Deficiency of gp91phox Inhibits Allergic Airway Inflammation

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Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, a multienzyme complex, is the major source for production of reactive oxygen species (ROS). ROS are increased in allergic diseases, such as asthma, but the role of ROS in disease pathogenesis remains uncertain. We hypothesized that mice unable to generate ROS via the NADPH oxidase pathway would have decreased allergic airway inflammation. To test this hypothesis, we studied gp91phox^{-/-} mice in a model of allergic airway inflammation after sensitization and challenge with ovalbumin. Serum, bronchoalveolar lavage fluid, and lungs were then examined for evidence of allergic inflammation. We found that mice lacking a functional NADPH oxidase complex had significantly decreased ROS production and allergic airway inflammation, compared with wild-type (WT) control animals. To determine the mechanism by which allergic inflammation was inhibited by gp91phox deficiency, we cultured bone marrow-derived dendritic cells from WT and gp91phox^{-/-} mice and activated them with LPS. IL-12 expression was significantly increased in the gp91phox^{-/-} bone marrow-derived dendritic cells, suggesting that the cytokine profile produced in the absence of gp91phox enhanced the conditions leading to T helper (Th) type 1 differentiation, while inhibiting Th2 polarization. Splenocytes from sensitized gp91phox^{-/-} animals produced significantly less IL-13 in response to ovalbumin challenge *in vitro* compared with splenocytes from sensitized WT mice, suggesting that NADPH oxidase promotes allergic sensitization. In contrast, inflammatory cytokines produced by T cells cultured from WT and gp91phox^{-/-} mice under Th0, Th1, Th2, and Th17 conditions were not significantly different. This study demonstrates the importance of NADPH oxidase activity and ROS production in a murine model of asthma.

Keywords: asthma; allergic airway inflammation; gp91phox; reactive oxygen species; NADPH oxidase

Asthma is a significant health problem, affecting up to 300 million children and adults around the world (1). In the United States alone, costs associated with asthma exceed \$50 billion each year due to health care use and lost productivity (2). The prevalence of this disease is increasing, without clear etiology (3, 4). Chronic

CLINICAL RELEVANCE

Our findings begin to fill an important gap in our knowledge of the actions of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and its homologs, and provide future direction for the investigation of the mechanisms by which chronic airway inflammation is established and maintained. Based on the data presented here, selective inhibition of NADPH oxidase homologs may deserve additional exploration as a therapeutic target for asthma.

inflammation of the airways is a hallmark of the disease (5), and yet, despite widespread research, the mechanisms of persistent allergic airway inflammation are poorly understood.

Reactive oxygen species (ROS) are increased in allergic diseases, such as asthma, and ROS production is associated with airway inflammation and airway hyperresponsiveness (6). Breath condensates from subjects with asthma have been shown to contain increased hydrogen peroxide, an ROS, and 8-isoprostane, an F₂-isoprostane that is a marker of oxidative stress, compared to control subjects without asthma (7–9). In addition, increased levels of peroxidation products are present in the serum, urine, and lung tissue of patients with asthma compared with those without asthma (10–12). Mice with allergic lung inflammation have higher levels of F₂-isoprostane compared with control animals (13).

The airway lining fluid contains high levels of antioxidants, such as ascorbic acid and α -tocopherol, and antioxidant enzymes, including glutathione, heme oxygenase, and superoxide dismutase, which are proposed to protect the lungs from oxidative stress (14–16). This has led to much investigation into the relationship between antioxidants and asthma. Dietary antioxidant supplementation attenuated established allergic airway inflammation in some animal models (17–22). However, clinical trials failed to show a definitive benefit from antioxidant supplementation in humans with asthma (23–27), and some have even shown harm (28). To delineate more accurately the role of ROS in the pathogenesis of allergic airway inflammation, we defined the role of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in a mouse model of allergic sensitization and challenge. NADPH oxidase, a multienzyme complex requiring assembly of both cytosolic and membrane components, is integral to the production of ROS by monocytes, neutrophils, eosinophils, and macrophages. The NADPH oxidase complex is composed of six subunits: gp91phox (also known as Nox2), p22phox, p40phox, p47phox, p67phox, and a Rho guanosinetriphosphatase (GTPase), usually Rac1 or Rac2. gp91phox has been proposed as a primary component of the microbicidal oxidase system of phagocytes.

