Acid aspiration-induced lung inflammation and injury are exacerbated in NADPH oxidase-deficient mice

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Segal BH, Davidson BA, Hutson AD, Russo TA, Holm BA, Mullan B, Habitzruther M, Holland SM, Knight PR 3rd. Acid aspiration-induced lung inflammation and injury are exacerbated in NADPH oxidase-deficient mice. Am J Physiol Lung Cell Mol Physiol 292: L760–L768, 2007. First published November 17, 2006; doi:10.1152/ajplung.00281.2006.—Increased reactive oxidant intermediates (ROI) or the mechanisms by which they are generated. Limitation of these studies is that they do not address the source of ROI scavengers, superoxide dismutase (SOD) and catalase, attenuated the acid aspiration-induced inflammatory pathogenesis of the lung injury (11, 15). We speculated that this downmodulating effect may be mediated by promoting the transition from production of cytokines and chemokines involved in neutrophil infiltration to a less injurious, chronic inflammatory response.

Acid aspiration pneumonitis; chronic granulomatous disease; reactive oxidants; acute respiratory distress syndrome

ACID ASPIRATION LUNG INJURY is a major cause of morbidity in the postoperative setting and in patients in the intensive care unit. Acute respiratory distress syndrome (ARDS) is the most ominous complication of aspiration pneumonitis and is associated with a high mortality rate (7, 17). Low pH and total volume of the aspirate as well as the presence of particulate material and bacterial content are important contributors to lung damage (37). Patients with ARDS have elevated alveolar concentrations of the proinflammatory cytokines IL-1β and TNF-α as well as IL-8, a CXC chemokine with potent neutrophil chemotactic activity (6). In these patients, low alveolar concentrations of the anti-inflammatory cytokines IL-10 and IL-1 receptor antagonist correlate with a poor survival, supporting the prognostic importance of downregulators of inflammation in controlling lung injury (6).

Prior studies have implicated reactive oxidant intermediates (ROI) as having a deleterious role following experimental lung injury. Capillary leak, systemic complement activation, and generation of proinflammatory cytokines and chemokines follow aspiration of low-pH gastric contents (14, 29). Acute influx of neutrophils into the alveolar space is required for the inflammatory pathogenesis of the lung injury (11, 15). We showed that pretreatment of rats with deferoxamine, an iron chelator that suppresses hydroxyl anion production, results in a reduction in hyperoxia-intensified lung injury after different types of gastric aspiration (27). We also demonstrated in rats with acid injury followed by hyperoxia an increase in superoxide production in leukocytes from bronchoalveolar lavage (BAL) and blood, increased oxidation of lipids and proteins in injured lungs, and a reduction in the antioxidant capacity of lungs (26). High-dose nitric oxide inhalation increased lung injury after gastric aspiration, and lung injury was further increased by hyperoxia (24). Intravenous treatment of rats after aspiration with the ROI scavengers, superoxide dismutase (SOD) and catalase, attenuated the acid aspiration-induced capillary leak and the lung wet/dry ratio but did not affect neutrophil sequestration (12). We previously showed that serine proteases play a role in degrading Cu/Zn SOD in rats following acid aspiration and hyperoxia, thereby decreasing pulmonary antioxidant capacity (23). Serine antiprotease administration did not affect lung injury, inflammatory cells, or cytokine profiles after acid aspiration and hyperoxia, indicating that there was not a straightforward relationship between antioxidant capacity and lung disease in this model (23). A limitation of these studies is that they do not address the source of ROIs or the mechanisms by which they are generated.

NADPH oxidase is responsible for the generation of the oxidative burst in neutrophils. The NADPH oxidase complex comprises a unique cytochrome component consisting of gp91phox (phox, phagocyte oxidase) and p22phox embedded in membranes. The NADPH oxidase is an emergency response pathway that is activated within a minute of stimulation by a...
variety of agents, including bacteria, latex particles, opsonized zymosan, and certain mitogens. The cytoplasmic subunits p47\textsuperscript{phox}, p67\textsuperscript{phox}, and p40\textsuperscript{phox} and rac translocate to the membrane-bound cytochrome upon activation of the oxidase.

