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Regulation of innate immunity by NADPH oxidase

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Abstract

NADPH oxidase is a critical regulator of both antimicrobial host defense and inflammation. Activated in nature by microbes and microbial-derived products, the phagocyte NADPH oxidase is rapidly assembled, and generates reactive oxidant intermediates (ROIs) in response to infectious threat. Chronic granulomatous disease (CGD) is an inherited disorder of the NADPH oxidase characterized by recurrent and severe bacterial and fungal infections, and pathology related to excessive inflammation. Studies in CGD patients and CGD mouse models indicate that NADPH oxidase plays a key role in modulating inflammation and injury that is distinct from its antimicrobial function. The mechanisms by which NADPH oxidase mediates killing of pathogens and regulation of inflammation has broad relevance to our understanding of normal physiological immune responses and pathological states, such as acute lung injury and bacterial or fungal infections.

Keywords

Chronic Granulomatous Disease; NADPH oxidase; inflammation

INTRODUCTION

The NADPH oxidase (NOX) family protein complexes generate superoxide anion and downstream reactive oxidant intermediates (ROIs). ROIs can be directly injurious to cells by damaging DNA, proteins and lipids. However, the rapid generation of ROIs is critical for host defense against certain bacteria and fungi, and ROIs have broad signaling functions. Thus, calibration of ROI generation and downstream pathways is important for effective antimicrobial host defense while averting excessive inflammation and injury. Chronic granulomatous disease (CGD) is an inherited disorder of the NADPH oxidase characterized by recurrent and severe bacterial and fungal infections and by excessive inflammation, such

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as Crohn's-like inflammatory bowel disease and obstructive granulomata of the genitourinary tract. Studies in CGD patients and engineered mouse models have identified that NADPH oxidase plays a key role in modulating inflammation and injury distinct from its antimicrobial function.

The phagocyte NADPH oxidase is the principal source of ROI generation in activated neutrophils and macrophages. In addition to the phagocyte NADPH oxidase (NOX2), isoforms of NADPH oxidase exist in several cell types and mediate diverse biological functions. The phagocyte NADPH oxidase complex is comprised of a cytochrome component consisting of gp91^{phox} (phagocyte oxidase) and p22^{phox} embedded in membranes. The cytoplasmic subunits p47^{phox}, p67^{phox}, and p40^{phox} and rac translocate to the membrane-bound cytochrome upon activation of the oxidase. NADPH is oxidized to NADP⁺, and electrons are transported down a reducing potential gradient that terminates when oxygen accepts an electron and is converted to superoxide anion.

Chronic granulomatous disease results from disabling mutations in genes encoding any of these phox proteins. X-linked CGD results from deficient gp91^{phox}, and accounts for about 65% of CGD cases. These phox proteins can have NADPH oxidase-independent signaling; however the major clinical features of CGD are similar among the different genotypes, with the level of functional NADPH oxidase being the strongest predictor or outcome (1). Thus, the major function of these phox constituents relates to NADPH oxidase activity.

The rapid consumption of oxygen in neutrophils following NADPH oxidase activation has been termed the “respiratory burst”. Neutrophil NADPH oxidase activation occurs in response to physiologic stimuli such as formylated peptides, opsonized particles, integrin-dependent adhesion (2, 3), and ligation of specific pathogen recognition receptors (e.g., dectin-1 (4)). Syk tyrosine kinase is a critical down-stream component of integrin signaling in neutrophils that mediates NADPH oxidase activation (2, 3, 5).

Rapid activation of NADPH oxidase constitutes an emergency response to invading pathogens. Paradoxically, although the immediate effects of NADPH oxidase lead to generation of ROIs and activation of neutrophil proteases that can damage tissue, NADPH oxidase can also dampen inflammation and limit tissue injury. This review will focus on NADPH oxidase both as a key mediator of antimicrobial host defense and as a modulator of inflammation.

