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Mechanical ventilation-induced apoptosis in newborn rat lung is mediated via FasL/Fas pathway

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Mechanical ventilation-induced apoptosis in newborn rat lung is mediated via FasL/Fas pathway. Mechanical ventilation induces pulmonary apoptosis and inhibits alveolar development in preterm infants, but the molecular basis for the apoptotic injury is unknown. The objective was to determine the signaling mechanism(s) of ventilation (stretch)-induced apoptosis in newborn rat lung. Seven-day-old rats were ventilated with room air for 24 h using moderate tidal volumes (8.5 ml/kg). Isolated fetal rat lung epithelial and fibroblast cells were subjected to continuous cyclic stretch (5, 10, or 17% elongation) for up to 12 h. Prolonged ventilation significantly increased the number of apoptotic alveolar type II cells during perinatal transition corresponds to maximal airway distension and increasing apoptosis (i.e., in type II cells; Refs. 12, 14); thus FasL-Fas may mediate stretch-induced apoptosis of late gestation. This is consistent with the finding that tracheal occlusion (a potent cause of lung stretch) increases type II cell apoptosis as well as FasL protein in the fetal lung (13). Finally, induction of lung-specific FasL causes increased lung epithelial apoptosis during postcanalicular remodeling and impaired alveolar development (9). However, the role of the Fas/FasL system in ventilation-induced apoptosis in preterm infants remains unknown. Mechanical ventilation (stretch) may also activate the mitochondrial apoptotic pathway, leading to activation of initiator caspase-9. This pathway has received little attention in preterm infants. Such infants, when ventilated, have increased levels of cleaved caspase-3 but not caspase-8 or -9 (33). Thus it is unclear by which pathway ventilation of neonatal mice increased lung epithelial cell apoptosis, which was paralleled by inhibition of alveolar development (35).

Apoptosis can occur through the extrinsic or intrinsic (mitochondrial) pathways, but the pathway related to ventilator-induced apoptosis in the preterm lung is not known. Emerging evidence suggests a role for the extrinsic [Fas ligand (FasL)/Fas] system in lung development (47). Fas is continuously expressed in fetal rabbit lung (14), in murine lung its expression increases at birth (12), and both Fas and FasL immunolocalize to bronchial epithelial cells and alveolar type II cells (12, 14). The temporal expression of FasL throughout development suggests transcriptional (or posttranscriptional) regulation of the FasL gene, and its expression in distal epithelial cells during perinatal transition corresponds to maximal airway distension and increasing apoptosis (i.e., in type II cells; Refs. 12, 14); thus FasL-Fas may mediate stretch-induced apoptosis of late gestation. This is consistent with the finding that tracheal occlusion (a potent cause of lung stretch) increases type II cell apoptosis as well as FasL protein in the fetal lung (13). Finally, induction of lung-specific FasL causes increased lung epithelial apoptosis during postcanalicular remodeling and impaired alveolar development (9). However, the role of the Fas/FasL system in ventilation-induced apoptosis in preterm infants remains unknown. Mechanical ventilation (stretch) may also activate the mitochondrial apoptotic pathway, leading to activation of initiator caspase-9. This pathway has received little attention in preterm infants. Such infants, when ventilated, have increased levels of cleaved caspase-3 but not caspase-8 or -9 (33). Thus it is unclear by which pathway ventilation of neonatal mice increased lung epithelial cell apoptosis, which was paralleled by inhibition of alveolar development (35).

Mechanical ventilation is frequently required in preterm infants. Although contemporary “protective” ventilation is designed to minimize volutrauma, this has not reduced the incidence of bronchopulmonary dysplasia (BPD; Ref. 36), a disease characterized by arrest of alveolar development. How mechanical ventilation adversely affects alveolar development is unknown, but a potentially important mechanism is apoptosis. For example, apoptotic cell (particularly alveolar epithelial cells) numbers are increased in ventilated preterm infants (24, 29, 33). Apoptosis is central to, and occurs in all stages of, lung development (12, 24, 43, 44), and abnormal apoptotic activity may reduce alveolar number. Indeed, prolonged mechanical ventilation of neonatal mice increased lung epithelial cell apoptosis, which was paralleled by inhibition of alveolar development (35).