Chronic granulomatous disease (CGD) is an inherited disorder of the NADPH oxidase complex in which phagocytes are defective in generating superoxide anion and its metabolites, hydrogen peroxide, hydroxyl anion, and hypohalous acid (35). As a result of the defect in this key host defense pathway, CGD patients suffer from recurrent life-threatening bacterial and fungal infections (35). CGD is also characterized by abnormally exuberant inflammatory responses leading to granuloma formation such as granulomatous enteritis, genitourinary obstruction, poor wound healing, and dehiscence. Knockout mouse models of CGD closely model the host defense impairments in the human condition (1, 3, 13, 21, 31, 32). These mice are an excellent tool to study the role of NADPH oxidase in the pathogenesis of acid aspiration lung inflammation and injury.

We evaluated experimental acid aspiration in the p47\textsuperscript{phox}\textsuperscript{−/−} knockout mouse model of CGD to test specific hypotheses about the interaction of NADPH oxidase and acid aspiration-induced lung injury. Reduction of acid aspiration-induced inflammation and vascular injury (capillary leak) in CGD compared with wild-type mice would implicate the NADPH oxidase as a potentiator of inflammation and injury. We also considered an alternative scenario. Based on our previous studies and those of colleagues showing enhanced neutrophilic responses in CGD patients (8) and mice (31, 34) in response to sterile irritants, we hypothesized a role of NADPH oxidase in attenuating the injurious acute neutrophilic response to acid aspiration. Yet another potential scenario is enhanced neutrophilic inflammation and vascular injury (capillary leak) in CGD induced lung injury. Reduction of acid aspiration-induced inflammation and vascular injury (capillary leak) in CGD compared with wild-type mice, a finding that would implicate NADPH oxidase in attenuating the injurious acute neutrophilic response to acid aspiration. Furthermore, the later occurring lung injury (48 h post-aspiration) clearly demonstrated interactions supporting our hypothesis regarding a role for NADPH oxidase-derived oxidants in regulating downstream inflammatory responses. These findings demonstrate a protective role of NADPH oxidase in acid lung injury and point to more complex interactions between ROIs and the sustained pathogenesis of acid aspiration.

**Acid aspiration and hyperoxia.** After induction of halothane anesthesia, a tracheotomy was performed by midline incision and blunt dissection. An angiocatheter was inserted into the trachea under direct visualization. Mice were injured by intratracheal instillation of 3.6 ml/kg of aspirate solution consisting of either isotonic saline or saline + HCl (pH 1.25). After recovery from anesthesia, mice were either exposed to air (normoxia) or to 98% O\textsubscript{2} (hyperoxia) while breathing spontaneously. Mice were killed at 5, 24, or 48 h after intratracheal challenge.

**BAL.** At 5, 24, or 48 h postaspiration, mice were reanesthetized with 2% halothane in 100% O\textsubscript{2}, and a midline abdominal/thoracic incision was made to allow exsanguination through inferior vena cava transection. The inferior vena cava was then clamped just below the diaphragm, and the thoracic and pulmonary vasculature flushed with 5 ml of HBSS with Ca\textsuperscript{2+} and Mg\textsuperscript{2+} (Life Technologies, Grand Island, NY) introduced through the right ventricle. After exposure, a 22-gauge cannula was secured in the trachea with a suture. BAL was performed with five 1-ml aliquots of sterile normal saline, as described previously (20). Recovered lavage fluid was pooled for cell and cytokine analysis. The percent recovery of instilled lavage fluid was 82 ± 1.70% (mean ± SE).

**Assessment of lung injury.** Pulmonary vascular permeability was quantified by measuring the leakage of albumin into the alveolar space. Albumin levels in BAL were assessed by ELISA using a rabbit anti-mouse albumin antibody (a generous gift from Dr. Dan Remick, Dept. of Pathology, Univ. of Michigan, Ann Arbor, MI) with horseradish peroxidase-conjugated goat anti-rabbit IgG as the secondary detection antibody, as previously described (28).

**Cell count.** Cells recovered by BAL were pelleted by centrifugation at 1,500 g for 3 min, and the total number of leukocytes were counted using a Multisizer 3 Coulter counter (Beckman Coulter, Fullerton, CA). The cell differential was determined by cytoplasm and Diff-Quik (Baxter, Miami, FL) staining of the leukocyte preparations, as previously described (36).