NADPH OXIDASE AND HOST DEFENSE

The types of infections that characteristically afflict CGD patients provide insight into the role of NADPH oxidase in host defense. The spectrum of infections is relatively narrow, principally involving *Staphylococcus aureus*, *Burkholderia cepacia*, *Serratia marcescens*, *Salmonella* species, *Bacille Calmette-Guerin*, *Nocardia* species, and *Aspergillus* species and rarer moulds (6, 7). Invasive fungal diseases, principally aspergillosis, are leading causes of mortality in CGD (8).

Although the key function of NADPH oxidase in host defense has been known for several decades, the mechanisms by which it kills pathogens are complex and incompletely understood. NADPH oxidase can mediate host defense via direct antimicrobial activity of ROIs. Superoxide anion, the direct product of NADPH oxidase activation, is unstable and is converted to hydrogen peroxide either spontaneously or by the enzyme superoxide dismutase. Hydrogen peroxide can undergo Fe²⁺-dependent conversion to hydroxyl anion. Using halides as substrates (e.g., Cl⁻), myeloperoxidase converts hydrogen peroxide to hypohalous acid. The myeloperoxidase-hydrogen peroxide-halide system in neutrophils has potent antimicrobial activity and cytotoxicity (9). Myeloperoxidase deficiency is common,

affecting 1 in 2,000 to 4,000 individuals in the general population (10). In contrast to the severe infectious complications in CGD, myeloperoxidase deficiency is usually asymptomatic in the absence of co-existing conditions such as diabetes mellitus (11). Thus, while myeloperoxidase can amplify NADPH oxidase-dependent killing, there is likely a redundancy in phagocytic host defense pathways, such that myeloperoxidase is dispensable in an otherwise normal host.

While the activated NADPH oxidase is the major source of ROIs in phagocytes, other ROI-generating systems exist. Xanthine oxidase, which is involved in purine metabolism, generates superoxide anion. Xanthine oxidase augments host defense against *B. cepacia* in NADPH oxidase-deficient mice, but is dispensable in wildtype mice (12). In addition, ROIs generated as products of mitochondrial respiration can modulate host defense. West et al. (13) showed that activation of specific toll-like receptors in macrophages leads to recruitment of mitochondria to phagosomes and generation of mitochondrial-derived ROIs that target intracellular bacteria.

ROI and reactive nitrogen intermediate (RNI) products interact to form reactive metabolites with microbicidal and inflammatory properties. For example, superoxide anion can directly interact with nitric oxide (NO) to form peroxynitrite anion, which is highly reactive and has microbicidal properties and toxicity to mammalian cells. CGD and inducible nitric oxide synthase (iNOS)-deficient mice have distinct susceptibility phenotypes to infectious pathogens. For example, iNOS^{-/-} mice are highly susceptible to *Mycobacterium tuberculosis* (14), whereas CGD mice are only transiently more susceptible than wildtype mice at early time points after challenge (15). In addition, NADPH oxidase, but not iNOS, is essential for early control of *Burkholderia cepacia* and *Chromobacterium violaceum* infection in mice (16). Mice deficient in both NADPH oxidase and iNOS activities are far more susceptible to infections than either single genedeficient mouse model alone, supporting distinct but interacting roles of these two pathways (17).

In addition to direct antimicrobial properties of ROIs and RNIs, neutrophil NADPH oxidase activation is coupled to intracellular and extracellular release of preformed antimicrobial proteases. In resting neutrophils, the flavocytochrome subunits gp91^{phox} and p22^{phox} are principally located within the membrane of the secondary granules (18, 19). Primary (azurophilic) and secondary granules fuse with the phagocytic vacuole, where their constituents can co-mingle. Reeves et al. (20) showed that activation of the NADPH oxidase in neutrophils is coupled to a rise in ionic strength that leads to release of cationic granule proteins, including neutrophil serine proteases, which, at rest are held in an inactivated state (20). Neutrophil NADPH oxidase activation is also linked to the extracellular release of granule proteins, DNA, and chromatin that co-mingle in a network to form neutrophil extracellular traps (NETs) (21, 22). These NETs bind to and kill bacteria, degrade bacterial virulence factors (23), and target fungi (22).