Mice (and humans) lacking a functional NADPH oxidase complex are unable to generate ROS. Although ROS can be beneficial to the immune system, for example in killing pathogens or in cell signaling, increased ROS have also been associated

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with inflammatory lung diseases, such as asthma. During allergic inflammation, myeloid cells, such as eosinophils and neutrophils, produce large amounts of ROS via activation of their NADPH oxidase systems, and are believed to contribute to asthma pathogenesis (29–31).

In this study, we used gp91phox^{-/-} mice to examine the role of ROS generated by NADPH oxidase in allergic airway inflammation. We hypothesized that the lack of a functional NADPH oxidase complex decreased ROS generation and resulted in decreased allergic airway inflammation.

MATERIALS AND METHODS

Mice

Pathogen-free 8- to 10-week-old C57BL/6 and gp91phox^{-/-} mice on a C57BL/6 genetic background were purchased from The Jackson Laboratory (Bar Harbor, ME) and breeding colonies established. gp91phox^{-/-} and wild-type (WT) control animals were maintained in sterile housing and their water supplemented with sulfamethoxazole and trimethoprim. Antibiotics were discontinued a week before the start of each experiment. Female mice were used for all experiments. In caring for the animals, the investigators adhered to guidelines of the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

Ovalbumin Sensitization and Challenge

On Day 0, gp91phox^{-/-} mice and the appropriate WT controls were sensitized to ovalbumin (OVA) with an intraperitoneal injection of 0.1 ml (10 µg) of OVA (chicken, grade V; Sigma-Aldrich, St. Louis, MO) complexed with 20 mg of Al(OH)₃ diluted in a volume of 100 µl of PBS. Nonsensitized mice were injected intraperitoneally with 20 mg of Al(OH)₃ diluted in a volume of 100 µl of PBS on Day 0. On Days 14–17, the mice were challenged with 1% OVA diluted in sterile PBS aerosolized by an ultrasonic nebulizer (Ultraneb 99; DeVilbiss, Somerset, PA) for 40 minutes each day (Figure 1).

Bioluminescence Imaging of ROS Production

An ROS-sensing probe, L-012 (Wako Chemicals, Richmond, VA), was used to detect ROS production in living mice, as previously described (32) and detailed in the online supplement.

Bronchoalveolar Lavage Analysis

Bronchoalveolar lavage (BAL) was performed by instilling 600 µl of 5% BSA diluted in normal saline through the tracheostomy tube and withdrawing the fluid via syringe. Additional details can be found in the online supplement.

ELISA

Protein levels of cytokines from BAL fluid and cell culture supernatants were measured by commercially available Duoset or Quantikine ELISA kits (R&D Systems, Minneapolis, MN) following the manufacturer's instructions.

Histological Analysis

Lungs were fixed in 10% buffered formalin overnight, transferred to 70% ethanol, embedded in paraffin blocks, cut, stained, and scored as detailed in the online supplement.

IgE Concentrations

On Day 18, sera were collected from sensitized mice and then analyzed by ELISA to determine levels of total IgE, as previously described (33).

Dendritic Cell Culture

Bone marrow from WT and gp91phox^{-/-} mice was harvested and then cultured for 8 days, as previously described (34) and detailed in the online supplement.

T Cell Isolation and Polarization

A single-cell suspension was prepared from spleens collected from WT and gp91phox^{-/-} mice, as previously described (34) and detailed in the online supplement. Purified CD4⁺ cells were positively selected and then polarized down the T helper (Th) type 0, Th1, Th2, or Th17 pathway, as previously described (35, 36).

Spleen Cell Culture and Stimulation

On Day 0, gp91phox^{-/-} mice and WT control animals were sensitized with OVA/aluminum hydroxide (alum) or alum as described above. On Day 14, a single-cell suspension was prepared, stimulated with OVA, BSA, or no protein, and cultured as described in the online supplement. The culture supernatant was collected on Day 19 and cytokines measured as above.

Statistical Analysis

Data are presented as means (± SEM). Measurements of photon counts, cell counts and differentials, cytokines, mucus score, and IgE were analyzed by ANOVA with Fisher's least significant difference performed as a *post hoc* analysis. Differences were considered significant when *P* was less than 0.05.