**Cytokine analysis.** Quantification of the cytokines IL-1β, IL-10, and IFN-γ, and the chemokines KC and macrophage inflammatory protein (MIP)-2, from cell-free BAL supernatants was performed by ELISA with rat monoclonal capture antibodies and goat polyclonal detection antibodies, per the manufacturer’s instructions (R&D Systems, Minneapolis, MN). Monocyte chemoattractant protein (MCP)-1 levels were quantitated by ELISA with a kit using hamster detection and capture antibodies (Pharmingen, San Diego, CA). TNF-α bioactivity was assessed in the BAL by a cytotoxicity assay using WEHI 164 subclone 13 cells derived from mouse fibrosarcoma (a generous gift from Dr. Steven Kunkel, Dept. of Pathology, Univ. of Michigan) as previously described (5).

**Statistics.** Descriptive statistics are expressed as means ± SE. The primary analysis consisted of fitting a standard 2 × 2 factorial ANOVA model with factors for injury (acid, saline), genotype (wild-type, p47\textsuperscript{phox}\textsuperscript{−/−}), and hyperoxia (no, yes) at each of three time points (5, 24, 48 h). Thus the following eight conditions were analyzed: 1) p47\textsuperscript{phox}\textsuperscript{−/−}/HCl/normoxia; 2) p47\textsuperscript{phox}\textsuperscript{−/−}/HCl/hyperoxia; 3) p47\textsuperscript{phox}\textsuperscript{−/−}/saline/normoxia; 4) p47\textsuperscript{phox}\textsuperscript{−/−}/saline/hyperoxia; 5) wild-type/HCl/normoxia; 6) wild-type/HCl/hyperoxia; 7) wild-type/saline/normoxia; and 8) wild-type/saline/hyperoxia. Thus, three two-way interactions (HCl-genotype, genotype-hyperoxia, and HCl-hyperoxia) and one three-way interaction (HCl-genotype-hyperoxia) were analyzed.

All statistical analyses were performed by one author (Hutson). Log\textsubscript{10} transformed values were used for the albumin, cell count, and cytokine data within the ANOVA model. Each factor in the model was characterized as a main effect, and the interactions within the regression model are given as products of indicator variables corresponding to the main effects.
The analysis strategy consisted of first examining the four total interactions via a stepwise regression model constrained to force the main effects into the model, i.e., only the interaction terms would possibly be eliminated via this approach. Once the stepwise procedures were carried out for each outcome variable, standard testing at the family-wise level (two-sided) was carried forth. Mean estimates of each of the effects (main and interactions) from the final model fit, along with the corresponding standard errors, are presented graphically as bar charts with standard errors attached.

RESULTS

Phagocyte NADPH oxidase-deficient (p47phox−/−) mice had increased lung injury compared with wild-type mice following acid aspiration. The amount of acidified saline (pH 1.25) administered intratracheally was specifically adjusted to produce a maximal nonlethal (<10%) lung injury in the C57BL/6 wild-type mice. There were no differences detected in mortality between wild-type and p47phox−/− mice. Total survival following experimental acid aspiration in the wild-type and p47phox−/− mice following acid aspiration was 100% and 95.7%, respectively (P = not significant).

The integrity of the alveolar capillary wall was assessed by the leakage of albumin into the air spaces utilizing a 2 × 2 × 2 ANOVA at each time point (5, 24, 48 h) following experimental aspiration with respect to the injury (saline or acid) and genotype (wild-type or p47phox−/−) (Fig. 1). Figure 1A displays the means of the raw data from each of the injury/genotype groups at each of the three time points. Figure 1B displays the mean estimates of the injury, genotype, and their interaction effects resulting from the ANOVA regression analysis. The values for these effects, along with the value of the “intercept,” can be used to predict the value of the log10 transformation of the raw data for any of the experimental groups by solving the following equation for each time point: y = A + (B × b) + (C × c) + (D × b × c), with y = log10([albumin]), A = intercept, B = injury effect, b = injury indicating variable (0 = saline, 1 = acid), C = genotype effect, c = genotype indicating variable (0 = wild-type, 1 = p47phox−/−), and D = injury/genotype interaction effect (values displayed in Fig. 1B are the values of the effects B, C, and D). The intercept values for the different time point models are: 1.86 ± 0.05 for 5 h, 1.64 ± 0.08 for 24 h, and 1.40 ± 0.10 for 48 h. For example, the prediction for saline-treated p47phox−/− mice at 48 h would be: log10([albumin])@ 48 h = A + (B × 0) + (C × 1) + (D × 0 × 1) = A + C = 1.40 + (0.93 × 0) + (−0.72 × 1) + (0.94 × 0 × 1) = 1.40 + (−0.72) = 0.68