Given the interactions between ROIs and neutrophil proteases, it has been unclear whether the host defense deficit in CGD is a direct consequence of impaired ROI production or is secondary to impaired neutrophil protease activation. We recently found that while NADPH oxidase plays a critical role in defense against *Aspergillus fumigatus* and *Burkholderia cepacia*, neutrophil serine proteases, neutrophil elastase, cathepsin G, and proteinase 3, are dispensable for host defense in the presence of intact NADPH oxidase (24). These results support a model in which NADPH oxidase-derived ROIs and neutrophil serine proteases have separable antibacterial and antifungal effector functions but protease activation is not the principal mechanism by which NADPH oxidase mediates host defense against these pathogens.

NADPH oxidase (NOX2) in macrophages, DCs, and MDSCs

Although NADPH oxidase is critical for neutrophil-mediated host defense, the role of NADPH oxidase in macrophages and dendritic cells (DCs), both of which harbor NOX2, is less clear. The strongest evidence for the role of macrophage NADPH oxidase in host defense is from the finding that mutations in gp91^{phox} that selectively affect macrophages lead to increased susceptibility to mycobacterial diseases (25). Mouse alveolar macrophages ingest and kill *Aspergillus* spores, whereas neutrophils principally target the hyphal stage (26). Studies of transgenic mice with macrophage-targeted NADPH oxidase function point to NADPH oxidase in macrophages limiting chronic inflammation and autoimmunity (27, 28). Data from our lab point to an important role for macrophage NADPH oxidase in regulating antifungal host defense and acute neutrophilic inflammation (unpublished).

The major function of DCs is to present antigen to T cells. DC-mediated T cell priming is influenced by the local milieu, including microbial and endogenous products that activate DCs. NOX2 in DCs modulates antigen processing and display, a function that is likely to be important in priming T cell activation (29–31).

In certain tumor-bearing mouse models, NADPH oxidase promotes the expansion of myeloid-derived suppressor cells (MDSCs) (32), which are heterogeneous immature myeloid cell populations that accumulate in the setting of chronic inflammation, including cancer. MDSCs may be particularly important in the cancer microenvironment where they can disable anti-tumor T cell responses.

THE ROLE OF NADPH OXIDASE IN REGULATING INFLAMMATION

The net effect of NADPH oxidase activation can be either pro-inflammatory or anti-inflammatory, depending upon the experimental model. In humans, CGD is characterized by abnormally exuberant inflammatory responses leading to granuloma formation, such as granulomatous enteritis resembling Crohn's disease (33) and genitourinary obstruction. In an experimental skin window model, neutrophil exudate was increased in CGD patients compared with normal volunteers (34). Consistent with these findings, in both the p47^{phox}-deficient (35) and X-linked (gp91^{phox}-deficient) (36) mouse models of CGD, increased neutrophil peritoneal leukocytosis occurs in CGD mice compared to wildtype littermates following intraperitoneal challenge with the sterile irritant, thioglycollate. These augmented inflammatory responses in both CGD patients and in CGD mouse models in response to sterile agents point to intrinsic dysregulation of inflammation in CGD as opposed to increased inflammation secondary to unresolved infection.

The immediate effects of the burst of ROIs following phagocyte NADPH oxidase activity and the release of microbicidal neutrophil granule proteases are expected to augment inflammation and injury. Indeed, genetically engineered deficiency of neutrophil elastase or cathepsin G reduces inflammatory responses in mice (37, 38). However, mounting evidence indicates that NADPH oxidase can also influence termination of innate immune responses through ROI-mediated regulation of intracellular signaling pathways and effects on inflammatory cell recruitment, activation, and survival. Substantial progress has been made in understanding molecular mechanisms by which NADPH oxidase regulates inflammation. Just as initiation of inflammation is an active process, the activation of anti-inflammatory pathways is required to protect the host from excessive inflammation. NADPH oxidase regulates innate immune responses through several mechanisms.

Neutrophil chemoattraction, apoptosis, and clearance

Neutrophils are front-line responders to infection and irritants. While neutrophils are essential for host defense, they also cause injury. Therefore, regulation of the neutrophil

response to achieve the appropriate balance of host defense while limiting tissue injury is required. NADPH oxidase regulates neutrophilic inflammation both at the level of neutrophil chemoattraction and apoptosis.

