The Chitinase-like Proteins Breast Regression Protein-39 and YKL-40 Regulate Hyperoxia-induced Acute Lung Injury

Myung Hyun Sohn[1], Min-Jong Kang[2], Hiroshi Matsuura[2], Vineet Bhandari[3], Ning-Yuan Chen[2], Chun Geun Lee[2], and Jack A. Elias[2]

[1]Department of Pediatrics, and Institute of Allergy, Severance Biomedical Science Institute, BK21 Project for Medical Science, Yonsei University College of Medicine, Seoul, Korea; [2]Section of Pulmonary and Critical Care Medicine, Department of Internal Medicine, and [3]Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut

***Rationale*: Prolonged exposure to 100% O_2 causes hyperoxic acute lung injury (HALI), characterized by alveolar epithelial cell injury and death. We previously demonstrated that the murine chitinase-like protein, breast regression protein (BRP)–39 and its human homolog, YKL-40, inhibit cellular apoptosis. However, the regulation and roles of these molecules in hyperoxia have not been addressed.**

***Objectives*: We hypothesized that BRP-39 and YKL-40 (also called chitinase-3–like 1) play important roles in the pathogenesis of HALI.**

***Methods*: We characterized the regulation of BRP-39 during HALI and the responses induced by hyperoxia in wild-type mice, BRP-39–null (−/−) mice, and BRP-39$^{-/-}$ mice in which YKL-40 was overexpressed in respiratory epithelium. We also compared the levels of tracheal aspirate YKL-40 in premature newborns with respiratory failure.**

***Measurements and Main Results*: These studies demonstrate that hyperoxia inhibits BRP-39 *in vivo* in the murine lung and *in vitro* in epithelial cells. They also demonstrate that BRP-39$^{-/-}$ mice have exaggerated permeability, protein leak, oxidation, inflammatory, chemokine, and epithelial apoptosis responses, and experience premature death in 100% O_2. Lastly, they demonstrate that YKL-40 ameliorates HALI, prolongs survival in 100% O_2, and rescues the exaggerated injury response in BRP-39$^{-/-}$ animals. In accord with these findings, the levels of tracheal aspirate YKL-40 were lower in premature infants treated with hyperoxia for respiratory failure who subsequently experienced bronchopulmonary dysplasia or death compared with those that did not experience these complications.**

***Conclusions*: These studies demonstrate that hyperoxia inhibits BRP-39/YKL-40, and that BRP-39 and YKL-40 are critical regulators of oxidant injury, inflammation, and epithelial apoptosis in the murine and human lung.**

Keywords: BRP-39; YKL-40; hyperoxygen; BPD; HALI

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Although supplemental oxygen is a commonly prescribed therapy, prolonged exposure to 100% oxygen is known to cause hyperoxic acute lung injury (HALI). However, the mechanisms that control HALI are inadequately understood.

What This Study Adds to the Field

These studies demonstrate that the chitinase-like proteins, breast regression protein–39 and YKL-40, are important regulators of HALI in the murine and human lung.

Supplemental oxygen is commonly administered to patients with significant pulmonary and cardiac disease to increase the delivery of oxygen to peripheral tissues. However, very high concentrations of oxygen (fractional inspired concentrations $\geq$ 50%) for prolonged periods cause hyperoxic acute lung injury (HALI). This response is characterized by endothelial and epithelial injury and enhanced alveolar capillary protein leak (1–6). Studies of this response have led to the free radical theory that suggests that, in 100% O_2, lung cells poison themselves by producing an excess of reactive oxygen species (7–9). Recent studies from our laboratory and others have added to this pathogenic paradigm by demonstrating that reactive oxygen species mediate their effects, in part, by inducing an endothelial and epithelial cell death response with features of apoptosis and necrosis (6, 7, 10–14). In spite of the obvious importance of pathways that regulate these toxic responses, and the well documented variations in the ability of inbred mice to withstand exposure to 100% O_2 (6, 7, 10, 15), the endogenous mechanisms that contribute to the control of these responses have not been adequately defined.

The evolutionarily conserved 18-glycosyl-hydrolase family contains true chitinases and molecules that lack chitinase activity (16–19). Much of the research in this area has focused on chitinases such as acidic mammalian chitinase, which plays a critical role in the life cycle of parasites and the pathogenesis of T helper cell (Th) 2 and antiparasite responses (18–20). However, the majority of the 18-glycosyl-hydrolase family members are chitinase-like proteins (CLPs), which, as a result of mutations in their highly conserved enzyme sites, do not contain chitinase activity. Breast regression protein (BRP)–39 and its human homolog, YKL-40 (also called chitinase-3–like 1 and human cartilage glycoprotein–39) (21–23) are the prototypes of these enzymatically deficient CLPs. They are produced by a variety of cells, including neutrophils, monocytes, and macrophages (18, 21, 24). In addition, increased levels of YKL-40 protein and/or mRNA have been noted in patients with a wide spectrum of pathologies, including bacterial infections, rheumatoid arthritis, osteoarthritis, giant cell arteritis, sarcoidosis, scleroderma, diabetes, atherosclerosis, inflammatory bowel disease, and a variety of malignancies (18, 21, 24–29). In many of these disorders, the levels of YKL-40 reflect the activity and natural history of the disease (17, 27–29). This is nicely illustrated

(*Received in original form December 1, 2009; accepted in final form June 16, 2010*)

Supported by National Institutes of Health grants RO1-HL-081639 and RO1-HL-064242, RO1-HL-093017 (J.A.E.), and RO1-HL-084225 (C.G.L.), and by grant A090399 from Korea Healthcare Technology R&D Project, Ministry for Health, Welfare, and Family Affairs, Republic of Korea, Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0001664), and faculty research grant 6-2009-0175 from Yonsei University College of Medicine (M.H.S.).

Correspondence and requests for reprints should be addressed to Jack A. Elias, M.D., Department of Internal Medicine, Yale University School of Medicine Boardman 110, 333 Cedar St., P.O. Box 208,057, New Haven, CT 06520-8057. E-mail: jack.elias@yale.edu

Am J Respir Crit Care Med Vol 182. pp 918–928, 2010
Originally Published in Press as DOI: 10.1164/rccm.200912-1793OC on June 17, 2010
Internet address: www.atsjournals.org

by studies from our laboratory and others, which have demonstrated that elevated levels of YKL-40 are seen in patients with asthma, which correlate with the levels of lung tissue YKL-40 and disease severity (17). These studies also highlighted polymorphisms in the *chitinase-3–like 1* gene that correlated with the levels of circulating YKL-40, the presence of asthma, and compromised lung function (30). Surprisingly, although oxidant-induced injuries are believed to contribute to the pathogenesis of many of these responses, the relationship(s) between BRP-39/YKL-40 and oxidant injury has not been investigated.

We hypothesized that BRP-39/YKL-40 plays a critical role in the pathogenesis of HALI. To test this hypothesis, we characterized the hyperoxia-induced responses in wild-type (WT) mice, mice with null mutations of BRP-39 (BRP-39$^{-/-}$), mice that overexpress YKL-40 in a lung-specific fashion, and mice that lack BRP-39 and produce transgenic YKL-40 only in the respiratory epithelium. To assess the applicability of our murine findings to humans, we also evaluated the levels of YKL-40 in tracheal aspirates from premature babies receiving oxygen supplementation for respiratory failure. These studies demonstrate that hyperoxia is a potent inhibitor of BRP-39 expression and production, and that BRP-39 and YKL-40 inhibit the toxic effects of hyperoxia. In accord with these findings, it was also demonstrated that the levels of tracheal YKL-40 are lower in premature babies that develop bronchopulmonary dysplasia (BPD) or die compared with those without these complications.

METHODS

Genetically Modified Mice

BRP-39$^{-/-}$ mice were generated and used as previously described (31). The mice were generated on a mixed 129/C57BL/6 background and subsequently bred for more than 10 generations onto a C57BL/6 background. Transgenic mice in which human YKL-40 was tightly and inducibly overexpressed (CC10-rtTA-tTS-YKL-40) in a lung-specific manner were generated with constructs and approaches that have been previously described by our laboratory (31). Mice that lacked BRP-39 and produced YKL-40 only in pulmonary epithelial cells (CC10-rtTA-tTS-YKL-40/BRP-39$^{-/-}$) were generated by breeding the CC10-rtTA-tTS-YKL-40 and BRP-39$^{-/-}$ mice. Mice with caspase-3–null mutations were kindly provided by Dr. Flavell (Dept. of Immunobiology, Yale University School of Medicine). Animal protocols were approved by the Yale University Institutional Animal Care and Use Committee, the guidelines of which were followed for all experiments.

Oxygen Exposure

Mice (4–6 wk old) were placed in cages in an airtight Plexiglas chamber (55 × 40 × 50 cm), as described previously (6, 10, 32). Throughout the experiment, they were given free access to food and water. Oxygen levels were constantly monitored by an oxygen sensor, which was connected to a relay switch incorporated into the oxygen supply circuit. The inside of the chamber was kept at atmospheric pressure, and mice were exposed to a 12-hour light–dark cycle.

Bronchoalveolar Lavage

Mice were killed, the trachea was isolated by blunt dissection, and a small-caliber tube was inserted into the airway and secured. Two volumes of 1 ml of phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin were instilled, gently aspirated, pooled, and processed as previously described (5, 6, 33).

Immunohistochemistry

Immunohistochemistry (IHC) was undertaken with a polyclonal anti–BRP-39, as previously described by our laboratory (34). Antibodies against surfactant apoprotein C (Millipore, Billerica, MA) and CC10 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) were used to identify alveolar type II cells and airway epithelial cells, respectively. The specificity of the staining was evaluated in experiments in which the primary antiserum was not used and experiments that compared tissue samples from WT and BRP-39$^{-/-}$ animals.

Histological Analysis

The lungs were removed *en bloc*, inflated at 25-cm pressure with PBS containing 0.5% low melting point agarose, fixed in Streck solution (Streck, Omaha, NE), embedded in paraffin, sectioned, and stained. Hematoxylin and eosin and Periodic acid-Schiff (PAS) stains were performed in the Research Histology Laboratory of the Department of Pathology at the Yale School of Medicine.