The value 0.68 is the value displayed in Fig. 1C for saline-treated p47phox−/− mice at 48 h (derived by effects estimates). Since no acid is present, both the injury and interaction terms drop out of the equation, and the only effect is that of the p47phox−/− genotype. The hyperoxia effects, along with its interactions with the other factors, have been omitted for clarity but were included as part of the 2 × 2 × 2 ANOVA...
regression analysis. The effects of hyperoxia in our injury model are presented in Fig. 5.

Figure 1C displays values for the log_{10} transformed means of the raw values of the BAL [albumin] from the animals in each injury/genotype/time point group and indicates that they are comparable to the corresponding predicted values derived from the effects estimates resulting from the ANOVA regression analysis. This analysis methodology allows for a clear display of effects of specific factors and their interactions and takes advantage of utilizing all of the data for comparisons, thereby enhancing its power to discern differences.

Both acid and the p47phox−/− genotype demonstrated main effects to increase BAL albumin levels at 5 h (407.8 ± 94.5 µg/ml and 596.2 ± 75.1 µg/ml for wild-type and p47phox−/−, respectively, \( P < 0.05 \)) and at 24 h (304.6 ± 127.6 µg/ml and 670.8 ± 133.3 µg/ml, wild-type and p47phox−/−, respectively, \( P < 0.0001 \)) postaspiration (Fig. 1A and B). At 48 h following acid aspiration, the picture was more complex. The main effect of acid was to increase BAL albumin, but the main effect of the p47phox−/− genotype was to decrease albumin levels, suggesting protection to the alveolar capillary wall. However, analysis also indicated an interaction between the acid injury and p47phox−/− genotype that resulted in an increase in BAL albumin levels (Fig. 1B). The net result was a lack of an observable difference in BAL albumin concentrations (Fig. 1A) between wild-type and p47phox−/− mice (480.6 ± 95.7 µg/ml compared with 384.6 ± 95.7 µg/ml, respectively).

**Effect of the p47phox−/− genotype on leukocyte infiltration into the lung.** With the use of the \( 2 \times 2 \times 2 \) ANOVA analysis, the main effects of acid and the p47phox−/− genotype were to increase neutrophil counts at 5 h (2.4 ± 2.5 \times 10^5 \) compared with 9.6 ± 1.7 \times 10^5 \) cells, respectively, \( P < 0.001 \)), at 24 h (8.5 ± 5.2 \times 10^4 \) compared with 2.0 ± 0.3 \times 10^5 \) cells, respectively, \( P < 0.005 \), and at 48 h (2.0 ± 0.8 \times 10^5 \) compared with 7.9 ± 0.8 \times 10^5 \) h, respectively, \( P < 0.0001 \)) in the BAL following experimental acid aspiration (Fig. 2A). There were no interactions between the p47phox−/− genotype and acid injury at 5 and 24 h following aspiration. However, there was a negative effect on BAL neutrophil numbers due to the interaction (\( P = 0.001 \)) between the genotype and acid injury at 48 h (Fig. 2B).

Macrophage recovery from BAL was, in general, similar in the wild-type and p47phox−/− mice at 5 and 24 h after saline or acid challenge (Fig. 3). However, a small main effect of the p47phox−/− genotype to decrease the number of macrophages in the BAL (1.9 ± 0.4 \times 10^5 \) compared with 3.1 ± 0.6 \times 10^5 \), \( P < 0.005 \)) could be detected 5 h postaspiration. No difference between the two genotypes could be detected at 24 h following aspiration. At 48 h post-acid or -saline aspiration, the main effect of the p47phox−/− genotype was to decrease the number of macrophages (Fig. 3) in the BAL (\( P < 0.0001 \)). Thus, in wild-type mice, both the saline and acid aspiration inflammatory injury evolved from an acute neutrophilic response to an increasing monocytic response by 48 h following aspiration. In the p47phox−/− animals, the neutrophilic response escalated dramatically at 48 h while the monocytic response was attenuated.