Leukotriene B₄ (LTB₄) and C5a are potent chemotactic molecules that accumulate early in inflamed tissue (39, 40). ROIs have been shown to inactivate proinflammatory chemotactic factors including leukotrienes, C5a, and N-formyl peptide *in vitro* (41–43). The lack of ROI generation by phagocytes from patients with CGD may allow for accumulation of these chemotactic factors at sites of inflammation, leading to increased neutrophil influx. Clearance of peritoneal LTB₄ was shown to be impaired in CGD mice in thioglycollate-elicited peritonitis (44). Similarly, increased tissue levels of LTB₄ were found in CGD versus wildtype mice in a cutaneous model of aspergillosis (45). However, inhibition of LTB₄ does not affect zymosan-induced arthritis in CGD mice (46), suggesting that the importance of LTB₄ in inflammation in CGD is stimulus-dependent.

IL-8 is a pro-inflammatory CXC chemokine induced by several signals (e.g., microbial constituents such as N-formyl peptide and LPS, cytokines, and toxins such as ozone (47)). IL-8 (and its murine homologs KC and MIP-2) ligates two receptors (CXCR1 and CXCR2) on neutrophils that mediate distinct functions including cytosolic free Ca²⁺ changes, release of granule enzymes, activation of phospholipase D, and NADPH oxidase activation (48). IL-8 has potent neutrophil chemoattractant activity and is implicated in the pathogenesis of several inflammatory disorders (e.g., acute lung injury). Lekstrom-Himes et al. (49) showed that IL-8 mRNA and protein production is augmented in human CGD compared to normal neutrophils. Addition of H₂O₂ to CGD neutrophils inhibits production of IL-8, and addition of catalase (an H₂O₂ scavenger) or diphenyleneiodonium (DPI; inhibitor of NADPH oxidase and other flavocytochromes) to normal neutrophils leads to IL-8 responses comparable to those of CGD neutrophils. Thus, there appears to be a feedback loop in neutrophils involving superoxide generation and IL-8 levels, by which IL-8 acts to recruit neutrophils and prime NADPH oxidase activity. In turn, further IL-8 production is downregulated by generation of ROIs after neutrophils arrive.

In addition to regulation of neutrophil influx, NADPH oxidase also appears to play an important role in clearance of neutrophils at sites of inflammation. Neutrophils and macrophages interact in the inflammatory environment; their cross-talk regulates the extent and nature of the inflammatory response. Impaired neutrophil apoptosis and clearance likely contribute to persistent inflammation in CGD. Coxon et al. (50) showed that phagocytosis-induced apoptosis is impaired in neutrophils from CGD patients. Neutrophils from CGD patients are more resistant to spontaneous apoptosis *in vitro* compared to normal neutrophils and produce less prostaglandin D₂ (PGD₂) (51), an inflammatory mediator with both pro-allergic and anti-inflammatory properties. During phagocytosis of apoptotic targets, CGD macrophages are also defective in production of (PGD₂) and transforming growth factor-beta (TGF-beta), which contribute to persistence of inflammation (51). Additional studies indicate that NADPH oxidase activation stimulates recognition and removal of apoptotic neutrophils (efferocytosis) by macrophages (52–55). Efferocytosis is linked to dampening of the inflammatory response both by direct clearance of neutrophils and limiting production of pro-inflammatory cytokines (56).

NADPH oxidase activation in neutrophils is linked to generation of NETS, which requires death of neutrophils and breakdown of cell membranes (21). While NET generation is expected to have an important host defense function against extracellular pathogens, we speculate that NADPH oxidase-mediated NET generation also functions to accelerate neutrophil cell death, thereby limiting neutrophil numbers at inflammatory sites. Additional

research is required to compare the relative effects of NADPH oxidase on apoptosis and NET generation as modes of neutrophil death at sites of inflammation.

Molecular regulation of oxidant-sensitive transcription factors

Transcription factors bind to specific consensus sequences in the promoter regions of target genes to activate or repress their expression. Regulation of effector gene expression can have profound effects on cellular phenotypic characteristics, including cell proliferation, differentiation, cytokine production, and apoptosis. A number of transcription factors are under redox-sensitive regulation (57), including NF- κ B and Nrf2.

