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Review

Nox enzymes in allergic airway inflammation ☆

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ABSTRACT

Chronic airway diseases such as asthma are linked to oxidative environmental factors and are associated with increased production of reactive oxygen species (ROS). Therefore, it is commonly assumed that oxidative stress is an important contributing factor to asthma disease pathogenesis and that antioxidant strategies may be useful in the treatment of asthma. A primary source of ROS production in biological systems is NADPH oxidase (NOX), originally associated primarily with inflammatory cells but currently widely appreciated as an important enzyme system in many cell types, with a wide array of functional properties ranging from antimicrobial host defense to immune regulation and cell proliferation, differentiation and apoptosis. Given the complex nature of asthma disease pathology, involving many lung cell types that all express NOX homologs, it is not surprising that the contributions of NOX-derived ROS to various aspects of asthma development and progression are highly diverse and multifactorial. It is the purpose of the present review to summarize the current knowledge with respect to the functional aspects of NOX enzymes in various pulmonary cell types, and to discuss their potential importance in asthma pathogenesis. This article is part of a Special Issue entitled: Biochemistry of Asthma.

1. Asthma—a disease associated with oxidative stress

Asthma is a chronic inflammatory disease of the airways, characterized by remodeling of the airways leading to enhanced airway hyperresponsiveness and increased mucus secretion. In addition to genetic factors, the occurrence and severity of asthma is determined by a variety of environmental factors, including allergens, bacterial or viral infection, physical stimuli such as exercise or cold air, or various airborne pollutants or occupational hazards, such as ozone, tobacco smoke, diesel particulates, isocyanates, etc. Because of the oxidative nature of most of these environmental pollutants, it is commonly thought that oxidative stress is an important contributing factor to asthma development and pathogenesis. Airway inflammation, a complex multi-cellular process that involves eosinophils, neutrophils, CD4+ T lymphocytes and mast cells, is also fundamental in asthma disease pathogenesis, and since many of these cell types are capable of production of reactive oxygen species (ROS), it is widely assumed that these endogenously produced ROS also contribute importantly to airway injury and disease pathogenesis. Analysis of several irreversible biomolecular oxidation markers that are diagnostic of oxidative chemistry by specific granulocytes (neutrophils, eosinophils) has indeed revealed their presence within airway secretions or lung tissue sections from asthmatic subjects, often in correlation with the extent of ongoing inflammation and with the severity of clinical symptoms [1,2]. While these findings clearly establish the presence of ongoing activation of neutrophils or eosinophils within the asthmatic airway, it has remained a matter of debate to what extent these ROS contribute to distinctive features of allergic airway inflammation. Indeed, direct evidence for an active contribution of specific oxidative events in asthma pathophysiology is still largely lacking, and attempts to curtail allergic inflammation or asthma symptoms by antioxidant supplementation strategies have so far been dissapointingly ineffective [3].

The concept of oxidative stress in asthma, and in inflammatory diseases in general, has been complicated by our growing appreciation of the diversity in biological sources of ROS production, with similarly diverse biological consequences. The NADPH oxidase system of activated granulocytes, which generates reactive oxygen species (ROS) as a key component of antimicrobial defense, is commonly considered the major source of oxidative stress during acute or chronic inflammation. However, with the discovery of other NADPH oxidase homologs over the past decade, and their presence in many diverse cell types and involvement in a broad range of physiological processes, ROS are increasingly appreciated as critical mediators in a broad range of cellular processes, such as cell proliferation, migration, differentiation, immunomodulation, and oxygen sensing, in virtually all aerobic organisms [4–7]. These actions are generally mediated by strictly controlled and localized production of ROS, which transmit signals through reversible oxidative modifications within specific target proteins, a process collectively known as redox signaling. Since

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different NOX isoforms are present within various different lung cell types that play key roles in asthma pathophysiology [8–11], it should not be surprising that NOX-derived ROS are not merely involved in pulmonary disease and injury, but may also have salutory roles in various aspects of lung biology and may even prevent injury associated with chronic inflammation. As a result, global antioxidant strategies or generic approaches to interfere with overall ROS production or NOX activation do not necessarily present effective therapeutic strategies to treat chronic diseases such as asthma, and development of more refined strategies based on selective targeting of NOX isoforms in specific cell types would be required. This review will discuss the general aspects of NOX biochemistry and biology, and summarize the current state of knowledge regarding their functional roles in within various cell types of the respiratory tract as well as their potential involvement in the development of allergic airway inflammation and remodeling.

2. General aspects of NADPH oxidases

2.1. The NOX family

Activated phagocytic cells produce ROS through assembly and activation of the NADPH oxidase complex [12,13], which comprises membrane-associated flavocytochrome b_{558} ($gp91^{phox}$) and $p22^{phox}$ and various cytosolic cofactors ($p47^{phox}$, $p67^{phox}$, and $p40^{phox}$, and the GTPase, Rac1), and mediates transmembrane electron transfer from the major cellular electron donor, NADPH, to reduce molecular O_2 to superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2). A number of homologs of the main business end of NADPH oxidase, $gp91^{phox}$, have been discovered, and mammalian systems are now known to contain seven NADPH oxidase (NOX) homologs, comprising NOX1-5 (NOX2 being the new name for $gp91^{phox}$) and two larger Dual Oxidases, DUOX1 and DUOX2, which are widely expressed in many diverse cell types to regulate a variety of biological functions, including cell mitosis, differentiation, migration, and immune regulation. Similar to NOX2, activation of NOX1 and NOX3 also require association with $p22^{phox}$, and assembly with Rac and cytosolic co-factors ($p47^{phox}$ and $p67^{phox}$ or their homologs, NOX organizer 1 (NOXO1) and NOX activator 1 (NOXA1)) [4,5,13]. NOX4 also requires $p22^{phox}$, but appears to be constitutively active without the need for activation of other co-factors [5,13]. NOX5 and DUOX1/2 differ from the other NOX homologs and contain additional intracellular Ca^{2+}-binding EF-hand domain regions, and are primarily regulated by Ca^{2+} signaling, without the need for association with $p22^{phox}$ or other cytosolic co-factors [14–17]. The two DUOX proteins contain an additional extracellular peroxidase homology domain, with functional peroxidase activity in lower organisms but as yet unclear function in mammalian homologs [4,5,18]. The molecular biology and regulation of the various NOX/DUOX enzymes have been summarized in several excellent recent reviews [4,5,13,19], and will not be further reviewed here.