**Effect of the p47phox−/− genotype on lung inflammatory regulator production.** To examine possible mechanisms that may be responsible for the observed differences in the loss of integrity of the alveolar capillary wall and pulmonary leukocyte infiltration between the wild-type and p47phox−/− mice, we examined several peptide regulators of neutrophil and macrophage recruitment and activation into the lung. Changes in cytokine levels were assessed utilizing a \( 2 \times 2 \times 2 \) ANOVA at each of three time points (5, 24, 48 h) following experimental aspiration with respect to the injury (saline or acid) and genotype (wild-type or p47phox−/−).

Acid demonstrated a main effect of increasing BAL IL-1β, MIP-2, MCP-1, and IL-10 levels at 5 and 24 h postaspiration (Fig. 4, B, C, D, F). TNF-α was also increased at 24 h following acid aspiration (Fig. 4A). At 48 h, the main effect of acid was to increase BAL MCP-1 and decrease BAL MIP-2.
mice to increased ambient oxygen concentrations (hyperoxia) following acid aspiration. We considered the hypothesis that by increasing the level of ambient ROIs, hyperoxia may attenuate the excessive inflammation and injury observed in CGD mice following acid injury. Hyperoxia may also increase the level of NADPH oxidase-derived ROIs from lung endothelial cells (2, 30). The effect of hyperoxia is to increase ambient levels of ROIs, whereas activation of the NADPH oxidase in phagocytes results in a rapid burst of superoxide anion generation and downstream ROI metabolites. We hypothesized that hyperoxia would exacerbate acid aspiration-induced lung injury in both wild-type and CGD mice.

The main effect of hyperoxia (Fig. 5A) was to further increase albumin levels in the BAL in both wild-type and p47phox−/− mice at 5 h (477.4 ± 94.5 μg/ml and 643.9 ± 75.1 μg/ml, respectively, P < 0.05) and 24 h (210.4 ± 118.2 μg/ml and 640.5 ± 133.3 μg/ml, respectively, P < 0.05). There was no specific interaction between hyperoxia and the p47phox−/− genotype on BAL albumin levels. There was no increase in mortality in any of the groups of animals following hyperoxia.

**Effects of hyperoxia on p47phox−/− genotype-induced leukocyte infiltration into the lung.** The main effect of hyperoxia was to decrease neutrophil counts in the BAL of mice 5 h (P < 0.001) following acid or saline aspiration (Fig. 5B). In the presence of acid injury, an interaction was also detected between the p47phox−/− genotype and increased ambient O2 resulting in a further decrease in the BAL neutrophil counts (P < 0.05). The net decrease in BAL neutrophils was from 9.6 ± 1.8 × 10^4 following acid aspiration in p47phox−/− mice exposed to air compared with 4.7 ± 1.8 × 10^4 cells in animals similarly injured and exposed to hyperoxic atmosphere. Hyperoxia produced no main effects on neutrophil counts at 24 and 48 h postaspiration in either the wild-type or p47phox−/− mice. Additionally, there were no specific interactions between hyperoxia, acid aspiration, and the p47phox−/− genotype.

**Effects of hyperoxia on p47phox−/− genotype-induced lung inflammatory regulator production in the BAL.** Hyperoxia demonstrated no main effect on BAL cytokine levels at the three time points (5, 24, 48 h) following experimental acid or saline aspiration. Additionally, there were no interactions between increased ambient O2, acid lung injury, and/or the p47phox−/− genotype on the BAL cytokine levels that we examined (data not shown).

**DISCUSSION**

We demonstrated that acute inflammatory lung injury secondary to acid aspiration is greater in p47phox−/− mice compared with wild-type animals. Specifically, we observed an increase in the accumulation of albumin in the airways of the p47phox−/− mice at 5 and 24 h following acid aspiration. p47phox−/− mice also developed a significantly greater alveolar neutrophilic leukocytosis compared with wild-type mice at all time points after acid injury, with the difference between genotypes being most marked at 48 h. These findings suggest that NADPH oxidase-derived oxidants (i.e., superoxide) play a role in the transition to a less injurious monocyctic response. Thus, direct or indirect products of activation of the NADPH oxidase complex play a role in downregulating the acute neutrophilic response, reducing capillary leak, and reducing levels of proinflammatory cytokines. These results demonstrate
a previously unrecognized protective role for NADPH oxidase in decreasing acid injury and point to a complex relationship between ROI production and lung inflammation and injury. p47\textsuperscript{phox}\textsuperscript{−/−} mice were backcrossed at least five generations in the C57BL/6 lineage; we acknowledge the possibility that background genetic factors unrelated to the targeted gene may have influenced the response to acid aspiration.