NF- κ B is a ubiquitous transcription factor that functions as a homo- or heterodimer of five members of proteins: c-Rel, RelA (p65), RelB, p50, and p52. The prototypical NF- κ B complex is the RelA (p65)/p50 heterodimer, which resides in cytoplasm by forming complexes with inhibitory κ B (I κ B) in unstimulated conditions (58). NF- κ B is activated by many signals, including microbial motifs that ligate specific pathogen recognition receptors, UV irradiation, various toxins, and cytokines (e.g., TNF- α). NF- κ B regulates gene expression of proinflammatory cytokines (TNF- α , IL-1 β , and IL-6), CXC chemokines (IL-8, MIP-2, and KC), enzymes (iNOS and COX), and apoptosis-regulating proteins (59, 60). In the canonical pathway of NF- κ B, activation, the I κ B kinase (IKK) complex is required to phosphorylate I κ B, leading to its dissociation from NF- κ B, and enabling NF- κ B, to translocate to the nucleus and activate target genes. The IKK complex contains two catalytic subunits, IKK α and IKK β , and controls the activation of NF- κ B. IKK β mediates NF- κ B activation by phosphorylation of I κ B- α in response to pro-inflammatory cytokines and microbial products (61).

One of the principal ways that ROIs mediate signal transduction is through oxidation of susceptible cysteine residues to cysteine sulfonic acid or disulfide. Redox-sensitive residues are present in a number of proteins that regulate NF- κ B activation, including IRAK-1, IRAK-4 (62), and IKK β (63). The DNA binding avidity of NF- κ B can also be modulated by changes in the cellular redox state (64). Differential effects of inflammatory stimuli on these targets as well as timing and duration of NADPH oxidase activity may determine the net impact of NADPH-derived ROIs on NF- κ B signaling. NF- κ B activation can also augment NADPH oxidase by inducing the expression of p47^{phox} and gp91^{phox} (65, 66). Interestingly, ROI production can exert opposing effects on NF- κ B, resulting in activation in the cytoplasm and inactivation in the nucleus. The Cys 62 residue of the p50 subunit within the p50/p65 heterodimer appears to play an important role in redox regulation of NF- κ B. Cys 62 must be reduced for optimal p50 subunit binding to DNA (67). In addition, modification of other cysteine residues or additional ROI-mediated post-translational modifications of NF- κ B may be important for regulating transcriptional activity of NF- κ B,

The interaction between NADPH oxidase and NF- κ B activation is complex and stimulus-dependent. For example, hepatic injury and NF- κ B activation were found to be reduced in CGD mice compared to similarly treated wildtype mice following challenge with a peroxisomal proliferator (68, 69) and in alcohol-induced hepatitis (70). In other models of inflammation, NADPH oxidase limited NF- κ B activation. CGD mice had greater cigarette smoke-induced lung injury and NF- κ B activation compared to wildtype mice (71). Zymosan or LPS treatment results in increased NF- κ B activation in isolated macrophages and in whole lungs of CGD mice compared to similarly treated wildtype mice (72). NF- κ B activation was increased in PBMCs from CGD patients compared to normal donors following stimulation with LPS or zymosan (72–74). These results underscore the complexity of the interaction of ROIs and NF- κ B activation that can be influenced by the specific stimulus, site of inflammation, and numerous redox-sensitive targets that regulate NF- κ B function.