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Methods

Animals. Seven-day old rat pups were ventilated (FlexiVent; Sci-req, Quebec, Canada) with room air and moderate tidal volume [inspired tidal volume (VT): 7–9 ml/kg; respiratory rate: 150 min–1; positive end-expiratory pressure: 2 cmH₂O] for 24 h as previously
described (25, 26), after which lung tissue was processed for histology or was fresh frozen. All animal procedures were in accordance with Canadian Council of Animal Care guidelines and approved by the Animal Care Review Committee of the Hospital for Sick Children.

Immunohistochemistry and terminal transferase dUTP end-labeling assay. Lungs were pressure fixed and processed for paraffin embedding as previously described (26). Apoptosis was visualized with terminal deoxynucleotidyl transferase dUTP-mediated nick-end labeling (TUNEL) analysis (A, a–c) and cleaved caspase-3 immunohistochemistry (A, d–f) revealed increased numbers of apoptotic (brownish) cells (arrows) in 7-day newborn rat lung ventilated for 24 h with VT of 7–9 ml/kg (A, b and e) compared with nonventilated 7 (A, a and d) and 8 (A, c and f)-day newborn rat lungs. B: quantification of number of TUNEL-positive cells in ventilated and nonventilated control rat lungs. MV, mechanical ventilation. *P < 0.05.

Fig. 2. Prolonged ventilation of newborn rat with moderate VT induces pulmonary early growth response (Egr1) and Fas ligand (FasL) expression. A 24-h ventilation of 7-day rat with VT of 7–9 ml/kg increased number of Egr1 and FasL transcripts (A, left and middle) and immunopositive (brownish) cells (B and A, right) in whole lung compared with 8-day nonventilated rat lung. C: FasL (green) immunolocalizes with pro-SPC (alveolar type II cell marker: red) in the ventilated newborn rat lung (merged yellow indicates colocalization). AW, alveolar walls; V, vessel. *P < 0.05.
VENTILATION INDUCES FASL-MEDIATED APOPTOSIS

A

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<tr>
<th>Control Lung</th>
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<tr>
<td>8-day</td>
<td>7-day</td>
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<td>Egr1 Transcripts</td>
<td>FasL Transcripts</td>
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B

8-day control lung

7-day ventilated lung

C

FasL/proSPC

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labeling (TUNEL; using in situ cell death detection-POD Kit; Roche, Montreal, QC, Canada), and sections were stained with anti-cleaved caspase-3 (1:200 dilution; Cell Signaling Technology, Danvers, MA), anti-early response growth (Egr)1 (1:50 dilution, Cell Signaling Technology) and anti-FasL (1:500 dilution; Thermo Scientific, Ottawa, ON, Canada) antibodies using the avidin-biotin (ABC) immunoperoxidase method (21). For immunofluorescence sections were stained with anti-FasL (1:500 dilution) and anti-prosurfactant protein C (1:1,000 dilution; Abcam, Cambridge, MA). For quantitative analysis, digital images were captured at ×20 magnification with random sampling of all tissue in an unbiased fashion. Images were captured randomly from 15 nonoverlapping fields/slide with 3 slides/animal and 4 animals/group.