RESULTS

NADPH Oxidase Is Required for ROS Production in the Lung

To test whether ROS production is dependent on NADPH oxidase in the setting of allergic airway inflammation, we performed alum sensitization alone or followed by OVA sensitization and challenge. ROS generation was measured using an *in vivo* bioluminescence imaging methodology (Figure 2). We found that, at baseline, there was significantly less ROS production in gp91phox^{-/-} mice compared with WT mice that were given alum alone. OVA sensitization and challenge significantly increased ROS production over the chest in WT mice compared with those that received alum, whereas there was no increase in ROS in OVA-sensitized and -challenged gp91phox^{-/-} mice compared with alum-sensitized gp91phox^{-/-} mice. These results reveal that allergic sensitization and challenge increases ROS in WT mice, but not in gp91phox^{-/-} mice, supporting the use of this model in defining the role of ROS in the allergic airway phenotype.

NADPH Oxidase Deficiency Decreases Allergen-Induced Airway Inflammation

Because a functional NADPH oxidase complex is necessary to generate ROS, and ROS are associated with allergic airway

Figure 1. Experimental design for the ovalbumin (OVA) model. Mice are sensitized to OVA conjugated to aluminum hydroxide (alum) on Day 0, followed by 4 days of OVA challenge by aerosolization (Days 14–17), and harvested on Day 18. i.p., intraperitoneal.

Figure 2. gp91phox deficiency abrogated reactive oxygen species (ROS) production during OVA-induced lung inflammation. In mice sensitized with alum alone, ROS production in the lung was observed in low levels in wild-type (WT), but not gp91phox^{-/-} mice *in vivo*. After OVA sensitization and challenge, ROS production increased in the WT mice, but not in gp91phox^{-/-} mice ($n = 14$ WT, $n = 15$ gp91phox^{-/-}; * $P < 0.05$ and ** $P < 0.01$). IP, intraperitoneal.

disease, we measured BAL fluid cell counts and differentials in WT and gp91phox^{-/-} mice to determine whether an inability to generate ROS resulted in decreased allergic airway inflammation in gp91phox^{-/-} mice. In mice given alum alone, cell counts and differentials were not significantly different between WT vs gp91phox^{-/-} mice (Figure 3A). However, after OVA sensitization and challenge, WT mice had significantly greater total cells ($P < 0.05$) and eosinophils ($P < 0.01$) in the BAL fluid than gp91phox^{-/-} mice (Figure 3B).

NADPH Oxidase Deficiency Attenuates IL-13 Production *In Vivo*

IL-13 is a Th2 cytokine critical to the allergic airway phenotype. Therefore, we quantified IL-13 levels in the BAL fluid of WT and gp91phox^{-/-} mice after OVA sensitization and challenge. IL-13 levels were significantly higher in the BAL fluid of WT mice compared with gp91phox^{-/-} mice ($P < 0.0001$; Figure 4A). Because of the increased numbers of eosinophils in WT mice after OVA sensitization and challenge, we measured IL-5 in BAL. Although there was a trend toward increased BAL IL-5 levels in WT mice compared with gp91phox^{-/-} mice, this difference was not statistically significant (Figure 4B). Because the difference in BAL eosinophils between WT and gp91phox^{-/-} mice was not fully explained by the differences in IL-5, we measured eotaxin, a chemokine known to recruit eosinophils by chemotaxis. We found that eotaxin was significantly higher in WT mice compared with gp91phox^{-/-} mice ($P < 0.05$; Figure 4C). Suppression of eotaxin expression is therefore likely one mechanism

responsible for reduced airway eosinophilia in NADPH oxidase-deficient mice.

NADPH Oxidase Deficiency Decreases Airway Mucus and Leads to Crystal Formation

As there was a significant decrease in BAL IL-13 protein expression in allergically sensitized and challenged gp91phox^{-/-} mice compared with WT mice, and IL-13 is an important factor in airway mucus expression, we postulated that gp91phox^{-/-} mice have decreased epithelial mucus production. Therefore, we harvested lungs after OVA sensitization and challenge, and performed histological analysis for mucus after periodic acid Schiff staining. Histopathology from OVA-sensitized and -challenged mice was quantitatively scored by a pathologist blinded to the experimental groups. There was significantly less mucus in the lungs of gp91phox^{-/-} mice sensitized and challenged with OVA compared with WT mice (Figures 5A and 5B). Large crystals were noted in the airways and lung parenchyma of the gp91phox^{-/-} mice on hematoxylin and eosin staining (Figure 5C). No crystals were observed in the WT lung.