Immunoblot Analysis

Bronchoalveolar lavage (BAL; 50 μg) fluids and/or lung lysates were subjected to immunoblot analysis with antibodies against inhibitor of *caspase*-activated deoxyribonuclease (Chemicon International, Billerica, MA), caspase-3 (Cell Signaling Technology, Danvers, MA) or β-tubulin (Santa Cruz Biotechnology, Inc.), and the polyclonal rabbit antiserum against BRP-39, as noted above. These samples were fractionated by polyacrylamide gel electrophoresis, transferred to membranes, and evaluated as described previously by our laboratory (35).

Quantification of BRP-39, CXCL-1, and CCL-2

The levels of BRP-39 in BAL or lung lysates were evaluated by ELISA with an anti–BRP-39 rabbit polyclonal IgG for capture and biotinylated anti–BRP-39, followed by horseradish peroxidase–labeled streptavidin (GE Healthcare, Piscataway, NJ) for detection. This assay detects as little as 50 pg/ml recombinant BRP-39. The levels of BAL fluid CXCL-1 and CCL-2 were measured by ELISA with commercial kits (R&D Systems, Minneapolis, MN), as directed by the manufacturer.

mRNA Analysis

mRNA levels were assessed by real-time reverse transcriptase–polymerase chain reaction, as previously described by our laboratory (36, 37). The sequences for the primers that were used were obtained from Primer Bank online (http://pga.mgh.harvard.edu/primerbank).

Terminal Deoxynucleotidyl Transferase–Mediated dUTP Nick End Labeling Staining

DNA fragmentation and cell death were evaluated with terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assays, as previously described by our laboratory (37).

8-Hydroxy-2′Deoxyguanosine Staining

Immunohistochemical detection 8-hydroxy-2′deoxyguanosine (8-OHdG) was done using paraffin-embedded sections. The primary antibody used was mouse anti−8-OHdG (Santa Cruz Biotechnology, Inc.). The DAKO ARK system (Carpinteria, CA) was used as per the manufacturer's instructions. Negative controls consisted of isotype-matched control or rabbit serum.

Measurement of Tracheal Aspirate YKL-40

The tracheal aspirate samples were collected from neonates admitted to the Yale–New Haven Children's Hospital Newborn Special Care Unit. All infants had respiratory distress syndrome (RDS), which required them to be intubated, and administered at least one dose of natural surfactant and ventilated for treatment, per standard nursery guidelines. BPD was defined as the need for oxygen with characteristic radiographic changes at 36-weeks postmenstrual age. Samples were stored at −70°C until assayed with ELISA. All human work was approved by the Human Investigational Committee at Yale University School of Medicine.

In Vitro Oxygen Exposure

Human bronchial epithelial cell line BEAS-2B cells (ATCC, Rockville, MD) were incubated in complete bronchial epithelial basal medium (Lonza, Walkersville, MD). They were placed in an airtight modular incubator chamber (Billups-Rothenberg, Del Mar, CA), which had been flushed continuously with 95% O_2/5% CO_2 until the oxygen level inside the chamber reached approximately 95%. The incubator chamber was then placed in a tissue culture incubator at 37°C, the O_2 inside the chamber was replaced every 24 hours, and the cells were harvested

at the desired time points (24–72 h). The responses in these cells were compared with events in cells incubated in 5% CO_2 and air. In select experiments, the cells were incubated with N-acetyl-L-cysteine (Sigma, St. Louis, MO), which was added at a dose of 10 mM 1 hour before oxygen exposure, or its vehicle control.

Statistical Analysis

All data were initially checked for normal/parametric distribution (Kolmogorov-Smirnov test). If parametric distribution was found, analysis of variance was applied to screen for differences among at least three groups. To compare two individual groups, Student's *t* test was applied. If nonparametric distribution was found, the Kruskal-Wallis test was applied to screen for differences among at least three groups, followed by the Mann-Whitney *U* test (Wilcoxon rank-sum test) to compare two individual groups. The survival rate was analyzed by the Kaplan-Meier method. Statistical significance was defined at a *P* value less than 0.05. All statistical analyses were performed with SPSS version 13.0 (SPSS Inc., Chicago, IL).

RESULTS

Hyperoxia Inhibition of BRP-39 *In Vivo*

To begin to address our hypothesis, we compared the expression of BRP-39 in lungs from WT mice exposed to room air (RA) and 100% O_2. BRP-39 mRNA was readily appreciated in lungs (Figure 1A), and BRP-39 protein was abundant in BAL fluids from mice breathing RA (Figures 1B and 1C). IHC demonstrated that this BRP-39 was most readily appreciated in pulmonary macrophages and alveolar type 2 cells from these mice (Figure 1D and data not shown). In contrast, hyperoxia caused a significant decrease in BRP-39 mRNA and lung, macrophage, and alveolar type 2 cell BRP-39 protein accumulation (Figures 1A–1D). This inhibition was seen after as little as 24 hours, and was most prominent after 72 hours of exposure to 100% O_2 (Figure 1E). It was only partially BRP-39 specific, because similar inhibition of chitotriosidase expression was noted (data not shown). These studies demonstrate that hyperoxia inhibits macrophage and epithelial cell BRP-39 expression and accumulation in the murine lung.

Hyperoxia Inhibition of BRP-39 *In Vitro*

To further define the mechanism by which hyperoxia inhibits BRP-39/YKL-40, we quantitated the expression of YKL-40 in BEAS-2B cells in RA or 95% oxygen, and evaluated the effects of antioxidants on these regulatory events. In keeping with our *in vivo* findings, hyperoxia inhibited YKL-40 expression in a time-dependent manner (Figure 1F). Importantly, pretreatment with the antioxidant, N-acetyl-L-cysteine, abrogated the YKL-40–inhibiting effects of hyperoxia (Figure 1G). These studies demonstrate that hyperoxia inhibits epithelial cell YKL-40 expression via an oxidant-dependent mechanism.

BRP-39 Inhibition of HALI

We next compared the effects of 100% O_2 in WT and BRP-39$^{-/-}$ mice. As previously described by our laboratory and others (6, 7, 10–14), exposure to 100% O_2 caused ALI, with alveolar capillary permeability alterations characterized by increased

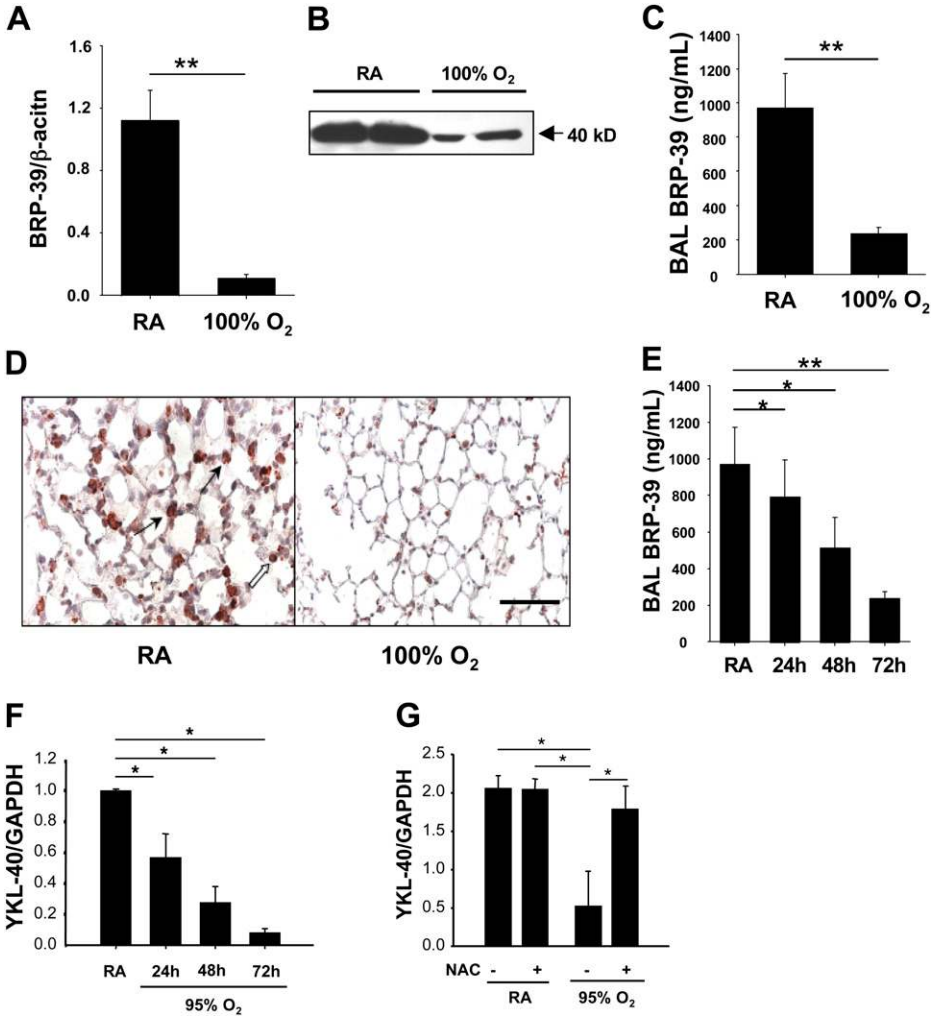

Figure 1. Hyperoxia regulation of breast regression protein (BRP)–39. Mice were exposed to room air (RA) or 100% O_2 for up to 72 hours (*A–E*). The levels of BRP-39 mRNA were evaluated by real-time reverse transcriptase–polymerase chain reaction (RT-PCR) (*A*). BRP-39 protein accumulation was assessed via Western analysis (*B*) and ELISA (*C* and *E*) with bronchoalveolar lavage fluid and lung lysates, respectively. Immunohistochemistry was used to localize the BRP-39 (*D*; *closed arrows*, alveolar type II cells; *open arrow*, macrophage). BEAS-2B cells were exposed to 95% O_2 in the presence or absence of N-acetyl-L-cysteine (NAC). Real-time RT-PCR was used to evaluate the effects of this exposure on YKL-40 expression (*F*) and the effects of NAC in this setting (*G*). The values in *A*, *C*, and *E* represent the mean (±SEM) of evaluations in a minimum of five animals. The values in *F* and *G* represent the mean (±SEM) of triplicate experiments. (*B*) Representative of two separate experiments. (*D*) Representative of four similar evaluations. *Scale bar* = 50 μm. $^*P < 0.05$, $^{**}P < 0.01$.

levels of BAL protein. This alveolar capillary protein leak was seen after as little as 24 hours, and progressed over the first 72 hours of 100% O_2 exposure (Figures 2A and 2B). With continued exposure, these lesions progressed, causing WT mice to begin to expire after 4 days of 100% O_2 exposure (Figures 2C and 2D). Interestingly, BRP-39$^{-/-}$ mice had an exaggerated response to hyperoxia. This manifested as enhanced protein leak (Figure 2B) and premature death after 100% O_2 exposure (Figures 2C and 2D). These responses were not strain specific, because augmented HALI and accelerated death were seen in BRP-39$^{-/-}$ mice on C57BL/6 and Balb/c backgrounds (Figures 2C and 2D and data not shown). Thus, BRP-39 is an important inhibitor of HALI.