**Cell stretch.** Rat fetal lung fibroblast (FLF) and fetal lung distal epithelial (FLDE) cells (day 19 of gestation) were isolated as previously described (5). Within 24 h of isolation, fibroblast and epithelial cells (10⁶ cells/well) were separately inoculated on Bioflex multwell plates precoated with collagen I and maintained in MEM + 10% FBS. Six hours before stretch medium was changed to MEM + 0.5% FBS. Cells received fresh MEM + 0.5% FBS and were then mounted in a Flexercell FX-4000 Strain Unit (Flexercell, Hillsborough, NC). Control cells were grown on the Bioflex collagen I plates, treated in the same manner as stretched cells, but were subjected to no stretch. Cyclic continuous radial elongations of 5, 10, or 17% were applied at intervals of 30 cycles per min for 2, 4, 6, or 12 h. In some cases cells were subjected to intermittent radial elongation of 5% at a frequency of 30 cycles per minute, for 15 min each hour, for 24–48 h. Cells treated for 3 h with staurosporine, which induced apoptosis, served as positive control. Following cyclic stretch, cells were assessed for nuclear fragmentation, DNA laddering, poly(ADP-ribose) polymerase-1 (PARP-1) activity, membrane permeability, mitochondrial membrane potential, and caspase activation.

**DNA fragmentation.** Following exposure to stretch, cells were stained with DAPI to visualize nuclear fragmentation. DNA laddering was measured using the ApoAlert LM-PCR Ladder Assay Kit (Clontech Laboratories, Palo Alto, CA) according to the manufacturer’s instructions. Amplified products were visualized by electrophoresis in 1.5% agarose ethidium-bromide gels.

**PARP activity.** After being stretched, cells were harvested and resuspended in 1 ml 56 mM HEPES, 28 mM KCl, 28 mM NaCl, 1.5% agarose ethidium-bromide gels. A 500-μl aliquot of cell suspension was added to premixed Eppendorf tubes containing 25 μl digitonin, 25 μl nicotinamide adenine dinucleotide (NAD), and 3 μl [3H]NAD (ICN Biomedicals, St. Laurent, PQ, Canada) and incubated at 37°C for 5 min. The reaction was stopped by addition of 200 μl of ice-cold 50% (vol/vol) trichloroacetic acid (TCA) and centrifugation at 10,000 g for 10 min. The supernatant was discarded, and the pellet was washed twice with ice-cold 20% (vol/vol) TCA. The final pellet was solubilized in 200 μl 2% (vol/vol) SDS and 0.1 M NaOH, placed in scintillation vials, and counted. PARP activity in counts per minute (cpm/10⁶ cells) was expressed as fold change of PARP activity vs. control samples.

**Membrane permeability.** After being stretched, cells were harvested and resuspended in 1 ml PBS and 1 μl of 100 μM YO-PRO-1 and 1.5 mM propidium iodine (Molecular Probes, Eugene, OR) was added. The cells were incubated on ice for 30 min in the dark and analyzed by flow cytometry (FACS Calibur using CELLQuest software; Becton Dickinson, Mountain View, CA), measuring the fluorescence emission at 530 and >575 nm.

**Mitochondrial membrane potential.** After being stretched, cells were harvested, resuspended in 2 ml PBS, and stained with 2 μg/ml JC-1 (5,5′,6′,6-tetrachloro-1,1′,3,3′-tetrabromphenylalanine-carboxylic acid iodide: Molecular Probes, Eugene, OR) dye for 30 min at 37°C. Following extensive washing, cells were analyzed by flow cytometry. Mitochondrial membrane depolarization was monitored as a result of a shift in emission spectra after 480 nm excitation from orange-red (590 nm, for JC-1 aggregates) to green (530 nm, for JC-1 monomers). The green and red fluorescence signals were resolved by detection in the conventional FL1 and FL2 channels, respectively.

**Caspase activities.** After stretch, cells were harvested and protein lysates were extracted. Lysates were incubated with and without either caspase-3 (DEVD-fmk), caspase-8 (IETD-fmk), or caspase-9 (LEHD-fmk) substrates (Biomol, Plymouth Meeting, PA) for 2 h at 37°C in 96-well bottom black-coated plates (Sigma, St. Louis, MO). Activity was measured with a fluorometer set a wavelength of 400 nm. The relative increase in caspase activity in stretched cells vs. static control cells was calculated after subtracting the background obtained from lysates containing no substrate.