Nrf2 is a cap “n” collar basic leucine zipper (b-ZIP) transcription factor. Nrf2 translocates to the nucleus, binds to Antioxidant Response Elements (ARE) present in the promoters of target genes, and induces Phase 2 enzymes that detoxify carcinogens and oxidants, as well as dampen inflammatory responses (75–79). Nrf2-regulated genes include almost all of the relevant antioxidants, such as heme oxygenase (HO)-1, γ -glutamyl cysteine synthase, and several members of the GST family (80). Under basal conditions, Nrf2 undergoes rapid ubiquitination by the ubiquitin ligase CUL3 with subsequent proteasome-dependent degradation (81–83). Keap1 is an oxidative stress sensor that functions as an adaptor for CUL3 (83). Oxidation or adduction of Keap1 at cysteine 151 induces a conformational change in Keap1 that inhibits its ability to bind to CUL3, thereby abrogating Nrf2 ubiquitination (82, 84). Oxidation or adduction of Keap1 at critical cysteine residues in the intervening domain induce conformational changes that distort the beta propeller/double glycine repeat domain that binds Nrf2, thereby perturbing Nrf2 ubiquitination (85). Alternatively, oxidants and electrophiles can initiate PKC-mediated phosphorylation of Nrf2, inhibiting the ability of Nrf2 to bind Keap1 (86).

Nrf2^{−/−} mice have increased inflammatory responses as well as increased susceptibility to carcinogens (77). They have decreased expression of antioxidant/phase II detoxifying enzymes (e.g., HO-1, NAD(P)H-quinone reductase-1, UDP-glucosyltransferase 1A1, and glutathione S-transferase), and, in general, increased production of pro-inflammatory cytokines. Nrf2 has a protective role in several experimental models, including LPS-induced inflammation and injury (87), colitis (76), allergic asthma (80), and tobacco-induced lung injury (88, 89). Lugade et al. showed that Nrf2 limits lung inflammation and B cell responses following repeated challenge with non-typeable *Haemophilus influenzae* (NTHI), a major cause of chronic obstructive pulmonary disease (COPD) exacerbations (90). In addition to modulating inflammation, Nrf2 activation can enhance the ability of alveolar macrophages to clear bacteria (91).

Studies using flavoenzyme inhibitors have shown that NADPH oxidase can be an upstream regulator of Nrf2 (92–94), raising the question whether increased inflammation in CGD might in part be explained by defective Nrf2 activation. We evaluated the interaction of NADPH oxidase and Nrf2 in modulating inflammation *in vivo*. After challenge with either intratracheal zymosan or LPS, NADPH oxidase-deficient p47^{phox}^{−/−} and gp91^{phox}-deficient mice developed exaggerated and progressive lung inflammation and elevated pro-inflammatory cytokines (TNF- α , IL-17, and G-CSF) compared to wildtype mice (72). Bone marrow chimeric studies with wildtype and CGD mice showed this inflammatory phenotype was conferred by hematopoietic cells. In the absence of a functional NADPH oxidase, zymosan failed to activate Nrf2. The triterpenoid, CDDOIm, activated Nrf2 independently of NADPH oxidase and reduced zymosan-induced lung inflammation in CGD mice. In addition, zymosan-treated peripheral blood mononuclear cells from X-linked CGD patients showed impaired Nrf2 activity. Together, available data support the concept that NADPH oxidase regulates inflammation by modulating redox-sensitive transcription factors, such as NF- κ B and Nrf2, and suggest that pharmacological targeting of these pathways may be a therapeutic strategy in CGD inflammation.

THE ROLE OF NADPH OXIDASE IN SELECTED DISEASES

NADPH oxidase in acute lung injury

Microbes and endogenous products of necrosis are both pro-inflammatory and activate innate immune responses through similar pathogen recognition receptors. We and others have therefore evaluated the role of NADPH oxidase in models of acute lung injury. Acute lung injury comprises a spectrum of lung diseases resulting from cellular damage, inflammation, and capillary leak that follow a variety of insults that include direct lung

injury (e.g., inhalation of caustic agents, lung infection, and aspiration of gastric contents) or indirect lung injury (e.g., sepsis, severe trauma, and pancreatitis). NADPH oxidase activation leads to rapid generation of ROIs and activation of neutrophil granular proteases (20) that are expected to be injurious; however, studies in NADPH oxidase-deficient mice point to a more complex interaction between NADPH oxidase and acute lung injury that is context-dependent. While NADPH oxidase worsened the severity of acute lung injury following H1N1 challenge in mice (95), wildtype and CGD mice had similar levels of lung injury following LPS challenge (96). In a model of *E. coli* sepsis, NADPH oxidase-deficient mice had greater lung neutrophil sequestration, but similar lung injury compared to wildtype mice (97). In contrast, CGD mice developed increased alveolar neutrophilic leukocytosis and worse acute lung injury following acid aspiration, demonstrating a protective role of NADPH oxidase (98). Studies *in vitro* have shown that H₂O₂ and hypochlorous acid (which are downstream metabolites of NADPH oxidase activation) induce Nrf2 activation in macrophages and airway epithelial cells (99–102), which is posited to protect cells from oxidative injury. Nrf2^{-/-} mice developed similar lung injury after acid aspiration compared to CGD mice (103), raising the possibility that NADPH oxidase may limit acid aspiration-induced lung injury through redox-dependent activation of Nrf2.