BRP-39 Inhibition of Hyperoxia-induced Inflammation and Cytokine Production

We also compared the BAL and tissue inflammatory responses in WT and BRP-39$^{-/-}$ mice breathing RA or 100% O_2. In WT mice, hyperoxia increased BAL total cell, macrophage, and neutrophil recovery, and induced a neutrophil- and macrophage-rich tissue inflammatory response (Figures 3A–3C). In accord with their chemotactic properties for neutrophils and macrophages, these alterations were associated with significant increases in the levels of keratinocyte chemoattractant (KC)/CXCL1 and monocyte chemoattractant protein (MCP)-1/CCL-2, respectively (Figures 3D and 3E). Importantly, BRP-39 played an important role in these alterations, because BAL total cell, macrophage and neutrophil recovery, tissue inflammation, and KC/CXCL1 and MCP-1/CCL-2 production were enhanced in BRP-39$^{-/-}$ mice breathing 100% O_2 (Figures 3A–3E). These studies demonstrate that BRP-39 also inhibits hyperoxia-induced tissue inflammation and chemokine production.

BRP-39 Inhibition of Hyperoxia-induced Oxidant Injury and Apoptosis

Because oxidant-induced DNA injury and cell death play important roles in the pathogenesis of HALI (6, 7), the roles of BRP-39 in these responses were evaluated. In accord with this concept, 100% O_2 caused oxidant injury that was readily apparent with 8-OHdG tissue staining (Figure 4A), and DNA injury and cell death that manifested as increased levels of tissue TUNEL staining and caspase-3 activation (Figures 4B–4D and data not shown). BRP-39 played a critical role in the pathogenesis of both responses, because oxidant-induced tissue injury (8-OHdG staining), TUNEL staining, and caspase-3 activation were all significantly increased in BRP-39$^{-/-}$ mice compared with WT mice (Figures 4A–4D and data not shown). Combined TUNEL and cell-specific IHC highlighted the alveolar and epithelial cell apoptosis in the BRP-39$^{-/-}$ mice in 100% O_2 (Figure 4D). Thus, BRP-39 is a critical mediator of hyperoxia-induced oxidant injury, DNA injury, and cell death.

Transgenic YKL-40 Inhibition of HALI

To further define the effector functions of BRP-39 and its human homolog, YKL-40, two approaches were used. In the first, we bred the CC10-rtTA-tTS-YKL-40 mice with the BRP-39$^{-/-}$ mice to generate CC10-rtTA-tTS-YKL-40/BRP-39$^{-/-}$ (BRP-39$^{-/-}$/YKL-40 Tg) mice that did not produce BRP-39, and only produced YKL-40 in the epithelium of the lung. This allowed us to define the effects of YKL-40 in hyperoxia in the absence of potentially confounding responses induced by endogenously produced BRP-39. Transgenic YKL-40 enhanced the survival of BRP-39$^{-/-}$ mice exposed to 100% oxygen (Figure 5A). It also gave us a clear window into the ability of YKL-40 to rescue the augmented hyperoxia-induced responses in BRP-39$^{-/-}$ animals. As noted previously here, 100% O_2 caused exaggerated HALI responses, with alveolar capillary permeability alterations, protein leak, tissue and BAL neutrophil- and macrophage-rich inflammation, KC/CXCL-1 and MCP-1/CCL2 production, a TUNEL-positive cell death response, and caspase-3 activation in BRP-39$^{-/-}$ mice (Figures 5B–5H). These augmented responses in BRP-39$^{-/-}$ mice were restored to levels comparable to those in WT animals by epithelial-targeted transgenic YKL-40 (Figures 5B–5H).

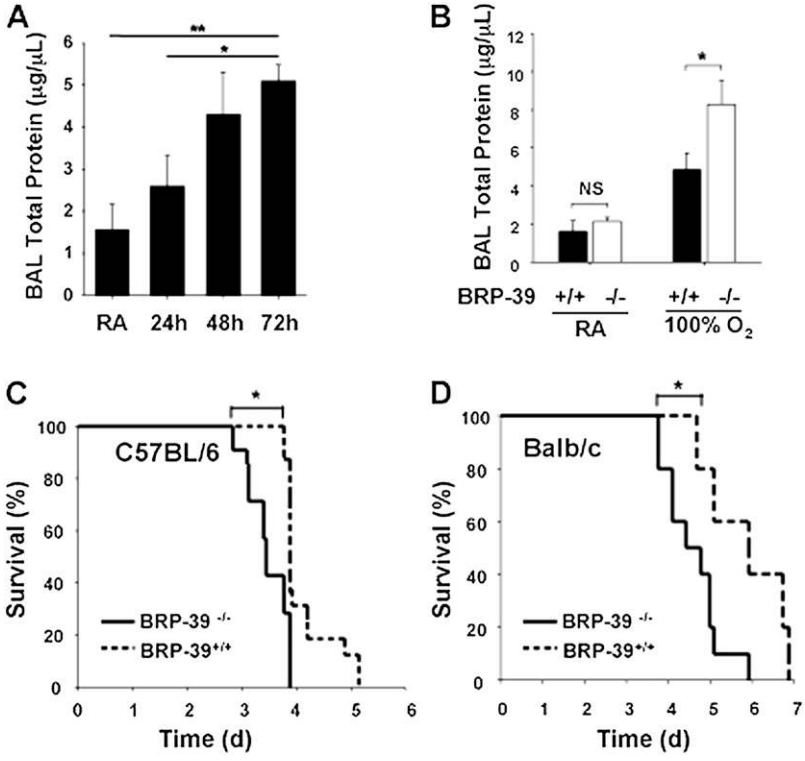

Figure 2. Role of breast regression protein (BRP)–39 in hyperoxia-induced vascular permeability and premature death. Wild-type (WT) (+/+) mice were exposed to 100% O_2 up to 72 hours, and bronchoalveolar lavage (BAL) protein was quantitated (*A*). WT and BRP-39$^{-/-}$ mice were exposed to 100% O_2 for 72 hours, and BAL protein was assessed (*B*). C57BL/6 (*C*) and Balb/c (*D*) mice were exposed to 100% O_2, and survival was assessed. The values in *A* and *B* are the mean (±SEM) of evaluations in a minimum of five animals, and are representative of two separate experiments. Data in *C* and *D* represent assessments in a minimum of eight mice. NS, not significant. $^*P < 0.05$, $^{**}P < 0.01$.