**Western blot analysis.** Total protein extracts were subjected to 10% SDS-PAGE, transferred to PVDF membranes, and immunoblotted as previously described (4, 26). Primary antibodies (all from Cell Signaling Technology, Danvers, MA) were rabbit anti-cleaved caspase-3 (dilution 1:500), rabbit anti-cleaved caspase-7 (dilution of 1:1,000), rabbit anti-cleaved caspase-8, (dilution of 1:500), rabbit anti-cleaved caspase-9 (dilution 1:500), and rabbit anti-Fasl (dilution of 1:1,000).

**Caspase inhibition.** Cells were stretched in medium containing either DMSO (vehicle control) or 5 μM caspase inhibitor benzoyl-carbonyl-Val-Ala-Asp-fluoromethylketone (z-VAD-fmk; R&D Systems Minneapolis, MN) or IDN6556 [Idun Pharmaceuticals; donated by Dr. Mingyao Liu (University Hospital Network, Toronto, ON, Canada)]. Following cyclic stretch, cells were analyzed for caspase-3 activation and DNA laddering.

**Neutralizing FasL.** Cells were stretched in the presence and absence of 3 μg/ml of either neutralizing hamster anti (mouse and rat)-CD178 (FasL) antibody (BD Biosciences, Pharmingen, San Jose, CA) or nonimmune IgG, followed by assessment of cleaved caspase-3 and-8 expression, caspase-8 activation, and DNA laddering.

**Quantitative RT-PCR.** Total RNA was extracted from lung tissues or cells and reverse transcribed (26). Complementary DNA was amplified for target genes Egr1, Fas, and FasL (25). For quantification, polymerase chain reaction signals were compared between groups after normalization using 18S as internal reference. Fold change was calculated (28).

**Statistical analysis.** All values of animal experiments are presented as means ± SE of at least four separate animals. All values of cell experiments are shown as means ± SD of at least three separate experiments carried out in triplicate. Statistical analysis was by Student’s t-test or, for comparison of more than two groups, by one-way ANOVA followed by Duncan’s multiple range comparison test. Significance was inferred where P < 0.05.

**RESULTS**

**In vivo stretch-induced apoptosis.** Prolonged (24 h) mechanical ventilation with moderate VT significantly increased the number of TUNEL-positive cells in lungs of 7-day old rat pups vs. nonventilated 8-day rat pups (Figs. 1A, a–c, and B). The increase in lung cell apoptosis by prolonged ventilation was confirmed by anti-cleaved caspase-3 immunohistochemistry (Fig. 1A, d–f). In adult rat lung, high VT rapidly induces Egr1, a transcription factor implicated in regulating FasL expression (2, 7). Therefore, we investigated the effect of prolonged ventilation with moderate VT on Egr1 and FasL expression in newborn rat lung. Prolonged ventilation increased significantly Egr1 and FasL mRNA and protein levels (Fig. 2A, left and middle, and B) as well as the number of FasL-positive cells (Fig. 2A, left and right). Immunohistochemical localization indicated upregulation of Egr1 primarily in alveolar walls (Fig. 2B), in agreement with previous findings for Egr-1 in adult lung (37). Colocalization of FasL and pro-SPC (type II cell marker) immunofluorescence demonstrated the presence of
Fig. 3. A continuous 17% cyclic stretch induces apoptosis in fetal rat lung epithelial cells. A: increases in nuclear fragmentation (left, arrows) and DNA laddering (right) were noted in fetal lung distal epithelial (FLDE) cells after 4 h of stretch. B: DNA laddering (left) and poly(ADP-ribose) polymerase-1 (PARP-1) activity (right) were increased after 6 h of stretch in FLDE cells, but not in fetal lung fibroblasts (FLF). C: stretch (6–12 h) increased membrane permeability in FLDE cells. NS, nonstretched; C, static control; S, stretch; St, staurosporine- positive control. *P < 0.05 and ++P < 0.05 vs. 6- or 12-h controls.
FasL in type II epithelial cells and in non-SPC expressing cells (Fig. 2C).

