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Comment on Boussetta et al, page 5795

Pin-ing down PMN priming

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In this issue of Blood, Boussetta et al provide novel insights into how TNFα primes human neutrophils and report that a specific conformational change switches the phagocyte NADPH oxidase into a more productive state.¹

The phagocyte NADPH oxidase generates an array of reactive oxidants that synergize with granule contents and circulating host proteins to mediate efficient antimicrobial activity in the neutrophil phagosome. In rest-
ing neutrophils, the phagocyte oxidase is unas-

sembled and inactive, as the individual com-
ponents are spatially segregated in naive phagocytes. Upon exposure to suitable stimuli, agonist–dependent changes in the cytoplasmic oxidase components p47phox and Rac2 result in their translocation to the phagosomal mem-
brane and culminate in the assembly of an ac-

tivized oxidase complex.²

However, activation of the phagocyte NADPH oxidase has nuances beyond simply toggling from resting to activated status; neutrophils can adopt a “primed” phenotype, whereby suboptimal concentrations of typical agonists or exposure to agents that do not directly stimulate neutrophils can elicit robust oxidase activity. For example, pretreat-
ment of neutrophils with tumor necrosis factor α (TNFα), an agent that does not directly trigger NADPH oxidase activity, renders neutrophils responsive to suboptimal concentra-
tions of formylated peptides, such as the formylated tripeptide, formyl-methionyl-leucylphenylalanine (fMLF). Agents that have the capacity to prime neutrophils include cy-
tokines, such as TNFα, microbial compo-
nents, β2 integrin agonists, extracellular ma-
tix proteins, and several pharmacologic 
agents (reviewed in El-Benna et al³). Not only have a variety of agents that prime neutrophils been identified, but several phenotypic changes in primed neutrophils have also been described, including transcriptional changes, increases in membrane flavocytochrome b558 expression, augmented G-protein activity, reorganization of plasma membranes, and partial phosphorylation of p47phox.

The El-Benna laboratory previously demon-
strated that phosphorylation of Ser345 in p47phox is required for TNFα-dependent priming of the phagocyte NADPH oxidase.⁴

In this issue, the same laboratory now reports that Pin1, a peptidyl prolyl cis-trans isomerase (PPI), catalyzes a conformational change in p47phox that is required for oxidase priming by TNFα. Appreciation of the beauty of this novel insight requires some background information.

Because of its ringed structure, proline can profoundly alter protein conformation, de-
dpending on whether it is in the cis or trans con-
mfiguration with respect to the peptide bond. Cis-trans isomerization of proline occurs at a very slow rate, on the order of minutes, unless catalyzed by a PPI. The conformational switch

In the cytoplasm of resting neutrophils (1), the conformation of p47phox renders cryptic its SH3 and PX domains, regions that have the potential to interact with targets on the phagosomal or plasma membrane. TNFα-mediated activation of p38/ERK (1/2) phosphatases ⁵⁶⁷⁸ in the autoinhibitory region (AIR) of p47phox (2), allowing the prolyl isomerase Pin1 to bind and catalyze a conformational change in p47phox (3), with subsequent phosphorylation of neighboring serines by protein kinase C (PKC). Now revealed after Pin1-induced conformational rearrangement, SH3 domains of p47phox can associate with the membrane-bound p22phox and complete the assembly and activation of the NADPH oxidase (5). See the complete figure in the article beginning on page 5795.

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provided by prolyl cis-trans isomerization participates in modulation of a wide range of cellular processes.5,6 The subfamily of PPI represented by Pin1 is unique in its ability to promote efficient isomerization of prolines adjacent to phosphorylated Ser or Thr residues,7 thereby coupling Pin1 binding and activity to regulation of signal transduction systems that rely on proline-directed phosphorylation.

Boussetta et al demonstrate for the first time that Pin1 is present in the cytoplasm of human neutrophils and is required for TNFα priming of the NADPH oxidase. Furthermore, phosphorylated in response to priming of the NADPH oxidase. Further, human neutrophils and is required for TNF activity to regulation of signal transduction systems.6

The report by Boussetta et al adds to our understanding another critical step in phagocyte oxidase activation that rests heavily on conformational changes in the adaptor protein p47

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The “age” of understanding VKA dose

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Dose response to vitamin K antagonists (VKA) exhibits large within- and between-individual variation in both adults and children, presenting challenges to clinicians attempting to maximize the safety and efficacy of these agents. In part to address limitations with VKAs, alternative oral anticoagulant agents (eg, direct Xa and thrombin inhibitors) are being developed and evaluated in clinical trials. However, for the foreseeable future, VKAs will continue to be used given their familiarity, cost, and ease of access. Thus, anticoagulant research must continue to focus on optimizing use of these agents.

Warfarin produces its anticoagulant effect through inhibition of vitamin K epoxide reductase, which results in diminished recycling of vitamin K and, thereby, impaired gamma-carboxylation of the vitamin K–dependent procoagulants (factors II, VII, IX, and X) and intrinsic anticoagulants (proteins C and S). The net effect of this process is measured by the prothrombin time, and calculated as an international normalized ratio.
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