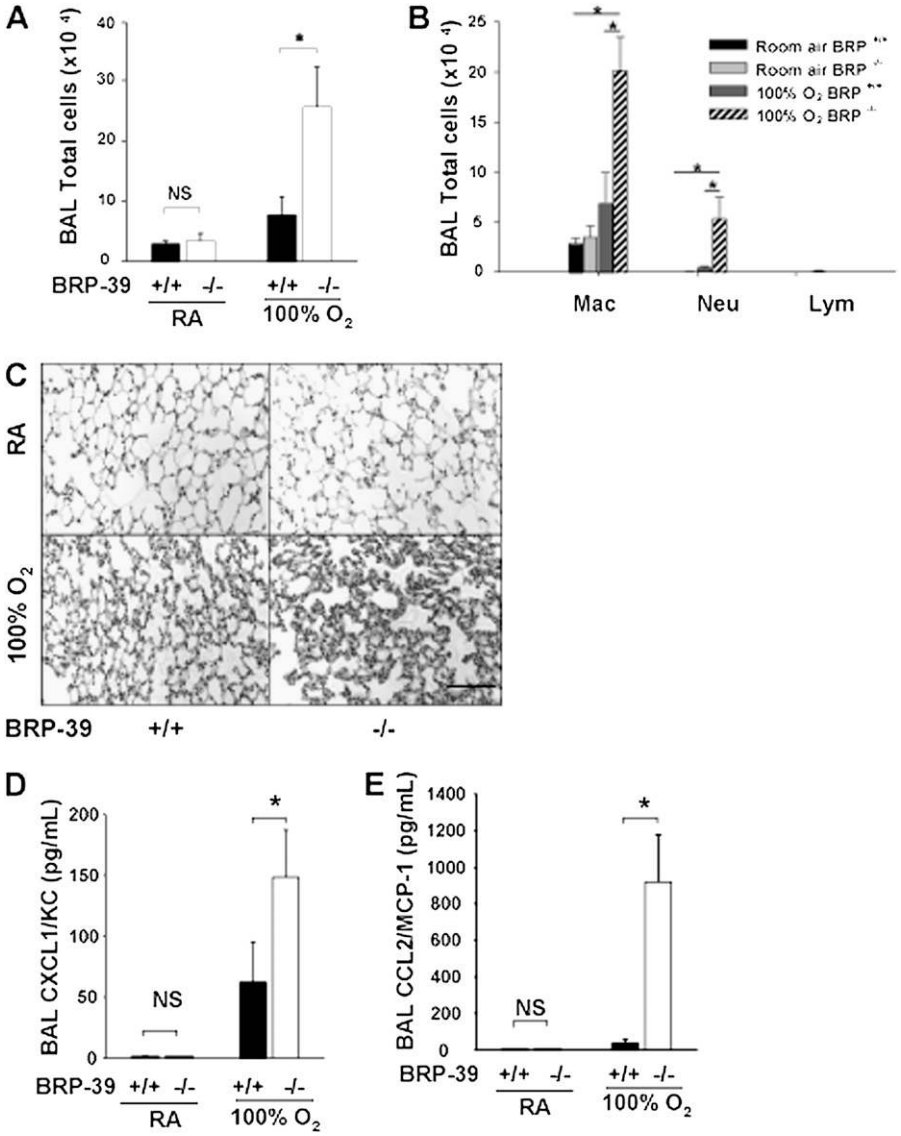

Figure 3. Role of breast regression protein (BRP)–39 in hyperoxia-induced inflammation and chemokine production. Wild-type (WT) (+/+) and BRP-39$^{-/-}$ mice were exposed to 100% O_2 for 72 hours, and bronchoalveolar lavage (BAL) total cell recovery (*A*), differential cell recovery (*B*), lung histology (*C*; hematoxylin and eosin stain), and the levels of BAL KC/CXCL1 (*D*) and MCP-1/CCL-2 (*E*) were assessed. The values in *A*, *B*, *D*, and *E* represent the mean (±SEM) of evaluations in a minimum of five animals, and are representative of two separate experiments. *C* is representative of five similar evaluations. NS, nonsignificant. *Scale bar*, 100 μm. *$P < 0.05$.

Experiments were also undertaken with CC10-rtTA-tTS-YKL-40 mice on a WT genetic background. These experiments compared the hyperoxia-induced responses in mice with physiologic and supraphysiologic levels of BRP-39/YKL-40. The features of the HALI that was seen in WT mice with physiologic levels of BRP-39 have been described previously here. Importantly, survival was enhanced (Figure 5I) and the alveolar capillary permeability alterations, protein leak, tissue and BAL inflammation, KC/CXCL-1 and MCP-1/CCL2 production, TUNEL-positive cell death, and caspase-3 activation were diminished in the YKL-40 Tg animals in 100% O_2 (Figures 5B–5H). When viewed in combination, these studies demonstrate that epithelial-targeted YKL-40 inhibits the toxic manifestations of 100% O_2 in the murine lung, and abrogates the exaggerated HALI in lungs from BRP-39$^{-/-}$ mice.

Caspase-3 Drives Inflammation in BRP-39$^{-/-}$ Mice Exposed to 100% O_2

To understand the relationship between the inflammation and permeability and caspase activation in our modeling system, we bred BRP-39$^{-/-}$ and caspase-3$^{-/-}$ mice and compared the effects of 100% O_2 in WT mice, single mutant mice, and mice with null mutations of BRP-39 and caspase-3 (BRP-39$^{-/-}$/caspase-3$^{-/-}$). As noted previously here, hyperoxia caused a neutrophil- and macrophage-rich inflammatory response in WT mice, which was exaggerated in BRP-39$^{-/-}$ mice. Interestingly, BAL and tissue inflammation were markedly ameliorated in the BRP-39$^{-/-}$/caspase-3$^{-/-}$ mice (Figures 6A–6C and data not shown). Similarly, hyperoxia caused alveolar capillary protein leak in WT mice, which was exaggerated in BRP-39$^{-/-}$ animals (Figure 6D). Interestingly, the hyperoxia-induced permeability changes in WT mice, and the exaggerated permeability alterations in BRP-39$^{-/-}$ mice, were markedly ameliorated in mice that lacked caspase-3 (Figure 6D). These studies demonstrate that caspase-3, a critical effector of apoptosis, plays an essential role in the pathogenesis of the exaggerated inflammation and permeability alterations in hyperoxia-exposed WT and BRP-39$^{-/-}$ animals.

Differences in the Levels of Tracheal Aspirate YKL-40 in Premature Newborns on Supplemental Oxygen

To determine if our murine findings are relevant to humans, we measured the levels of YKL-40 in tracheal aspirates from premature newborns on mechanical ventilation and supplemental oxygen due to respiratory failure. Because oxidant injury is known to contribute to the pathogenesis of BPD (38), we compared the tracheal YKL-40 in infants that developed BPD

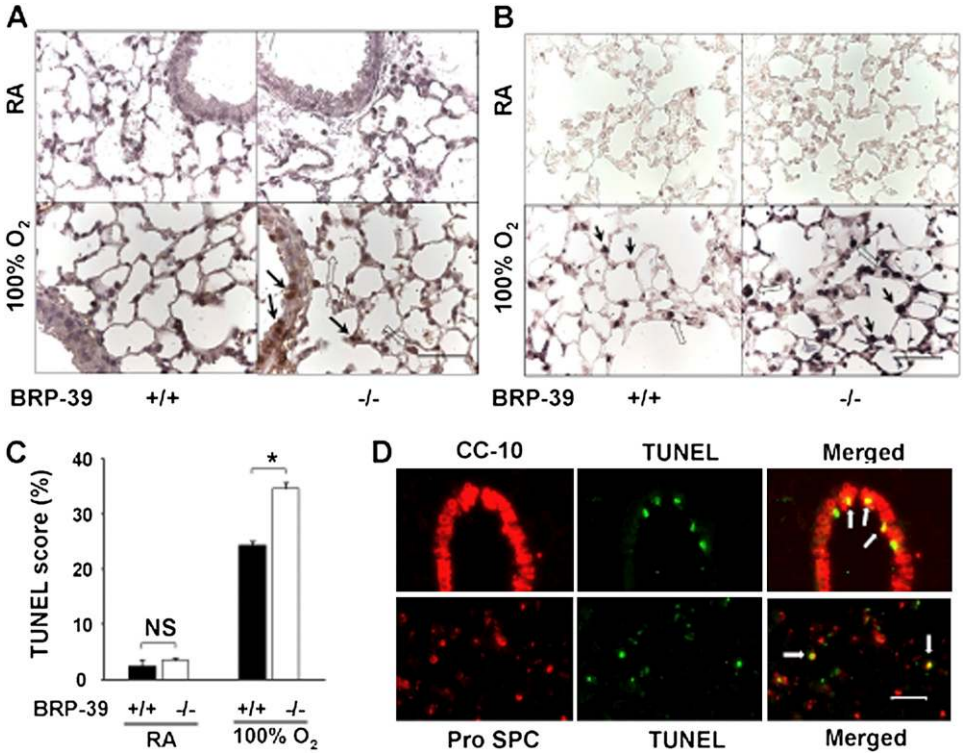

Figure 4. Role of breast regression protein (BRP)–39 in hyperoxia-induced oxidant and DNA injury. Wild-type (WT) (+/+) and BRP-39$^{-/-}$ mice were exposed to room air (RA) or 100% O_2 for 72 hours, and subjected to 8-hydroxy-2′deoxyguanosine (8-OHdG) (*A*; *closed arrows*, airway and alveolar type II epithelial cells; *open arrows*, macrophage) and terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) evaluation (*B*; *closed arrows*, alveolar type II cells; *open arrows*, macrophage). TUNEL-positive cells were counted and scored (*C*). TUNEL-positive apoptotic cells were further localized via double-label immunohistochemistry with cell-specific antibodies (CC10, airway epithelial cells; pro-SPC, alveolar type II cells) and TUNEL staining (*D*; *arrows*, double-stained cells). (*A* and *B*) Representative composites of five similar evaluations. The values in *C* are the mean (±SEM) of evaluations in a minimum of five animals, and are representative two separate experiments. *D* is representative of two separate experiments. *Scale bar*, 50 μm. NS, nonsignificant. *$P < 0.05$.

or died with those that did not develop these complications. Although there were no statistically significant difference between the "no BPD" ($n = 4$) and BPD/death ($n = 5$; two deaths) groups in the use of antenatal steroids, delivery route, percent male sex, gestational age, and the degree of oxygen supplementation (maximum $F_{I_{O_2}}$), their birth weights were significantly different (Table 1). There were also no significant differences in the two groups in terms of Apgar scores at 1 and 5 minutes and the time of collection of tracheal aspirate samples. Expectedly, babies in the BPD/death group had higher indices of severity of lung disease, as exemplified by more doses of surfactant and longer duration of invasive mechanical ventilation and exposure to supplemental oxygen (Table 1). YKL-40 was readily apparent in aspirates from premature babies being ventilated for RDS. Interestingly, the levels of tracheal aspirate YKl-40 were significantly lower in the babies that subsequently developed BPD or death compared with the babies that did not develop these outcomes ($P = 0.01$) (Figure 7). These studies demonstrate that elevated levels of tracheal aspirate YKL-40 are associated with improved pulmonary outcomes in premature neonates that are on supplemental oxygen due to respiratory failure.

DISCUSSION

ALI and its most severe form, acute RDS (ARDS), are devastating clinical syndromes affecting greater than 200,000 patients per year in the United States alone (39). Despite recent advances in therapy, the mortality for ARDS remains in the 25–50% range (40). On the other end of the spectrum, BPD, in which HALI is a critical contributing factor, is the commonest chronic lung disease in infants (41). There are currently no specific or effective interventions that prevent or ameliorate established BPD, and no established biomarkers that predict its occurrence in premature infants (41). In addition, although significant effort has been directed at the mechanisms that underlie this disorder(s), our understanding of the pathogenic mechanisms that are responsible for these adverse events is highly incomplete. To address this issue, we initiated studies that were designed to determine if the CLPs, BRP-39 and YKL-40, regulate the ALI induced by the prototypic oxidant, 100% O_2. These studies demonstrate that hyperoxia inhibits BRP-39 expression and production in the otherwise naive murine lung and in epithelial cells in cluture. They also demonstrate that mice that lack BRP-39 have exaggerated responses to 100% O_2, manifested by augmented alveolar–capillary permeability and protein leak, tissue oxidation, neutrophil- and macrophage-rich inflammation, chemokine elaboration, epithelial apoptosis, and premature death. Lastly, they demonstrate that transgenic YKL-40 ameliorates HALI, prolongs survival in 100% O_2, and rescues the exaggerated injury response in BRP-39$^{-/-}$ animals. These studies highlight novel relationships between BRP-39/YKL-40 and oxidants in the lung, including the demonstration that oxidant injury decreases the expression and production of BRP-39, and that BRP-39 and YKL-40 are important inhibitors of oxidant-induced lung injury, permeability, and structural cell apoptosis.