Although the number of neutrophils in the air spaces was still increased in p47\textsuperscript{phox}\textsuperscript{−/−} mice at 48 h following acid aspiration compared with wild-type, leakage of albumin across the alveolar capillary wall injury was similar at this time point. Analysis using a 2 × 2 × 2 ANOVA provides an explanation for these somewhat paradoxical findings. A direct effect of the p47\textsuperscript{phox}\textsuperscript{−/−} genotype that increases albumin levels at this time was detected. We predict that this is due to the increase in neutrophil infiltration in the p47\textsuperscript{phox}\textsuperscript{−/−} animals. However, an interaction between the acid injury and the p47\textsuperscript{phox}\textsuperscript{−/−} genotype that is responsible for decreasing albumin levels in the air spaces was also detected. The decrease in the number of macrophages in the lung in the NADPH oxidase-deficient mice at 48 h after aspiration is a possible cause for this observation. Thus, NADPH oxidase-derived ROIs may have a dual effect on acid lung injury: they may reduce lung injury by attenuating the acute neutrophilic response by increasing downstream responses that attenuate acute inflammation or promote neutrophil apoptosis but may also increase lung injury by direct insult to the alveolar epithelial and/or endothelial cells of the alveolar capillary wall.

We also quantitated several peptide regulators of neutrophil and macrophage recruitment and activation into the lung to examine possible mechanisms for the observed differences in capillary leak and pulmonary leukocyte infiltration. At 24 h
following acid aspiration, the proinflammatory cytokines TNF-α and IL-1β and the neutrophil chemoattractant MIP-2 were increased due to a main effect of the p47phox–/– genotype and/or an interaction between the p47phox–/– genotype and the acid lung injury. These findings can clearly account for the increased neutrophilic response and lung injury in the p47phox–/– mice. Additionally, IFN-γ, a cytokine associated with augmentation of the acute inflammatory cells, and the anti-inflammatory cytokine IL-10 were both increased at 24 h in p47phox–/– mice.

At 48 h following acid aspiration, MIP-2 levels remained elevated in the p47phox–/– mice, consistent with the large number of neutrophils in the airways of these animals. However, the mononuclear chemokine MCP-1 was also elevated in the airways at this time despite a much lower number of macrophages in the BAL of the p47phox–/– animals compared with the wild-type. We speculate that a decreased ability to transition from the acute neutrophilic response to a chronic inflammatory response in p47phox–/– mice may be responsible for this finding despite the presence of elevated levels of this monocyte chemokine. Consistent with this hypothesis, BAL levels of IL-10, an anti-inflammatory cytokine that promotes transition to a more mononuclear response, were decreased in p47phox–/– mice compared with wild-type at 48 h following acid aspiration.

To examine the role of NADPH oxidase-independent ROI in acid aspiration-induced lung injury, we exposed p47phox–/– and wild-type mice to increased ambient oxygen levels following acid aspiration. Although this is not directly analogous to the intense generation of superoxide by NADPH oxidase produced following activation of neutrophils (and monocytes/macrophages), there is an increase in superoxide production through alternative pathways (i.e., by mitochondria). The main effect of hyperoxia was to decrease neutrophil counts in the BAL of mice at 5 h and macrophage counts in the BAL at 5 and 48 h post-acid aspiration. More importantly, an interaction between the p47phox–/– genotype and increased ambient O2 resulted in a further decrease of the BAL neutrophils, but not macrophages, at 5 h post-acid aspiration. These findings suggest an important role for superoxide and/or downstream oxidants (i.e., H2O2 or the hydroxyl radical) in regulating neutrophil numbers. This could occur directly by enhancing apoptosis or indirectly by affecting agents that are responsible for neutrophil recruitment. We observed no changes in any of the cytokines examined by exposing the injured animals to increased ambient oxygen levels. Interestingly, lung injury as assessed by leakage of albumin into the air spaces was increased at 5 and 24 h following acid aspiration when either p47phox–/– or wild-type mice were exposed to hyperoxia. This may be secondary to the interaction of acid inflammatory injury and hyperoxia-induced oxidant generation by cells of the alveolar capillary wall despite the decrease in neutrophil numbers. We have demonstrated such a finding previously in several animal species exposed to increased ambient oxygen following acid aspiration (16, 25–27).