**In vitro stretch-induced apoptosis.** Next, we utilized rat FLDE cells in to investigate the underlying mechanism of ventilation (stretch)-induced apoptosis. Nuclear fragmentation and DNA laddering (Fig. 3) were detected in FLDE cells subjected to continuous 17% cyclic stretch (mimics moderate/high V̇T ventilation) for 4 h, compared with static cultured control cells. This cell stretch regimen for 6 h also increased PARP activity in FLDE cells but did not trigger DNA fragmentation or changes in PARP activity in FLF cells (Fig. 3B). Additionally, FLDE membrane permeability (i.e., fluorescent dye YO-PRO-1 absorbance) was increased compared with control cells (Fig. 3C). After 6 h of stretch, membrane transport became transiently defective and the rate of YO-PRO-1 uptake was significantly increased (1.5 × fold increase vs. static control; \( P < 0.001 \)) and was further increased (1.7 × fold vs. control; \( P < 0.001 \)) after 12 h of stretch. This stretch-induced permeability increase over time correlated with increased DNA fragmentation (data not shown). Together, these data suggest that continuous 17% cyclic stretch rapidly induces apoptosis in FLDE cells. To determine if lower levels of stretch would also initiate an apoptotic response in FLDE cells, cells were subjected to lesser stretch regimens. Neither intermittent stretch (5%; mimics fetal breathing movements) or continuous cyclic stretch (mimics regular breathing) induced DNA fragmentation nor did the membrane permeability change in FLDE cells (data not shown). Longer duration of stretch (17% cyclic stretch for 72 h) also induced apoptosis in FLF (data not shown).

**Stretch-induced apoptosis: role of caspases.** Universal inhibitors of caspases (ZVAD-fmk and IDN6556) abrogated stretch-induced DNA laddering in FLDE cells (Fig. 4A). Indeed, stretch increased the amount of cleaved caspase-3, -7, and -8 (Fig. 4B) as well as the activities of caspase-3 and -8.

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**Fig. 4.** Stretch-induced apoptosis in fetal rat lung epithelial cells is caspase-dependent. **A:** universal caspase inhibitors, z-VAD-fmk and IDN-6556, abrogate DNA fragmentation in E19 fetal rat lung epithelial cells subjected to a continuous 17% cyclic stretch of 6 h. **B:** cleaved caspase-3, -7, and -8 content increases with duration of 17% stretch. **C:** increases of caspase-3 and -8 activities after 6 h of 17% stretch. \( *P < 0.05 \).
activation (Fig. 5A, bottom), suggesting no or minimal activation of the mitochondrial (intrinsic) death pathway by stretch. There was a small shift in stretched FLDE cells from red fluorescence (JC-1 aggregates) to green fluorescence (JC-1 monomers) compared with static control cells, suggesting some mitochondrial membrane depolarization in cells subjected to stretch (Fig. 5A, top) but insufficient to activate caspase-9. Both universal caspase inhibitors (ZVAD-fmk and IDN6556) abrogated 17% stretch-induced caspase-3 activation (Fig. 5B).

Stretch-induced apoptosis: role of Fas/FasL. FasL mRNA levels were increased in FLDE cells subjected to stretch (Fig. 6A, middle) while Fas mRNA expression was not changed (Fig. 6A, left). Western blotting revealed a small increase in FasL protein in stretched FLDE cells (Fig. 6A, right), and a neutralizing anti-rat FasL monoclonal antibody attenuated the stretch-induced levels of cleaved caspase-3 and -8 (Fig. 6B, left) as well as stretch-induced caspase-8 activity (Fig. 6B, right) and stretch-induced DNA laddering (Fig. 6C). A nonimmune IgG control had no effect (not shown).