2.2. General mechanisms of NOX-dependent signaling

The primary product of activated NOX/DUOX is superoxide anion ($O_2^{\bullet-}$), which subsequently dismutates to hydrogen peroxide (H_2O_2), although DUOX1/2 and NOX4 appear to generate H_2O_2 without apparent intermediate production of $O_2^{\bullet-}$ [4,15,20]. Therefore, the biological actions of NOX/DUOX are thought to be mediated by either or $O_2^{\bullet-}$ or H_2O_2. In granulocytes, the primary target for NOX2-derived $O_2^{\bullet-}/H_2O_2$ is a locally secreted heme peroxidase, such as neutrophil myeloperoxidase or eosinophil peroxidase, which catalyzes H_2O_2-mediated oxidation of various anionic substrates to generate antimicrobial oxidants [21]. Similar cooperative actions exist between DUOX proteins and locally secreted heme peroxidases within the thyroid gland or at mucosal surfaces within the respiratory or gastrointestinal tract, with DUOX-derived H_2O_2 activating thyroperoxidase to promote the synthesis of thyroid hormone [22] or lactoperoxidase (LPO) to produce antimicrobial oxidants within the airway or intestinal lumen [4,23,24]. In most cases, however, the biological actions of NOX-derived $O_2^{\bullet-}/H_2O_2$ are related to interactions with other target proteins at redox-sensitive metal centers or cysteine residues.

Collective studies towards the diverse biological actions of NOX-derived ROS indicate that these are largely related to modulation of a limited set of broadly used signaling mechanisms, primarily protein tyrosine phosphorylation and intracellular Ca^{2+} signaling. Following pioneering studies by Finkel and Rhee [25,26], who first indicated a relationship between oxidant signaling and tyrosine phosphorylation through transient inactivation of a protein tyrosine phosphatase (PTP) by reversible oxidation of its invariant catalytic cysteine residue, a range of PTPs or other cysteine-containing phosphatases have since been identified as the oxidant target in NOX/DUOX-dependent cytokine and/or growth factor signaling [6,27–30]. NOX/DUOX activation also appears to be intimately associated with Ca^{2+}-mediated signaling, by oxidative cysteine modifications within Ca^{2+} channels [31,32] or by increasing voltage-dependent Ca^{2+} channel opening [33,34]. Since Ca^{2+}-mobilizing stimuli are capable of activating various NOX enzymes, the ability of NOX/DUOX activation to regulate Ca^{2+} signaling may represent a positive feedback mechanism allowing for rapid signal amplification in discreet cellular regions.

Specificity in oxidative signaling is ensured by strict spatial localization of NOX activation in association with the cytoskeleton [35,36] or membrane rafts [37–39] and by direct interactions with locally present oxidant-sensitive proteins targets [6,39,40], which allows for effective oxidative signaling within discrete cellular regions in spite of the abundant presence of cytosolic antioxidant systems (Cu/Zn SOD, GSH peroxidase). In this regard, ROS can have opposing effects on certain biological processes depending on the location or extent of ROS production. For example, while a number of studies have demonstrated the ability of NOX enzymes to activate inflammatory signaling by promoting the activation of nuclear factor (NF)-κB [41,42], several steps within the NF-κB pathway are also subject to inhibition by oxidative modification of critical protein cysteine residues [27]. Similarly, NOX-derived ROS production has also been intimately linked to regulation of matrix metalloproteinases MMPs), which often involve stimulatory effects on MMP expression or direct oxidative MMP activation (e.g. [28]), but inhibitory effects by elevated ROS production [43].

Although it is commonly assumed that the biological actions of NOX are related to ROS production, an aspect of NOX activation that is often overlooked is the fact that NOX activation is electrogenic and induces membrane depolarization resulting in compensatory activation of various ion channels. Oxidation of NADPH by NOX/DUOX activation also results in localized intracellular acidification, which promotes the activation of voltage-gated H^+ channels, Na^+/H^+ exchangers (NHE) and/or alternative ion channels. Various lines of evidence indicate that these ROS-independent actions contribute importantly to the biological activity of NOX enzymes [44,45]. For example, NOX2-mediated antimicrobial activity in phagocytes has been demonstrated to involve ROS-independent mechanisms that are related to activation of K^+ or H^+ channels within the phagosomal membrane to regulate the intraphagosomal activity of secreted proteases [46].

3. NOX enzymes in lung cell biology and asthma pathology

Sensitization and progression towards asthma is influenced by a delicate balance between the airway epithelium, innate immune cells and the induction of adaptive immunity [47,48]. Interactions between airway epithelial cells and dendritic cells (DCs) are instrumental