gp91phox Deficiency Does Not Regulate Serum IgE Levels

To assess whether NADPH oxidase expression and oxidative stress impacts the levels of serum IgE, we determined serum IgE levels in WT mice and gp91phox^{-/-} mice after OVA sensitization and challenge. Serum IgE levels were not significantly different in gp91phox^{-/-} mice compared with WT control animals (8.6 ± 0.8 versus 10.9 ± 1.4 $\mu\text{g/ml}$; $P = 0.21$).

Figure 3. Reduced airway inflammation in gp91phox^{-/-} mice. (A) Bronchoalveolar lavage (BAL) cell counts and differentials from WT versus gp91phox^{-/-} mice with mock sensitization ($n = 3$ WT, $n = 3$ gp91phox^{-/-}). (B) Total cell count and eosinophils in the BAL fluid of gp91phox^{-/-} mice after OVA sensitization and challenge compared with WT mice. Data shown incorporate four independent experiments ($n = 17$ WT, $n = 13$ gp91phox^{-/-}; * $P < 0.05$ and ** $P < 0.01$). PMN, polymorphonuclear neutrophils.

Figure 4. Decreased T helper (Th) type 2 cytokine and chemokine in the lungs of gp91phox^{-/-} mice. (A) IL-13 is decreased in gp91phox^{-/-} mice after OVA sensitization and challenge compared with control animals. Data shown incorporate five independent experiments ($n = 31$ WT, $n = 24$ gp91phox^{-/-}; ** $P < 0.01$). (B) IL-5 is decreased in gp91phox^{-/-} mice after OVA sensitization and challenge compared with control animals ($n = 24$ WT, $n = 18$ gp91phox^{-/-}; $P = 0.06$). (C) Eotaxin is decreased in gp91phox^{-/-} mice after OVA sensitization and challenge compared with control animals ($n = 23$ WT, $n = 17$ gp91phox^{-/-}; * $P < 0.05$).

NADPH Oxidase Deficiency Results in Increased Dendritic Cell Production of IL-12

Dendritic cells are critical in antigen presentation to naive T cells, and the cytokines produced by dendritic cells have an important role in CD4 T cell differentiation. Phenotypic and functional dendritic cell maturation can be induced by oxidative stress—specifically, superoxide anion (37). This observation led us to investigate whether dendritic cells play a role in the cytokine protein expression observed in gp91phox-deficient mice. Bone marrow–derived dendritic cells (BMDCs) were cultured from WT and gp91phox^{-/-} mice for 8 days and then activated with LPS to determine the cytokine secretion profile of the activated dendritic cells. Activated BMDCs from gp91phox^{-/-} mice produced significantly more IL-12, a cytokine critical for CD4 Th1 differentiation, than those from WT mice ($1,361 \pm 28$ versus $1,188 \pm 48$ pg/ml; $P = 0.005$). There was no difference in IL-10 or IL-23 produced by BMDCs from gp91phox^{-/-} mice compared with WT mice (data not shown). This significant increase in IL-12 production by activated gp91phox^{-/-} BMDCs is perhaps one mechanism by which these mice have decreased Th2 cell differentiation.

NADPH Oxidase Deficiency Decreases *In Vivo* Th2 Differentiation

CD4 T cell differentiation is regulated by the conditions under which antigens are presented. Based on our results revealing that activated gp91phox^{-/-} BMDCs express significantly greater IL-12 than similarly activated WT BMDCs, we hypothesized that there would be decreased Th2 differentiation as a result of antigenic sensitization in mice with a defective NADPH signaling pathway. To test this hypothesis, we sensitized WT and gp91phox^{-/-} mice, and harvested their spleens 14 days later. We cultured the splenocytes for 5 days with OVA protein and then collected the cell culture supernatant. We found that there was a statistically significant 3-fold decrease in IL-13 production by the splenocytes from OVA-sensitized gp91phox^{-/-} mice in response to OVA stimulation *in vivo* compared with splenocytes from similarly treated WT mice (Figure 6). The IL-13 production was antigen specific, as culture with BSA did not result in detectable IL-13. There was also a nonsignificant 3-fold decrease in IL-5 production by OVA-stimulated splenocytes from gp91phox^{-/-} mice compared with WT splenocytes ($P = 0.06$). These results reveal that gp91phox deficiency impairs Th2 differentiation *in vivo*.