DISCUSSION

Apoptosis is critical for normal lung development and function (24, 45) and plays a vital role in remodeling of lung tissue by clearing excess epithelial and mesenchymal cells after injury (40, 45). Dysfunctional apoptotic mechanisms likely contribute to the pathophysiology of many lung diseases, including BPD. Mechanical ventilation is a well-recognized risk factor for development of BPD (3). In the present study, we demonstrate that mechanical ventilation of a newborn rat for 24 h with moderate VT and in the absence of supplemental O2 increases apoptosis of alveolar type II cells, possibly via activation of the FasL/Fas (extrinsic apoptotic) pathway.

Several in vitro studies have shown that mechanical stress stimulates apoptosis in isolated lung cells (15, 17, 42). Stretch-induced apoptosis in vivo was first demonstrated in fetal rabbits after tracheal occlusion (11). A recent report demonstrated that mechanical ventilation of newborn mice for 24 h led to a fivefold increase in the number of apoptotic lung cells (35) and, in keeping with the current data, a concomitant increase in cleaved caspase-3 protein (35). The number of caspase-3-positive cells, mainly interstitial cells, was also increased after in utero ventilation of fetal sheep for 6 h (1). In both studies, ventilation-induced apoptosis coincided with a significant reduction in alveolarization (1, 35), in agreement with our findings using the newborn rat model (26). The death pathway(s) by which mechanical ventilation reduces alveolarization were not investigated. In the present study, we demonstrated that the extrinsic pathway (caspase-8 activation) was predominantly activated by mechanical stretch (mimicking moderate/high VT ventilation) in E19 fetal lung epithelial cells, resulting in activation of effector caspases-3 and -7 and subsequent DNA fragmentation. In contrast, the intrinsic pathway (caspase-9 activation) appeared not to contribute to stretch-induced apoptosis. The 17% cyclic stretch regimen increased apoptotic activity of E19 fetal rat lung epithelial cells but not FLF. In contrast, mechanical stretch simulating fetal breathing movements (5% cyclic stretch) stimulated apoptosis of E19 fetal rat lung fibroblasts (canalicular stage) but not epithelial cells, compared with control (nonstretched) cells (42). Thinning of the mesenchyme is essential during this stage of lung development to create a close link between the capillary network and airway epithelium (41) and mesenchymal apoptosis during the canalicular stage of lung development has been reported previously (4, 24, 43). These observations and our present findings suggest that during normal development, physiological stretch initiates apoptosis in fibroblasts that contributes to structural alignment in the canalicular stage; in contrast, during mechanical ventilation, pathological stretch initiates apoptosis in epithelial cells that contributes to lung injury and abnormal dep-
development. Indeed, pathological (20% cyclic) stretch has been reported to increase apoptosis and cell death of E19 fetal rat type II epithelial cells (27, 42).

In adults with acute respiratory distress syndrome epithelial cell apoptosis appears to be induced by activation of the FasL/Fas pathway (31, 39). In the setting of an inflammatory response, secreted FasL leads to lung epithelial cell apoptosis by binding to and activating Fas antigen in humans (35) and in newborn mice (10). In the present study, ventilation-induced apoptosis was accompanied by increased FasL expression in epithelial cells and stretch-induced FasL/Fas pathway activation was confirmed in FLDE cells. A 17% cyclic stretch of these cells increased expression of FasL, but not Fas, and neutralizing anti-rat FasL monoclonal antibody reduced stretch-increased levels of cleaved caspases-3, -7, and -8. These findings suggest that mechanical ventilation and cell stretch activate the FasL/Fas pathway by increased FasL expression, leading to activation of upstream initiator caspase 8, which in turn activates effector caspases-3 and -7. Alternatively, it is possible that mechanical stretch increases the proteolytic release of soluble FasL from the membrane by activation of sheddases (ADAMs) or membrane-bound metalloproteinases (e.g. MMP14), thereby promoting binding to Fas-bearing epithelial cells and inducing apoptosis. Another
possibility is that stretch causes sustained clustering of FasL and Fas thereby promoting epithelial cell apoptosis (6, 46).