Thus, NADPH oxidase plays a complex role in mediating the response to acute injury, including the potential to activate pathways that limit inflammation and injury. Understanding mechanisms by which NADPH oxidase and its regulated pathways modulate inflammation and injury may identify novel therapeutic approaches.

NADPH oxidase in fungal pneumonia

Aspergillus and other moulds are ancient organisms that are ubiquitous in the environment. The mammalian immune system, therefore, evolved in their presence and has developed recognition of them through specific pathogen recognition receptors allowing for immune control of fungal growth, but also restraint of injurious inflammation (104). Knowledge gained from CGD patients and mouse models show that NADPH oxidase, in addition to its critical antifungal host defense function, also has a role in restraining the inflammatory response to fungal cell wall products.

“Mulch pneumonitis” is a life-threatening hyperinflammatory response to high level fungal inhalation in CGD, requiring both antifungal therapy and systemic corticosteroids (105). In CGD mice, even low virulence strains of *Aspergillus nidulans* cause excessive inflammation and death from pulmonary aspergillosis (106). Consistent with this concept, intratracheal administration of heat-killed *Aspergillus* hyphae elicited mild self-limited inflammation in wildtype mice, but robust and persistent inflammation in CGD mice (107). In addition, fungal cell wall beta-glucan-induced pneumonitis is augmented in CGD compared to wildtype mice (108). Taken together, these results show that invasive aspergillosis in CGD is both a disease of impaired host defense and dysregulated inflammation to fungal products.

There has been important knowledge gained about the interaction between pathogen recognition receptors and NADPH oxidase in mediating antifungal immunity (109). Fungal motifs, such as cell wall constituents and DNA, ligate specific pathogen recognition receptors. Dectin-1 is a receptor and immunomodulator of beta-glucans, which are ubiquitous cell wall constituents of fungi and plants (110, 111). Dectin-1 signals through the tyrosine kinase Syk and the caspase recruitment domain, CARD9 (112–114). Activation of dectin-1 by beta-glucans can stimulate NADPH oxidase activation (4). *Aspergillus fumigatus* spores are relatively immunologically silent, likely due to a hydrophobic protein that masks pro-inflammatory cell wall products (115). During germination (transition from spores to hyphae), cell wall beta-glucans of *A. fumigatus* become unmasked and can ligate dectin-1, leading to NADPH oxidase activation and production of pro-inflammatory

cytokines (116–118). Dectin-1 in macrophages is activated by particulate, but not soluble beta-glucans, which during natural infection likely enables the immune system to distinguish cell wall-associated beta-glucans from soluble products that do not pose a threat (119). The ability of host cells to recognize fungal cell wall products displayed in a stage-specific fashion likely assists in calibrating the inflammatory response to avert excessive inflammation following inhalation of ubiquitous fungal spores.

Activation of dectin-1 by fungal beta-glucans can lead to NADPH oxidase activation and induction of pro-inflammatory IL-23 and IL-17 responses (120–122). An inherited deficiency in dectin-1 signaling is associated with familial mucocutaneous candidiasis, but not invasive fungal diseases (120–122). Dectin-1-deficient mice have increased susceptibility to aspergillosis in association with reduced, but not absent, *Aspergillus*-stimulated NADPH oxidase activity, and impaired production of IL-17 and other pro-inflammatory cytokines and chemokines (123). The *A. fumigatus* inoculum required to cause mortality in dectin-1-deficient mice was several-thousand fold greater than that reported for CGD mice, indicating that dectin-1 deficiency does not recapitulate the severity of immune impairment caused by complete NADPH oxidase deficiency. Data in mice support the notion that dectin-1 and NADPH oxidase have coordinated effects in regulating antifungal host defense and the inflammatory response to inhaled fungi (113, 121–126).

