The controlled enzymatic generation of reactive oxygen species (ROS) by phagocytic leukocytes is an integral component of the innate immune response. While high-level formation of ROS by phagocytes was long recognized as a means to kill ingested pathogens, we now know that ROS at lower levels can have intracellular signaling activity. The formation of these short-lived, but reactive oxygen metabolites, which include superoxide anion, hydrogen peroxide, hydroxyl radical and others, is catalyzed in leukocytes by the membrane-associated enzyme, NADPH oxidase. This multi-component enzyme consists of both membrane-bound components (Nox2, p22phox) and cytosolic regulatory proteins (p40phox, p47phox, p67phox and Rac2 GTPase). While much is understood about the assembly and activation of the phagocyte enzyme during the innate immune response, our knowledge remains far from complete.

It is now recognized that the phagocyte NADPH oxidase is a member of a ubiquitous family of NADPH oxidases (or Nox). The 7 Nox family members (Nox1, Nox2, Nox3, Nox4, Nox5, Duox1 and Duox2) are homologous, each with 6-membrane-spanning domains and a cytoplasmic NADPH/FAD-containing electron transfer domain [1]. Nox1, Nox2, Nox3 and Nox4 form natural heterodimers with membrane-associated p22phox, but Nox5 and the Duox appear to not require p22phox for either stability or function. While the Nox family members are found throughout many tissues and cell types, it is of particular interest that several Nox are abundant in so-called ‘barrier’ tissues or epithelia. Thus, Nox1 is abundant in the gastrointestinal epithelium, Nox4 in the vasculature and Duox in the respiratory epithelium. The location of these ROS-generating enzymes in the barrier tissues has prompted the expectation of their participation in host defense against invading pathogens in these tissues. Indeed, recent studies have begun to support this expectation, demonstrating roles in bacterial killing, cytokine production, apoptosis, gene expression and signaling, including by pattern-recognition receptors [2–6].

The papers in the current Journal of Innate Immunity thematic issue on ‘NADPH oxidases and innate immunity’ address a variety of aspects of Nox family function and regulation. The initial review by Quinn and Schlepetkin [7] provides an interesting and comprehensive exploration of a little-known and poorly understood aspect of the innate immune response: the role of Nox-generated ROS in macrophage fusion. Depending on the tissue and the inflammatory stimulus, phagocytic macrophages can fuse to form giant, multinucleated cells that serve as important mediators of tissue remodeling and repair, as well as of the removal of bacteria and other pathogens. This review provides an up-to-date overview of the role(s) of multinucleated macrophages in inflammation and autoimmune disorders.

The following papers focus on aspects of the phagocyte NADPH oxidase, Nox2. Munafò et al. [8] investigate the phenomena of neutrophil extracellular trap (NET) formation and their interaction with Nox2. NETs consist of extracellular complexes of DNA fibers, histones and granule-derived bactericidal proteins extruded from subsets of activated neutrophils [9]. NET formation requires a specialized form of cell death that is distinct from apop-
tosis and which is dependent on NADPH oxidase activity. Munafo et al. [8] show that a late phase of Nox2-mediated ROS production is sensitive to DNase treatment and suggest a role for myeloperoxidase-derived ROS in NET-dependent extracellular killing.

Making use of a biophysical approach, Yuzawa et al. [10] utilized small-angle X-ray scattering analysis to evaluate the conformation of the Nox2 cytosolic regulatory activator protein, p67phox. These studies reveal an elongated structure of p67phox with a ‘beads-on-a-string’ alignment of interacting domains for Rac GTPase, p40phox and p47phox, with little or no interaction between domains. Importantly, the positioning of the individual protein interaction domains is critical for NADPH oxidase activation. Campion et al. [11] characterize 6 monoclonal antibodies generated against purified Nox2/p22phox complex. All 6 antibodies detect various epitopes on p22phox and had distinct effects on NADPH oxidase activity. One of the antibodies, 12E6, preferentially binds to the activated state of Nox2. Since p22phox is a common partner of several of the Nox family enzymes, these antibodies may prove to be useful tools to investigate maturation, activation and activity of multiple Nox during the innate immune response.

The next 2 papers investigate non-phagocytic Nox family members. Li et al. [12] investigate the action of microglial Nox4 in the generation of the cytokine IL-6. The possibility of ‘Nox isotype switching’ as a means to modulate the balance between neuroprotection and neuroinflammatory damage is considered. Haselmayer and colleagues [13] probe the interactions between the Toll-like receptor 4 and the triggering receptor expressed on myeloid cells (TREM) to stimulate ROS formation by neutrophil Nox2. Concurrent activation of Toll-like receptor synergistically enhances TREM-mediated activation of neutrophils and monocytes. This appears to occur via distinct upstream mechanisms that converge on common downstream signaling cascades involving PI3 kinase/phospholipase C and p38 MAP kinase.

Finally, the influence of Nox2-derived ROS on phagocytosis, the process of engulfment of bacterial and/or apoptotic neutrophils from infected or damaged tissue into the macrophage for clearance, was investigated by Brown et al. [14]. Interestingly, they observe differential effects of ROS on the uptake of apoptotic cells versus bacteria. Thus, Nox-derived ROS contribute to the resolution of an inflammatory response both by directly killing ingested bacteria, as well as by enhancing the removal of dying phagocytes.

Overall, while the participation and contributions of members of the NADPH oxidase family to innate immune responses, especially in non-phagocyte barrier tissues, still remains an open question, this thematic issue underscores the many aspects of the innate immune process that may be modulated by Nox-derived ROS.

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References