NADPH Oxidase Does Not Regulate *In Vitro* Th1, Th2, or Th17 Polarization

T cells have been found to express a functional NADPH oxidase that regulates elements of T cell receptor signaling (38). To further elucidate the mechanism by which allergic airway inflammation is attenuated in gp91phox^{-/-} mice, culture of CD4⁺ T cells from WT and gp91phox^{-/-} mice was performed under Th0, Th1, Th2, and Th17 conditions.

Under Th1 polarizing conditions, IFN- γ protein expression was not different between CD4⁺ T cells from WT and gp91phox^{-/-} mice. Similarly, under Th2 polarizing conditions, there was no difference in the expression of IL-4 and IL-13 protein in CD4⁺ T cells between WT and gp91phox^{-/-} mice. There was also no difference between the WT and gp91phox^{-/-} mice in IL-17A protein secretion from CD4 T cells under Th17 polarizing conditions. Therefore, there was no difference between the groups in the ability of polarized CD4⁺ T cells to generate the signature cytokines for those subsets (data not shown). This finding suggests that T cell differentiation is not NADPH oxidase dependent.

DISCUSSION

The gp91phox subunit is required for NADPH oxidase complex function, and therefore for the production of ROS. ROS have a critical function in host defense, cell signaling, and oxidant stress. In this study, we found that allergen sensitization and aerosol challenge resulted in increased ROS in WT mice, but not gp91phox^{-/-} mice. Allergically sensitized and challenged gp91phox-deficient mice had significantly decreased airway eosinophilia, reduced airway IL-13 and eotaxin protein expression, and blunted airway mucus expression compared with WT mice. There were no differences in serum IgE levels between the groups. This is one of the first studies to address the importance of a functional defect in NADPH oxidase activity, and specifically gp91phox deficiency, in a mouse model of allergic airway disease.

Our findings are in contrast to previous studies using gp91phox^{-/-} mice in models of chronic inflammation. There was increased and prolonged inflammation in response to *Aspergillus fumigatus* (39), branched fungal β -glucan (40), and zymosan or LPS (41) in mice lacking gp91phox. These studies suggest that gp91phox deficiency does not protect against these forms of inflammation. Our results suggest the opposite for

Figure 5. Reduced mucus metaplasia in gp91phox^{-/-} mice after OVA sensitization and challenge. (A) Mucus scores. OVA-sensitized and -challenged gp91phox^{-/-} mice produce significantly less mucus than WT mice ($n = 7$ WT, $n = 7$ gp91phox^{-/-}; $*P < 0.05$). (B) Lung histology, periodic acid Schiff. WT versus gp91phox^{-/-} mice. Original magnification, $\times 20$. (C) Hematoxylin and eosin. WT versus gp91phox^{-/-}. Crystal deposition in the gp91phox^{-/-} lung is marked with arrows. No crystals were observed in the WT lung. Original magnification, $\times 40$.

allergic inflammation. In addition, despite the fact that patients with chronic granulomatous disease, the human clinical correlate of dysfunctional NADPH oxidase, are at increased risk of developing noninfectious hyperinflammatory disorders, there is no clear association between chronic granulomatous disease and asthma (42) or other lung diseases (43).

Interestingly, a recent report by Banerjee and colleagues (44) also examined allergic airway inflammation in gp91phox^{-/-} mice, and found that these mice had increased inflammation

compared with WT mice. However, their gp91phox^{-/-} mice had significantly increased neutrophilia in the lungs at baseline as well as after OVA sensitization and challenge, suggesting the possibility of pre-existing infection, to which these mice are prone. For this reason, we maintained our mouse colony on antibiotics before their use in experiments.