Similar to *Aspergillus* challenge, intratracheal administration of killed hyphae and zymosan caused dramatically augmented lung inflammation and IL-17 production in CGD mice, emphasizing the intrinsic regulatory role of the NADPH oxidase on inflammation (72, 126). George-Chandy et al. (127) showed that NADPH oxidase-deficient DCs induce higher levels of interferon- γ and IL-17 in responding T cells after antigen-specific or superantigen-induced activation compared to wildtype DCs. CGD mice have more severe IL-17-driven autoimmune arthritis compared to wildtype mice. Consistent with observations in mice, the frequency of circulating Th17 cells and production of Th17-derived cytokines was significantly higher among CGD patients compared to normal donors (128). Studies in mice point to NADPH oxidase inducing the development of regulatory T cells (27, 126, 129), which dampen T cell responses, thereby potentially averting allergy and autoimmunity. Taken together, these results demonstrate an important role of NADPH oxidase in regulating Th17 and point to modulation of the IL-23/IL-17 (IL-23 expands Th17 cells) and Treg pathways as potential therapeutic targets in CGD. An important limitation is that these results were derived principally from mouse models of inflammation, and important differences in the role of NADPH oxidase in regulating inflammation can exist between mice and humans. Specifically, while NADPH oxidase is an important activator of tryptophan catabolism leading to development of regulatory T cell responses during inflammation in mice, this pathway is maintained in leukocytes from CGD patients (130, 131). The requirement for superoxide in tryptophan catabolism varies among different cell types and species (132), highlighting the need for correlative studies in humans

CONCLUSIONS

CGD is a rare inherited disorder of the NADPH oxidase from which we can learn about the function of this enzyme, both as a mediator of host defense and of inflammation. From CGD patients and CGD mouse models, we have learned that NADPH oxidase, in addition to defending against a spectrum of bacterial and fungal pathogens, also limits neutrophilic inflammation through several mechanisms. Effective calibration of neutrophilic inflammation is critical in protecting the host from infection, while averting injury associated with excessive or persistent inflammation.

There are several gaps in current knowledge regarding the role of NADPH oxidase in host defense and inflammation. At the most fundamental level, it is still unclear how NADPH oxidase mediates antimicrobial host defense. The relative contribution of oxidant (and nitrogen) free radicals versus downstream signaling with activation of other host defense pathways remains uncertain. Our view is that NADPH oxidase and its downstream activated pathways (such as protease activation and NET generation in neutrophils) have distinct, non-redundant functions. Another gap in knowledge relates to the impact of NETotic and apoptotic neutrophil cell death, both of which are NADPH oxidase-modulated, on neutrophil-mediated injury. Other areas of active research include the role of NADPH oxidase in DC function (including antigen display) and generation of myeloid-derived suppressor cells. The role of NADPH oxidase in these processes may be important in understanding the microenvironment in tumors and other chronic diseases. In addition, NADPH oxidase plays an important role in modulating the balance between Th17 and regulatory T cells. This function is likely to be particularly important to our understanding of chronic inflammation and autoimmunity. Seen in this light, it is noteworthy that about one-third of CGD patients develop Crohn's-like inflammatory bowel disease. Knowledge gained from future studies regarding mechanisms by which NADPH oxidase and its downstream pathways modulate host defense and inflammation may lead to novel therapeutic targets both for CGD patients, and, more broadly, for diseases associated with excessive inflammation and inflammation-induced organ injury.

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Highlights

- NADPH oxidase is the major source of reactive oxidants in activated phagocytes
- CGD, a disorder of NADPH oxidase, is characterized by infections and inflammation
- NADPH oxidase is a critical mediator of antimicrobial host defense
- NADPH oxidase calibrates innate and adaptive immunity through a variety of mechanisms
- NADPH oxidase-regulated pathways may be therapeutic targets for inflammatory diseases