BRP-39/YKL-40 is a product of the *CH3L1* gene on chromosome 1 in mice and humans that is found in significant quantities in the circulation and tissues of normal humans and other animal species. BRP-39 and YKL-40 are also highly inducible, with elevated levels being seen in the serum and or tissues from patients with a variety of diseases, and elevated levels being noted in epithelial cells and/or macrophages after stimulation with IL-13 (31) and during late stages of macrophage activation (42). Transcriptional mechanisms have been shown to contribute to some of these stimulatory events (42). Surprisingly, our *in vitro* and *in vivo* studies are the first to highlight a circumstance in which the production and/or expression of BR-39/YKL-40 is decreased. They are also the first to define the relationship between this inhibition and oxidant injury, and to associate this decrease with a pathologic tissue response. When combined with our demonstration that BRP-39 inhibits HALI, one can envision a scenario in which the decrease in BRP-39 that is seen contributes to the initiation and/or perpetuation of this oxidant injury response. In light of our demonstration that transgenic YKL-40

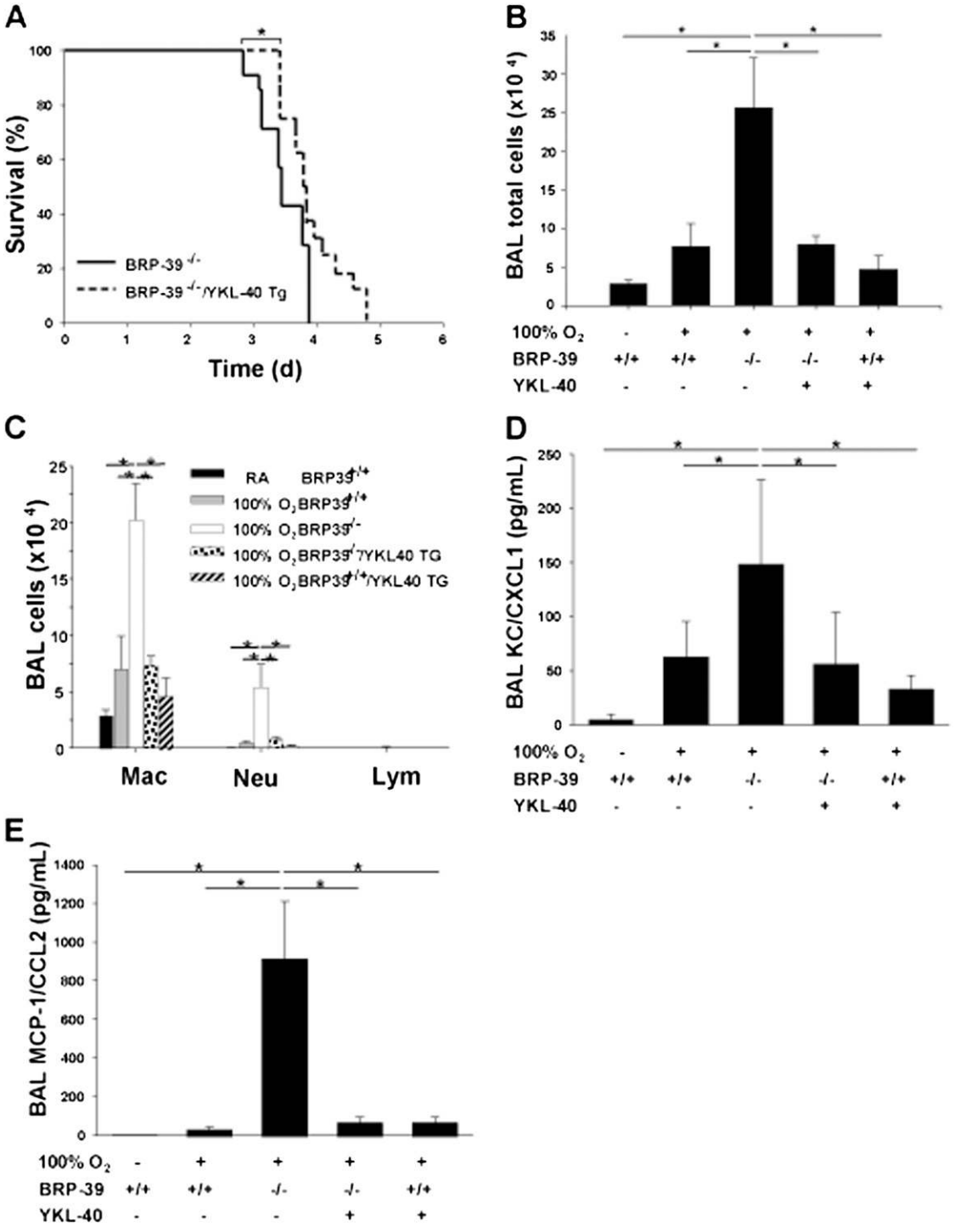

Figure 5. Effects of transgenic YKL-40 on hyperoxia-induced bronchoalveolar lavage (BAL) and tissue responses. Wild-type (WT) (+/+) mice, mice with null mutations of breast regression protein (BRP)–39 (BRP-39$^{-/-}$) mice, and BRP-39$^{-/-}$ mice that express transgenic YKL-40 only in respiratory epithelium (BRP-39$^{-/-}$/YKL-40^{+} mice) were exposed to room air (RA) or 100% O_2. (*A* and *I*) Survival was assessed. After 72 hours of hyperoxia, BAL total cell recovery (*B*), differential cell recovery (*C*), the levels of BAL KC/CXCL1 (*D*) and MCP-1/CCL-2 (*E*), lung tissue histology (*F*; hematoxylin and eosin stain), and the percentage of terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL)–positive cells (*G*) were also assessed. Capase-3 activation and ICAD cleavage were evaluated (*H*). The data in *A* and *I* represent assessments in a minimum of eight mice. The values in *B–E* and *G* are the mean (±SEM) of evaluations in a minimum of five animals, and are representative of two separate experiments. (*F* and *H*) Representative of four similar evaluations. *Scale bars*, 100 μm. *$P < 0.05$.

inhibits HALI and reverses the exaggerated injury phenotype in BRP-39$^{-/-}$ mice in 100% O_2, one can also hypothesize that the production of BRP-39/YKL-40 could increase during the later phase of HALI, where it could feed back to inhibit the tissue injury response. If future studies confirm these findings, one can also speculate that interventions that increase BRP-39/YKL-40 may be therapeutically useful in the treatment of these disorders.

The glycosyl hydrolase (GH) 18 family contains chitinases and CLPs, such as BRP-39 and YKL-40, which lack enzymatic activity (43). They are widely expressed, being seen in archea, prokaryotes, and eurkaryotes (44). Mammals do not contain or synthesize chitin, yet the human genome has eight GH-18 family members (44). Although it is presumed that the physiologic roles of these chitinases and CLP have resulted in their conservation over these vastly different species, the roles of these molecules in biology are poorly defined (43). Recent studies, however, have shed light on important roles that chitinases and CLPs play that may account for this conservation. Specifically, we demonstrated that BRP-39 and YKL-40 inhibit the apoptosis of and CD95 expression by inflammatory cells at sites of Th2- and IL-13–induced inflammation (31). We also demonstrated that acidic mammalian chitinase, a true chitinase, can also inhibit epithelial cell apoptosis, and that this inhibition is independent of the chitinolytic effects of the enzyme (45). The present studies add to

