^{2+}$ influx blockage appeared to have intercepted the priming process. A role of $Ca^{2+}$ signaling upregulation in enhanced $O_2^-$ response has also been shown in neutrophils primed with growth hormone and subsequently stimulated with f-MLP (12). $Ca^{2+}$ signaling upregulation in the primed neutrophils can apparently contribute to enhanced $O_2^-$ response via potentiation the PKC pathway to NADPH oxidase activation. In addition, in the case of stimulation of previously primed neutrophils by a serpentine receptor agonist, NADPH oxidase may understandably be activated to a maximum possible level due to potential parallel activation of both $Ca^{2+}$-dependent and $Ca^{2+}$-independent pathways. A somewhat similar maximum potentiation of the $O_2^-$ response may also occur due to parallel activation of the $Ca^{2+}$-dependent and -independent pathways after the stimulation of the primed neutrophils with the Fc receptor ligands.

Figure 4 shows a diagrammatic representation of the above-described potential stimulations of neutrophils in a
milieu of mediators such as those found in the inflammatory phase of burn/trauma injury. The resting neutrophil might be initially stimulated by ligands binding to the single- and/or seven-transmembrane-domain receptors leading to neutrophil priming. During priming, the signaling pathways involved would primarily be the PTK-dependent and Ca$^{2+}$-independent pathways if single peptide receptors were activated. On the other hand, if both the single and seven-peptide receptors were to be triggered, there is the possibility that both Ca$^{2+}$-independent and Ca$^{2+}$-dependent pathways could be activated in a parallel manner. Yet in both scenarios, O$_2^-$ production would be no more than a marginal response as observed in neutrophils harvested from burn rats before they were stimulated with f-MLP in vitro. The primed neutrophils might be activated, in vivo, via actions of the seven-transmembrane-domain ligands or Fc/CR/b$_2$-integrin ligands to lead to an overt and potentially intense O$_2^-$ generation due to cumulative activations of the Ca$^{2+}$-dependent and Ca$^{2+}$-independent pathways (as shown in Fig. 4). The finding of the dependency of the O$_2^-$ response in burn rat neutrophils on Ca$^{2+}$ influx allows for a speculation that not only a cumulative but also an interactive/synergic activation of the Ca$^{2+}$-dependent and Ca$^{2+}$-independent pathways leads to the overt O$_2^-$ response. Whereas the maximum potentiation of primed neutrophils after stimulation with the serpentine receptor agonist could plausibly occur in circulation where both priming and “nonpriming” mediators might be present, the potentiation with Fc receptor stimulation is likely with the formation of immune complexes in inflammatory conditions.

The efficacy of diltiazem treatment of postburn rats in modulating Ca$^{2+}$ signaling upregulation and respiratory burst has provided clues to potential therapeutic regimens for abrogating neutrophil priming in injury conditions (13). Diltiazem’s effect was likely due to its ability to block Ca$^{2+}$ influx in neutrophils. The efficacy of Ca$^{2+}$ entry blockers may be limited to neutrophil priming phenomena in which respiratory burst is sensitized by Ca$^{2+}$ influx into the primed neutrophils. An alternative way of abrogating neutrophil priming would be to target the initial priming process itself resulting from the actions of primer agonists on resting neutrophils. Inasmuch as initial priming may be triggered in vivo via activation of a variety of neutrophil receptors, namely Fc, b$_2$-integrin, serpentine, and single-transmembrane-domain receptors, therapeutic abrogation of initial priming would seem at the outset to require multiple regimens. However, as discussed in the preceding section, priming through all of these receptor systems seems to be more or less universally dependent on activation of PTKs. Thus PTK blockers and Ca$^{2+}$ blockers both remain as prospective therapeutic agents against neutrophil priming in pathophysiological conditions prevailing in early inflammatory phase of trauma/burn injuries.

FIGURE 4. Schema showing priming of resting neutrophils and activation of primed neutrophils. Resting neutrophil receptors identified are 7- and single-transmembrane-domain, Fc, and b$_2$-integrin (also known as CR3 or CD11b/CD18) types. Neutrophil priming can occur via actions of TNF-α, GMCSF, and/or GCSF receptors (A and C) to prime neutrophils, or via f-MLP, LTB4, C5a, IL-8, PAF (B); whereas former ligands act primarily via activation of PTK, latter presumably activate both PTK and PLC. PLC activation leads to Ca$^{2+}$-dependent signaling, whereas PTK activation can lead to Ca$^{2+}$-independent priming of NADPH oxidase without actual O$_2^-$ production. Activation of primed cells can occur via 7-transmembrane-domain (A and B) or FcR/b$_2$-integrin receptors (C), leading to NADPH oxidase activation, resulting in overt O$_2^-$ production. PLC on open background indicates PLC-β, and PLC on shaded background indicates PLC-γ.
Concluding remarks

Neutrophil intracellular signaling triggered by neutrophil activating agents have been extensively studied in vitro. The signaling pathways leading to neutrophil O$_2$ generation include both the classical PLC-mediated Ca$^{2+}$/PKC sequence as well as a variety of novel protein kinases not dependent on the Ca$^{2+}$ signal, namely PTK, MAPK, and PI3K. The recent burst of knowledge about the roles of the novel kinases in modulating target proteins implicated in the O$_2$ response generation and the extensive presence of the Ca$^{2+}$-independent pathways has appropriately taken the limelight away from Ca$^{2+}$ signaling. The Ca$^{2+}$-independent pathways do play critical roles not only in actual generation of O$_2$ by neutrophils but also in priming neutrophils and enhancing their potential to produce O$_2$ without an actual production. Nevertheless, the activation of preprimed neutrophils could potentially occur via either the Ca$^{2+}$-independent or the Ca$^{2+}$-dependent sequences as redundant pathways. Recent studies in neutrophils harvested from inflammatory conditions prevailing after burn/trauma injuries indicate that the activation of the primed neutrophils, in vivo, may result from potentiating interactions between the Ca$^{2+}$-linked and the Ca$^{2+}$-independent pathways.

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References