Together with the upregulation of FasL, we found an increase of Egr1 expression in alveolar type II cells of newborn rats subjected to prolonged mechanical ventilation. Previous in vivo studies using adult rats (7), mice (20), and preterm fetal sheep (19) and in vitro studies using fetal rat lung epithelial cells (8) have demonstrated that Egr1 expression is upregulated in response to mechanical stress while its downregulation by carbon monoxide attenuates ventilator-induced lung injury (20). Thus Egr1 is an important proinflammatory transcriptional regulator that coordinates pro-inflammatory responses (38, 48), including transcription of genes involved in growth and apoptosis (38), including FasL (22). Increased expression of both Egr1 and FasL in alveolar type II cells after ventilation suggests that Egr1 may activate the extrinsic caspase pathway via upregulation of FasL. Another possible mediator is interleukin (IL)-8. It is known that Egr1 regulates IL-8 expression (30) and that elevated levels of IL-8 induce apoptosis via increased FasL expression (16). We have shown that mechanical ventilation of newborn rats increases pulmonary CXCL-2 (GR02/MIP-2: macrophage inflammatory protein-2, a functional rodent homolog of human IL-8) expression (25) and that the ventilation-induced upregulation of CXCL-2 expression is partly mediated via Egr1 (37). Whether CXCL-2 affects FasL expression remains to be elucidated.

Only a few studies have investigated the effect of ventilation on apoptosis in infants with BPD. In preterm infants (gestational weeks 24–37), elevated apoptotic activity was found in bronchial and epithelial cells but not in cells of mesenchymal origin (18). In lung sections from ventilated preterm infants (22–36 wk), the number of apoptotic epithelial cells was significantly greater than in lungs of stillborn fetuses of similar gestational age (32), but in contrast to our experiments, these infants were also treated with oxygen. While newborn rats have immature lungs at birth, they do not need mechanical ventilation to survive and do not lack surfactant. However, rat lungs at birth have a saccular appearance (as in preterm infants of 24–30 wks) and alveolarization is an exclusively postnatal process. In preterm infants with BPD, ventilation-induced lung injury results in defective alveolarization (20), enlarged alveoli, and an increased number of type II cells (8) have demonstrated that Egr1 expression is upregulated in response to mechanical stress while its downregulation by carbon monoxide attenuates ventilator-induced lung injury (20). Thus Egr1 is an important proinflammatory transcriptional regulator that coordinates pro-inflammatory responses (38, 48), including transcription of genes involved in growth and apoptosis (38), including FasL (22). Increased expression of both Egr1 and FasL in alveolar type II cells after ventilation suggests that Egr1 may activate the extrinsic caspase pathway via upregulation of FasL. Another possible mediator is interleukin (IL)-8. It is known that Egr1 regulates IL-8 expression (30) and that elevated levels of IL-8 induce apoptosis via increased FasL expression (16). We have shown that mechanical ventilation of newborn rats increases pulmonary CXCL-2 (GR02/MIP-2: macrophage inflammatory protein-2, a functional rodent homolog of human IL-8) expression (25) and that the ventilation-induced upregulation of CXCL-2 expression is partly mediated via Egr1 (37). Whether CXCL-2 affects FasL expression remains to be elucidated.

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In summary, our findings suggest a central role for the Fasl-dependent pathway in ventilation-induced apoptosis of alveolar type II cells, which may have therapeutic potential for preventing or treatment of BPD.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: A.A.K., V.D., and M.P. conception and design of research; A.A.K., V.D., and I.T. performed experiments; A.A.K. and V.D. analyzed data; A.A.K., V.D., and M.P. interpreted results of experiments; A.A.K. and V.D. prepared figures; A.A.K. drafted manuscript; A.A.K., B.P.K., and M.P. approved final version of manuscript; B.P.K. and M.P. edited and revised manuscript.

REFERENCES

VENTILATION INDUCES FASL-MEDIATED APOPTOSIS