The difference in allergic airway inflammation between the WT and gp91phox^{-/-} mice observed in our study appears to be due to events occurring at sensitization. When BMDCs were

Figure 6. Impaired Th2 differentiation in gp91phox^{-/-} mice. Splenocytes from gp91phox^{-/-} mice sensitized to OVA/alum had decreased IL-13 and a trend toward decreased IL-5 when stimulated with OVA allergen *in vitro* compared with splenocytes from similarly treated WT mice. BSA did not induce IL-13 or IL-5 production ($n = 8$ WT, $n = 8$ gp91phox^{-/-}; three stimulation conditions per spleen; $**P < 0.01$).

cultured from gp91phox^{-/-} mice and stimulated *in vitro*, we found that IL-12 production was increased compared with BMDCs cultured from WT mice. Because IL-12 is a key inducer of the Th1 response, our findings suggest the possibility that dendritic cell function is altered in the absence of a functional NADPH oxidase complex, driving T cell differentiation away from the Th2 pathway and providing a potential mechanism for the decreased allergic airway inflammation in these animals.

The concept that NADPH oxidase modulates sensitization is supported by our finding that splenocytes from sensitized gp91phox^{-/-} mice had significantly decreased antigen-specific IL-13 production compared with splenocytes from sensitized WT mice. These results suggest that perhaps the increased IL-12 produced by gp91phox-deficient dendritic cells is inhibiting Th2 differentiation. There were no differences in serum IgE levels between allergically sensitized and challenged WT and gp91phox^{-/-} mice, revealing that Th2 differentiation is decreased without affecting IgE levels. Although T cells are known to express a functional NADPH oxidase (38), our investigation of T cell differentiation revealed that T cell function was not affected by the lack of a functional NADPH oxidase complex *in vitro*. Whether this is also true *in vivo*, where conditions may be less strongly polarizing, remains to be proven.

Emerging evidence suggests an ever-more important role for dendritic cells in the immune response to environmental allergens (45–47). Many of the cell types involved in asthma pathogenesis, including eosinophils, neutrophils, and T cells, are normally capable of producing ROS. We found that allergic airway inflammation is decreased in mice unable to produce ROS, as we hypothesized. Interestingly, dendritic cells also express NADPH oxidase, and can generate ROS, though less efficiently than other phagocytes (48). One group showed that the activity of NADPH oxidase in dendritic cells is almost insignificant compared with that in neutrophils (49), and suggested that ROS generation by dendritic cells is “cryptic” due to the presence of endogenous inhibitors. They proposed that, under certain conditions, such as prolonged contact between dendritic cells and proinflammatory ligands, substantial production of ROS may occur and affect dendritic cell responses. Other studies have shown that low ROS production by dendritic cells can be enhanced by interaction with antigen-specific T cells (50) or Toll-like receptor ligands (51). Our results reveal a functional consequence of gp91phox deficiency in dendritic cells, despite the low levels of ROS that they produce.

It may be that dendritic cell NADPH oxidase plays a role other than the robust ROS generation for microbial killing seen in neutrophils, and, to a lesser extent, in macrophages. Savina and colleagues (52) showed that the phagosomal alkalization normally observed in dendritic cells was lost when the dendritic cells were derived from the bone marrow of gp91phox^{-/-} mice. It has been suggested that the gp91phox component itself may function as a hydrogen ion channel (53), though this has been debated (54). In any case, dendritic cells lacking gp91phox activity have lower phagosomal pH (52, 55), resulting in enhanced degradation of proteins and decreased efficiency of antigen cross-presentation. Another intriguing finding in our study was the presence of large crystals in both the airways and the interstitium of gp91phox^{-/-} mice after OVA sensitization and challenge. These crystals were not found in allergic WT mice. Although similar crystals have been reported in p47phox^{-/-} mice (56), this is the first report of crystal formation in gp91phox^{-/-} mice. Their exact significance is unclear at this time, and deserves further investigation.

In summary, we found that gp91phox is necessary for a complete allergic airway phenotype. In the absence of this molecule, there was decreased ROS production in the lung, airway inflammation, IL-13 and eotaxin expression, and mucus production.

Our findings begin to fill an important gap in our knowledge of the actions of NADPH oxidase and its homologs, and provide future direction for the investigation of the mechanisms by which chronic airway inflammation is established and maintained. It may be difficult to pin down the consequences of inhibiting a molecule that has multiple and sometimes contrary actions. However, based on the data presented here, selective inhibition of NADPH oxidase homologs may deserve additional exploration as a therapeutic target for asthma.

Author disclosures are available with the text of this article at www.atsjournals.org.

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