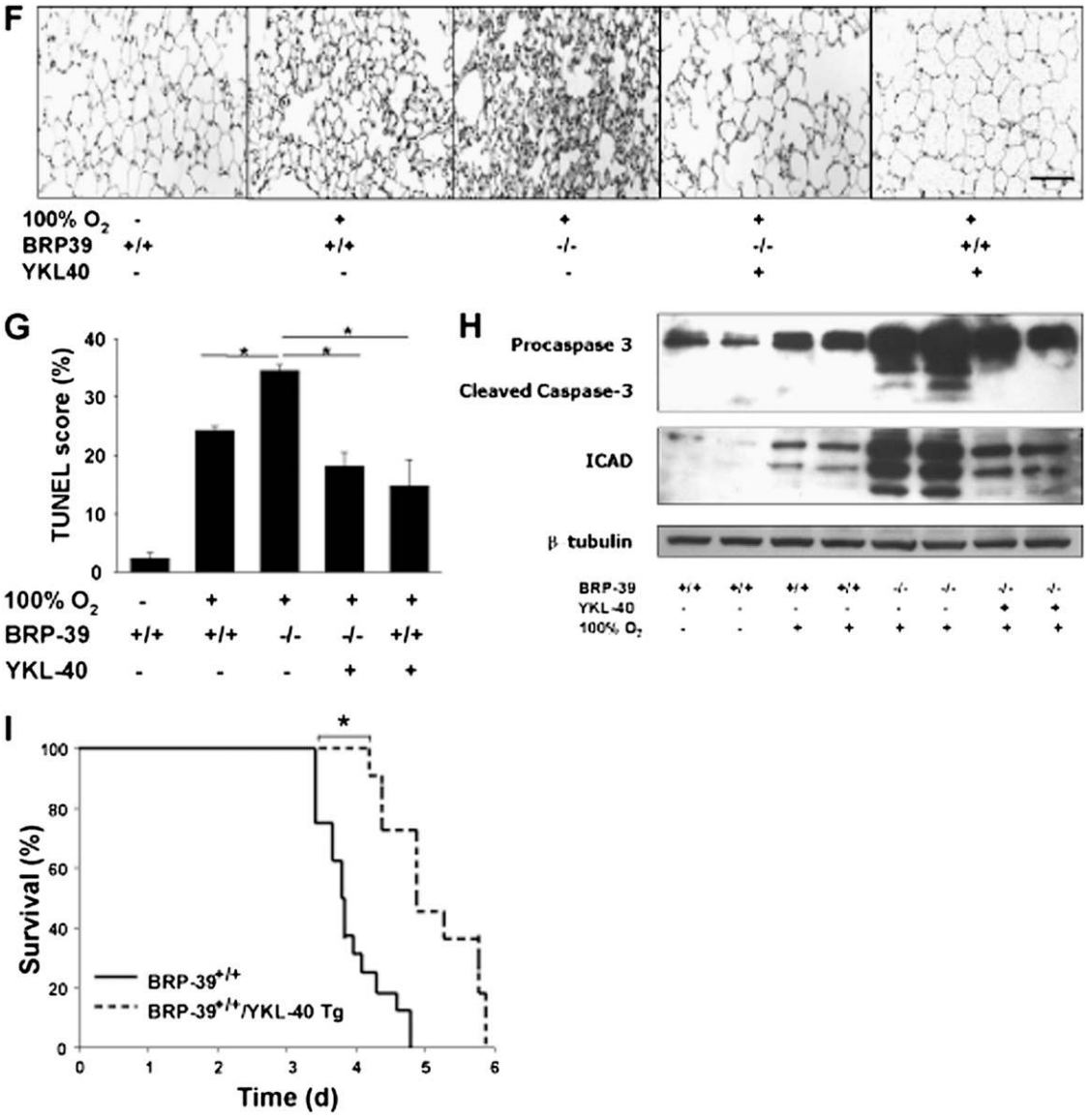

Figure 5. (*Continued*).

our understanding of the relationship(s) between BRP-39/YKL-40 and apoptosis by demonstrating that these chilectins inhibit the oxidant-induced cell death of alveolar type II cells. Although the mechanism of this protection is not clear, it is tempting to speculate that the phosphatidylinositol 3 kinase–Akt pathway is involved, because studies from our laboratory have demonstrated that BRP-39/YKL-40 is a potent activator of Akt (31), and Akt can confer cytoprotection in HALI (46–49). In combination, these studies allow for the speculation that BRP-39 and YKL-40 are critical regulators of cell death that inhibit oxidant injury and confer structural cell cytoprotection at physiologic concentrations, and prolong the survival of inflammatory cells, and contribute to antigen sensitization, chronic inflammation, and tissue remodeling when elevated.

IL-13 is a critical effector at sites of Th2-driven pathologies. As a result, anti–IL-13–based therapies are being developed to treat a variety of diseases and disorders. To fully understand the consequences of these interventions, studies have been undertaken to define the beneficial effects of IL-13 in the lung. These studies demonstrated that IL-13 is an important inhibitor of oxidant-induced lung injury (50). The mechanism of this response, however, is poorly understood. Recent studies from our laboratory demonstrated that IL-13 is a potent stimulator of BRP-39 (31). This raises the intriguing possibility that the protective effects of IL-13 in hyperoxia are mediated, at least in part, by BRP-39/YKL-40.

ALI and ARDS are complex, multigenic, and multifactoral disorders, with profound clinical heterogeneity (40). Nevertheless, progress has been made in the identification of genetic variants that contribute to these responses and enhance our understanding of the pathways that are involved in the pathogenesis of these disorders. Using predominantly a candidate gene approach, investigators have highlighted the importance of polymorphisms in a wide variety of genes, including those that encode cytokines, blood pressure regulators, immune regulators, gene transcription, coagulation, and antioxidants (40). Our studies demonstrate that BRP-39 is inhibited during HALI and, in turn, feeds back to inhibit HALI. Recent studies from our laboratory and others have demonstrated that polymorphisms in the *CH3L1* gene correlate with the levels of circulating YKL-40, the presence of asthma, and asthma severity (17, 30, 51). The present studies raise the possibility that polymorphisms in YKL-40 also play an important role in the pathogenesis of ALI and/or ARDS. This is an intriguing possibility, because polymorphisms in the mannose-binding lectin, which, like YKL-40, is a circulating carbohydrate-binding protein, have recently been shown to correlate with

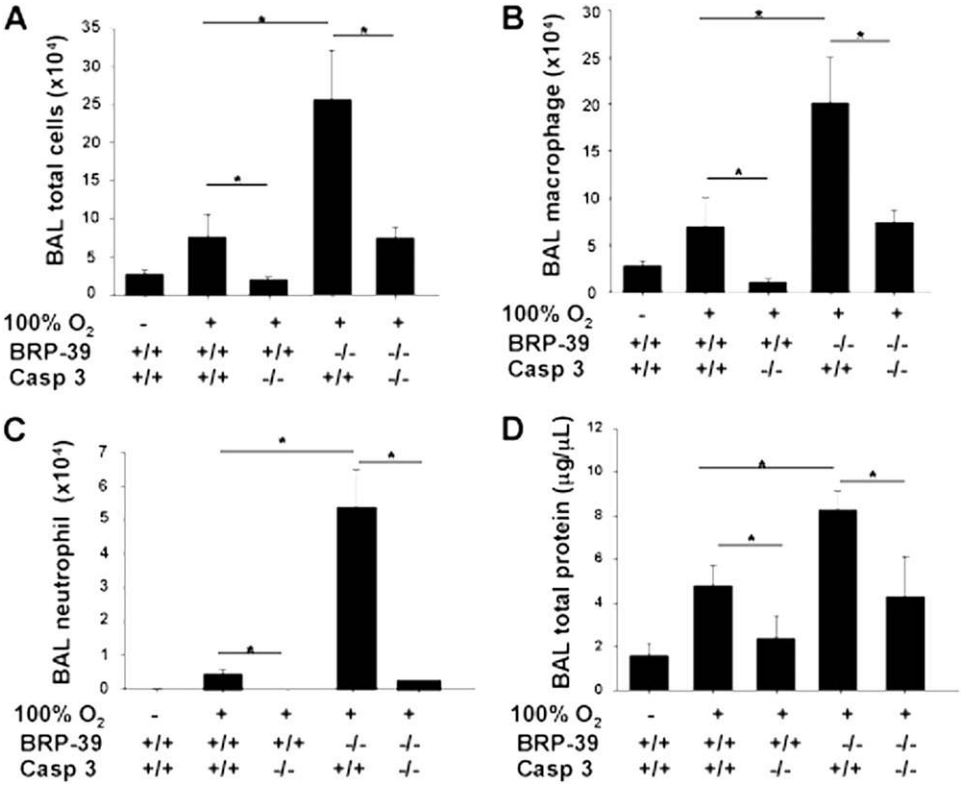

Figure 6. Roles of caspase-3 in breast regression protein (BRP)–39 regulation of hyperoxia-induced responses. Wild-type (WT) (+/+) mice, BRP-39$^{-/-}$ mice, caspase-3$^{-/-}$ mice, and BRP-39$^{-/-}$/caspase-3$^{-/-}$ mice were exposed to room air (RA) or 100% O_2 for 72 hours, and bronchoalveolar lavage (BAL) total cell recovery (*A*), BAL macrophage recovery (*B*), BAL neutrophil recovery (*C*), and BAL protein (*D*) were assessed. The values represent the mean (±SEM) of evaluations in a minimum of five animals, and are representative of two separate experiments. Casp 3 = caspase-3. *$P < 0.05$.

ARDS (52–54). In addition, if *CH3L1* polymorphisms correlate with asthma and HALI, this would add to the mounting evidence supporting the "common variant/multiple disease hypothesis," which suggests that certain disease genes may not be disease specific, and may contribute to related clinical phenotypes (55).

In animal models of ALI, inflammation and lung injury are frequently juxtaposed. This led to studies investigating the mechanisms of hyperoxia-induced inflammation, and the relationship between injury and inflammation in this disorder (11, 56, 57). Our studies have added to our understanding in this area by highlighting an interesting relationship between the cell death and inflammatory responses in this modeling system. Specifically, they demonstrate that null mutations of caspase-3 diminish the inflammatory response induced by hyperoxia. This suggests that cell death drives, at least in part, the inflammation in the lungs of mice breathing 100% O_2. Under classic conditions, apoptosis was proposed to be an inflammation-independent form of cell death (58). However, it is now clear that cells die via complex mechanisms, and that apoptotic cells can undergo secondary necrosis if not rapidly cleared by phagocytes (59). It is also clear that, during these responses, injured cells elaborate a number of danger signals, which can induce inflammation by activating Toll-like receptor 3 and C-type lectin receptors (59). In accord with this scenario, the cell

TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE STUDY GROUPS (NO BRONCHOPULMONARY DYSPLASIA AND BRONCHOPULMONARY DYSPLASIA OR DIED)

	No BPD ($n = 4$)	BPD or Died ($n = 5$)	*P* Value
Prenatal steroids, %	3 (75)	5 (100)	0.44
C-section delivery, %	3 (75)	5 (100)	0.44
Male sex, %	1 (25)	3 (60)	0.52
Gestational age, wk	26.2 ± 1.8	26.3 ± 1.6	0.92
Birth weight, g	898 ± 111	704 ± 81	0.02
Apgar @ 1 min ⩽ 3, %	1 (25)	2 (40)	1.00
Apgar @ 5 min ⩽ 7, %	1 (25)	1 (20)	1.00
TA sample collected, d	2.3 ± 1.3	2.4 ± 1.7	0.89
Maximum FI_{O_2} (on TA sample collection day)	0.27 ± 0.02	0.30 ± 0.04	0.49
Survanta, doses	1.0 ± 0.0	2.2 ± 0.8	<0.05
ETT PPV, d	7.3 ± 8.8	43.8 ± 11.4	0.001
Oxygen, d	28.8 ± 10.1	68.0 ± 16.9	0.005
Length of hospitalization, d	65.0 ± 14.5	68.4 ± 16.9	0.76

Definition of abbreviations: BPD = bronchopulmonary dysplasia; ETT PPV = endotracheal tube positive pressure ventilation; TA = tracheal aspirate.