Evidence of abnormal neutrophil inflammatory responses exists in both patients with CGD and in genetically engineered animal models. In an experimental skin window model, neutrophil exudate was increased in male CGD patients compared with normal volunteers (8). X-linked CGD mice generate enhanced inflammatory responses to intratracheal challenge with killed Aspergillus fumigatus hyphae (22). Both p47phox–/– and X-linked NADPH oxidase mice develop granulomatous synovitis and increased connective tissue destruction compared with wild-type in experimental arthritis models (39). NADPH oxidase-deficient mice also generated greater thioglycollate-elicited peritoneal neutrophil leukocytosis than wild-type mice (34); this enhanced response correlated with impaired clearance of leukotriene B4, a potent neutrophil chemoattractant. These studies and ours indicate that excessive inflammation in CGD is not solely the result of unresolved infection but results from an intrinsic defect in the control of inflammation.
NADPH oxidase-deficient mice have been evaluated in a number of models of acute lung injury. The NADPH oxidase is the principal source of ROI production after intratracheal endotoxin-mediated acute lung injury (33). In both the p47phox−/− and X-linked CGD mice, intraperitoneal challenge with Escherichia coli increased neutrophil sequestration in lungs and increased MIP-2 concentration in lung tissue compared with wild-type animals (9). Capillary leak was similar in p47phox−/−, X-linked CGD, and wild-type mice despite the increased neutrophil accumulation in NADPH oxidase-deficient animals (9). These studies and ours point to a key role of the NADPH oxidase in attenuating the acute neutrophilic inflammatory response in lungs in different pathological models.

The NADPH oxidase (Nox) family is found in phagocyte and non-phagocytic leukocyte populations as well as in several non-hematopoietic cells and mediate both host defense and diverse physiological functions (10). One limitation of our studies is the lack of knowledge about the NADPH oxidase isoform(s) that attenuates acid aspiration-induced lung injury and inflammation. In addition, the levels of ROI production in CGD mice are not nil. Endothelial-derived xanthine oxidase (XO) is an important potential source of superoxide and hydroxyl anion production. Kubo et al. (18) showed that the XO inhibitor allopurinol was protective against complement-dependent cobra venom factor-induced lung injury in both wild-type and in X-CGD mice, suggesting a role for endothelial XO in the pathogenesis of lung injury. In addition, catalase was only protective in wild-type mice, whereas inhibition of nitric oxide was only protective in X-CGD mice. These data indicate that cobra venom toxin-induced lung injury is mediated by alternative reactive oxidant and nitrogen intermediate generating pathways in wild-type and CGD mice (18).

Our results demonstrate a protective role of NADPH-derived products or coupled signaling in acid lung injury. This anti-inflammatory action appears to be mechanistically involved with downregulation of the neutrophilic response and reduction of the proinflammatory cytokines. Consistent with this notion, Lekstrom-Himes et al. (19) showed that regulators downstream of the activation of NADPH oxidase negatively regulate IL-8 mRNA in normal human neutrophils; their absence in CGD cells resulted in prolonged IL-8 mRNA elevation and enhanced IL-8 levels that may mediate enhanced recruitment of neutrophil to inflammatory sites in vivo. The protective effect of NADPH oxidase may be linked to ROI production or indirectly to signaling events coupled to NADPH oxidase assembly following activation. NADPH oxidase-derived ROIs may attenuate neutrophil accumulation following injury by priming neutrophil apoptosis (4). In addition, activation of the NADPH oxidase in neutrophils leads to the release of cationic granule proteins, including elastase and cathepsin G, which are likely to be the principal effectors of host defense against microbes (32, 38); the role of these proteases in modulating inflammation following NADPH oxidase activation merits investigation. Dissecting the mechanisms by which NADPH oxidase attenuates acid lung injury will be relevant to developing novel therapeutic strategies to decrease the morbidity and mortality associated with the aspiration of acidic gastric contents.

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