Values are expressed as means (±SD).

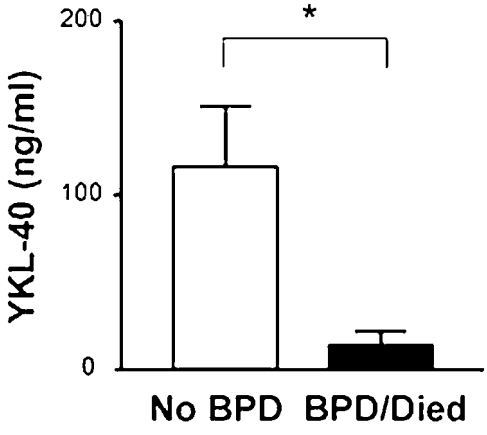

Figure 7. YKL-40 protein in tracheal aspirates from premature babies with respiratory failure requiring mechanical ventilation and O_2 supplementation. The levels of YKL-40 were evaluated by ELISA. The values represent the mean (±SEM) of evaluations in patients that developed bronchopulmonary dysplasia (BPD) or died ($n = 5$) and those that did not develop these complications (No BPD; $n = 4$). *$P < 0.05$.

death response in this oxidant injury model has been extensively studied and shown to have features of both apoptosis and necrosis (14, 60, 61).

RDS and respiratory failure are problematic consequences of premature birth. Patients with these conditions are commonly treated with mechanical ventilation, supplemental oxygen, and surfactant preparations (62), and, in many cases, rapidly recover. However, in a subset of patients, oxidant injury contributes to the development of BPD with chronic respiratory failure, and death can ensue (41). To determine if our murine findings are relevant to human disease, we compared the levels of tracheal aspirate YKL-40 in a cohort of premature babies with RDS that developed BPD or died, and premature infants with milder disease that did not experience these adverse consequences. In this cohort, the premature infants with the milder disease had higher levels of this CLP. These observations are in accord with our finding that YKL-40 inhibits HALI. If they are confirmed in subsequent, larger studies, they also raise the possibility that the elevated levels of YKL-40 are causally related to the milder disease in these individuals. Oxidant injury also plays a major role in the pathogenesis of interstitial lung diseases, asthma, and chronic obstructive pulmonary disease and can worsen the effects of pulmonary infections (63–67). When viewed in combination, these observations allow for the speculation that BRP-39/YKL-40 may be able to be manipulated to control oxidant-induced pulmonary responses, and that the levels of circulating and or organ YKL-40 might be useful biomarkers that can predict the severity and or course of these disorders. For example, in premature newborns, YKL-40 might be able to be used as a therapeutic in infants with RDS to prevent or ameliorate BPD, and the levels of tracheal aspirate YKL-40 might predict who will develop BPD and who will not. These studies also suggest that genetic polymorphisms, environmental exposures, or pharmacologic interventions that alter the levels and/or effects of BRP-39/YKL-40 can have major effects on an individual's ability to tolerate an oxidative load, and thus contribute to the severity and/or natural history of these disorders. Additional investigations of the regulation and roles of chitinases and CLP in oxidant-induced injury and subsequent repair, and the feasibility and utility of CLP-based therapeutic manipulations, are warranted.

Author Disclosure: M.H.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.-J.K. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. H.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. V.B. received grant support from the National Institutes of Health (>$100,001) and the American Thoracic Society ($50,001–$100,000). N.-Y.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. C.G.L. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. J.A.E. served as a consultant for Promedior Inc. ($5,001–$10,000) and served on the Scientific Advisory Board for Intermune Inc. ($1,001–$5,000). He has patents through Yale University on chitinases in lung Inflammation, microRNA (mir) in VEGF tissue responses, IL-18 in chronic obstructive pulmonary disease, and VEGF in asthma. He owns stocks or options of Merck, Inc. ($1,001–$5,000) and Intermune Inc ($5,001–$10,000).

References

1. Klekamp JG, Jarzecka K, Perkett EA. Exposure to hyperoxia decreases the expression of vascular endothelial growth factor and its receptors in adult rat lungs. *Am J Pathol* 1999;154:823–831.
2. Warner BB, Stuart LA, Papes RA, Wispe JR. Functional and pathological effects of prolonged hyperoxia in neonatal mice. *Am J Physiol* 1998;275:L110–L117.
3. Horowitz S, Davis JM. Lung injury when development is interrupted by premature birth. In: McDonald JM, editor. Lung growth and development. New York: Marcel Dekker, Inc.; 1997. pp. 577–610.
4. Slutsky AS. Lung injury caused by mechanical ventilation. *Chest* 1999; 116:9S–15S.
5. Corne J, Chupp G, Lee CG, Homer RJ, Zhu Z, Chen Q, Ma B, Du Y, Roux F, McArdle J, *et al.* Il-13 stimulates vascular endothelial cell growth factor and protects against hyperoxic acute lung injury. *J Clin Invest* 2000;106:783–791.
6. Waxman AB, Einarsson O, Seres T, Knickelbein RG, Warshaw JB, Johnston R, Homer RJ, Elias JA. Targeted lung expression of interleukin-11 enhances murine tolerance of 100% oxygen and diminishes hyperoxia-induced DNA fragmentation. *J Clin Invest* 1998; 101:1970–1982.
7. He CH, Waxman AB, Lee CG, Link H, Rabach ME, Ma B, Chen Q, Zhu Z, Zhong M, Nakayama K, *et al.* Bcl-2–related protein A1 is an endogenous and cytokine-stimulated mediator of cytoprotection in hyperoxic acute lung injury. *J Clin Invest* 2005;115:1039–1048.
8. Barazzone C, White CW. Mechanisms of cell injury and death in hyperoxia: role of cytokines and Bcl-2 family proteins. *Am J Respir Cell Mol Biol* 2000;22:517–519.
9. Freeman BA, Crapo JD. Biology of disease: free radicals and tissue injury. *Lab Invest* 1982;47:412–426.
10. Ward NS, Waxman AB, Homer RJ, Mantell LL, Einarsson O, Du Y, Elias JA. Interleukin-6–induced protection in hyperoxic acute lung injury. *Am J Respir Cell Mol Biol* 2000;22:535–542.
11. Mantell LL, Horowitz S, Davis JM, Kazzaz JA. Hyperoxia-induced cell death in the lung—the correlation of apoptosis, necrosis, and inflammation. *Ann N Y Acad Sci* 1999;887:171–180.
12. Barazzone C, Horowitz S, Donati YR, Rodriguez I, Piguet PF. Oxygen toxicity in mouse lung: pathways to cell death. *Am J Respir Cell Mol Biol* 1998;19:573–581.
13. O'Reilly MA, Staversky RJ, Huyck HL, Watkins RH, LoMonaco MB, D'Angio CT, Baggs RB, Maniscalco WM, Pryhuber GS. Bcl-2 family gene expression during severe hyperoxia induced lung injury. *Lab Invest* 2000;80:1845–1854.
14. Wang X, Ryter SW, Dai C, Tang ZL, Watkins SC, Yin XM, Song R, Choi AM. Necrotic cell death in response to oxidant stress involves the activation of the apoptogenic caspase-8/bid pathway. *J Biol Chem* 2003;278:29184–29191.
15. Paine R III, Wilcoxen SE, Morris SB, Sartori C, Baleeiro CE, Matthay MA, Christensen PJ. Transgenic overexpression of granulocyte macrophage-colony stimulating factor in the lung prevents hyperoxic lung injury. *Am J Pathol* 2003;163:2397–2406.
16. Boot RG, Blommaart EF, Swart E, Ghauharali-van der Vlugt K, Bijl N, Moe C, Place A, Aerts JM. Identification of a novel acidic mammalian chitinase distinct from chitotriosidase. *J Biol Chem* 2001;276: 6770–6778.
17. Chupp GL, Lee CG, Jarjour N, Shim YM, Holm CT, He S, Dziura JD, Reed J, Coyle AJ, Kiener P, *et al.* A chitinase-like protein in the lung and circulation of patients with severe asthma. *N Engl J Med* 2007; 357:2016–2027.
18. Kawada M, Hachiya Y, Arihiro A, Mizoguchi E. Role of mammalian chitinases in inflammatory conditions. *Keio J Med* 2007;56:21–27.
19. Zhu Z, Zheng T, Homer RJ, Kim YK, Chen NY, Cohn L, Hamid Q, Elias JA. Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. *Science* 2004;304:1678–1682.
20. Reese TA, Liang HE, Tager AM, Luster AD, Van Rooijen N, Voehringer D, Locksley RM. Chitin induces accumulation in tissue of innate immune cells associated with allergy. *Nature* 2007;447:92–96.
21. Hakala BE, White C, Recklies AD. Human cartilage GP-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein family. *J Biol Chem* 1993;268:25803–25810.
22. Rejman JJ, Hurley WL. Isolation and characterization of a novel 39 kDa whey protein from bovine mammary secretions collected during the nonlactating period. *Biochem Biophys Res Commun* 1988;150:329–334.
23. Shackleton LM, Mann DM, Millis JT. Identification of a 38-kDa heparin-binding glycoprotein (gp39k) in differentiating vascular smooth muscle cells as a member of a group of proteins associated with tissue remodeling. *J Biol Chem* 1995;270:13076–13083.
24. Johansen JS. Studies on serum YKL-40 as a biomarker in diseases with inflammation, tissue remodelling, fibroses and cancer. *Dan Med Bull* 2006;53:172–209.
25. Johansen JS, Christoffersen P, Moller S, Price PA, Bendtsen F. Serum YKL-40 is increased in patients with hepatic fibrosis. *J Hepatol* 2000; 32:911–920.
26. Ostergaard C, Johansen JS, Benfield T, Price PA, Lundgren JD. YKL-40 is elevated in cerebrospinal fluid from patients with purulent meningitis. *Clin Diagn Lab Immunol* 2002;9:598–604.

27. Knudsen LS, Ostergaard M, Baslund B, Narvestad E, Petersen J, Nielsen HJ, Ejbjerg BJ, Szkudlarek M, Johansen JS. Plasma IL-6, plasma VEGF, and serum YKL-40: relationship with disease activity and radiographic progression in rheumatoid arthritis patients treated with infliximab and methotrexate. *Scand J Rheumatol* 2006;35:489–491.
28. Kucur M, Isman FK, Karadag B, Vural VA, Tavsanoglu S. Serum YKL-40 levels in patients with coronary artery disease. *Coron Artery Dis* 2007;18:391–396.
29. Rathcke CN, Vestergaard H. YKL-40, a new inflammatory marker with relation to insulin resistance and with a role in endothelial dysfunction and atherosclerosis. *Inflamm Res* 2006;55:221–227.
30. Ober C, Tan Z, Sun Y, Possick JD, Pan L, Nicolae R, Radford S, Parry RR, Heinzmann A, Deichmann KA, *et al.* Effect of variation in CHI3L1 on serum YKL-40 level, risk of asthma, and lung function. *N Engl J Med* 2008;358:1682–1691.
31. Lee CG, Hartl D, Lee GR, Koller B, Matsuura H, Da Silva CA, Sohn MH, Cohn L, Homer RJ, Kozhich AA, *et al.* Role of breast regression protein 39 (BRP-39)/chitinase 3–like–1 in Th2 and IL-13–induced tissue responses and apoptosis. *J Exp Med* 2009;206: 1149–1166.
32. Wang J, Chen Q, Corne J, Zhu Z, Lee CG, Bhandari V, Homer RJ, Elias JA. Pulmonary expression of leukemia inhibitory factor induces B cell hyperplasia and confers protection in hyperoxia. *J Biol Chem* 2003;278:31226–31232.
33. Crapo JD. Morphologic changes in pulmonary oxygen toxicity. *Annu Rev Physiol* 1986;48:721–731.
34. Homer RJ, Zhu Z, Cohn L, Lee CG, White WI, Chen S, Elias JA. Differential expression of chitinases identify subsets of murine airway epithelial cells in allergic inflammation. *Am J Physiol Lung Cell Mol Physiol* 2006;291:L502–L511.
35. Kang HR, Lee CG, Homer RJ, Elias JA. Semaphorin 7A plays a critical role in TGF-beta1–induced pulmonary fibrosis. *J Exp Med* 2007;204: 1083–1093.
36. Ma B, Blackburn MR, Lee CG, Homer RJ, Liu W, Flavell RA, Boyden L, Lifton RP, Sun CX, Young HW, *et al.* Adenosine metabolism and murine strain–specific IL-4–induced inflammation, emphysema, and fibrosis. *J Clin Invest* 2006;116:1274–1283.
37. Lee CG, Cho SJ, Kang MJ, Chapoval SP, Lee PJ, Noble PW, Yehualaeshet T, Lu B, Flavell RA, Milbrandt J, *et al.* Early growth response gene 1–mediated apoptosis is essential for transforming growth factor beta1–induced pulmonary fibrosis. *J Exp Med* 2004;200: 377–389.
38. Davis JM. Role of oxidant injury in the pathogenesis of neonatal lung disease. *Acta Paediatr Suppl* 2002;91:23–25.
39. Rubenfeld GD, Caldwell E, Peabody E, Weaver J, Martin DP, Neff M, Stern EJ, Hudson LD. Incidence and outcomes of acute lung injury. *N Engl J Med* 2005;353:1685–1693.
40. Gao L, Barnes KC. Recent advances in genetic predisposition to clinical acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 2009;296: L713–L725.
41. Bhandari A, Bhandari V. Pitfalls, problems, and progress in bronchopulmonary dysplasia. *Pediatrics* 2009;123:1562–1573.
42. Rehli M, Niller HH, Ammon C, Langmann S, Schwarzfischer L, Andreesen R, Krause SW. Transcriptional regulation of CHI3L1, a marker gene for late stages of macrophage differentiation. *J Biol Chem* 2003;278:44058–44067.
43. Bussink AP, Speijer D, Aerts JM, Boot RG. Evolution of mammalian chitinase(-like) members of family 18 glycosyl hydrolases. *Genetics* 2007;177:959–970.
44. Funkhouser JD, Aronson NN Jr. Chitinase family GH18: evolutionary insights from the genomic history of a diverse protein family. *BMC Evol Biol* 2007;7:96.
45. Hartl D, He CH, Koller B, Da Silva CA, Kobayashi Y, Lee CG, Flavell RA, Elias JA. Acidic mammalian chitinase regulates epithelial cell apoptosis via a chitinolytic-independent mechanism. *J Immunol* 2009; 182:5098–5106.
46. Waldow T, Witt W, Ulmer A, Janke A, Alexiou K, Matschke K. Preconditioning by inhaled nitric oxide prevents hyperoxic and ischemia/reperfusion injury in rat lungs. *Pulm Pharmacol Ther* 2008; 21:418–429.
47. Waxman AB, Kolliputi N. IL-6 protects against hyperoxia induced mitochondrial damage via Bcl-2 induced Bak interactions with mitofusions. *Am J Physiol Lung Cell Mol Physiol* 2009;41:385–396.
48. Xu D, Guthrie JR, Mabry S, Sack TM, Truog WE. Mitochondrial aldehyde dehydrogenase attenuates hyperoxia-induced cell death through activation of ERK/MAPK and PI3K–Akt pathways in lung epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 2006;291:L966–L975.
49. Lu Y, Parkyn L, Otterbein LE, Kureishi Y, Walsh K, Ray A, Ray P. Activated Akt protects the lung from oxidant-induced injury and delays death of mice. *J Exp Med* 2001;193:545–549.
50. Bhandari V, Choo-Wing R, Homer RJ, Elias JA. Increased hyperoxia-induced mortality and acute lung injury in IL-13 null mice. *J Immunol* 2007;178:4993–5000.
51. Ober C, Chupp GL. The chitinase and chitinase-like proteins: a review of genetic and functional studies in asthma and immune-mediated diseases. *Curr Opin Allergy Clin Immunol* 2009;9:401–408.
52. Garred P, Strøm J, Quist L, Taaning E, Madsen HO. Association of mannose-binding lectin polymorphisms with sepsis and fatal outcome, in patients with systemic inflammatory response syndrome. *J Infect Dis* 2003;188:1394–1403.
53. Gordon AC, Waheed U, Hansen TK, Hitman GA, Garrard CS, Turner MW, Klein NJ, Brett SJ, Hinds CJ. Mannose-binding lectin polymorphisms in severe sepsis: relationship to levels, incidence, and outcome. *Shock* 2006;25:88–93.
54. Sutherland AM, Walley KR, Russell JA. Polymorphisms in CD14, mannose-binding lectin, and Toll-like receptor–2 are associated with increased prevalence of infection in critically ill adults. *Crit Care Med* 2005;33:638–644.
55. Gao L, Grant AV, Rafaels N, Stockton-Porter M, Watkins T, Gao P, Chi P, Munoz M, Watson H, Dunston G, *et al.* Polymorphisms in the myosin light chain kinase gene that confer risk of severe sepsis are associated with a lower risk of asthma. *J Allergy Clin Immunol* 2007; 119:1111–1118.
56. Bustani P, Kotecha S. Role of cytokines in hyperoxia mediated inflammation in the developing lung. *Front Biosci* 2003;8:s694–s704.
57. Lian X, Qin Y, Hossain SA, Yang L, White A, Xu H, Shipley JM, Li T, Senior RM, Du H, *et al.* Overexpression of STAT3C in pulmonary epithelium protects against hyperoxic lung injury. *J Immunol* 2005; 174:7250–7256.
58. Fink SL, Cookson BT. Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. *Infect Immun* 2005;73: 1907–1916.
59. Pinheiro da Silva F, Nizet V. Cell death during sepsis: Integration of disintegration in the inflammatory response to overwhelming infection. *Apoptosis* 2009;14:509–521.
60. Hoshino Y, Mio T, Nagai S, Miki H, Ito I, Izumi T. Cytotoxic effects of cigarette smoke extract on an alveolar type II cell–derived cell line. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L509–L516.
61. Bhandari V, Choo-Wing R, Lee CG, Zhu Z, Nedrelow JH, Chupp GL, Zhang X, Matthay MA, Ware LB, Homer RJ, *et al.* Hyperoxia causes angiopoietin 2–mediated acute lung injury and necrotic cell death. *Nat Med* 2006;12:1286–1293.
62. Ramanathan R. Optimal ventilatory strategies and surfactant to protect the preterm lungs. *Neonatology* 2008;93:302–308.
63. Tateda K, Deng JC, Moore TA, Newstead MW, Paine R III, Kobayashi N, Yamaguchi K, Standiford TJ. Hyperoxia mediates acute lung injury and increased lethality in murine legionella pneumonia: the role of apoptosis. *J Immunol* 2003;170:4209–4216.
64. Tuder RM, Zhen L, Cho CY, Taraseviciene-Stewart L, Kasahara Y, Salvemini D, Voelkel NF, Flores SC. Oxidative stress and apoptosis interact and cause emphysema due to vascular endothelial growth factor receptor blockade. *Am J Respir Cell Mol Biol* 2003;29:88–97.
65. Andreadis AA, Hazen SL, Comhair SA, Erzurum SC. Oxidative and nitrosative events in asthma. *Free Radic Biol Med* 2003;35:213–225.
66. Rahman I. Oxidative stress, chromatin remodeling and gene transcription in inflammation and chronic lung diseases. *J Biochem Mol Biol* 2003;36:95–109.
67. Saleh D, Barnes PJ, Giaid A. Increased production of the potent oxidant peroxynitrite in the lungs of patients with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 1997;155:1763–1769.