

Chapter 2

Invasive Mechanical Ventilation in the Pathogenesis of Bronchopulmonary Dysplasia

Lauren M. Ramos, Tanbir Najrana, and Juan Sanchez-Esteban

Introduction

Many premature infants born with underdeveloped lungs are exposed to excessive, non-physiological levels of stretch. Invasive mechanical ventilation is life saving for preterm infants with respiratory failure. However, mechanical ventilation uses positive pressure that may result in ventilator-induced lung injury (VILI). It is estimated that around 30 % of patients born before 32 weeks of gestation will develop bronchopulmonary dysplasia (BPD) [1]. BPD is a chronic lung disease characterized by alveolar hypoplasia and impaired pulmonary vascular development [2]. BPD is a devastating condition that disrupts not only normal lung development but is also associated with long-term neurodevelopmental sequelae [3]. Although the etiology of BPD is multifactorial, resulting from the interaction between genetic and environmental factors [4], invasive mechanical ventilation plays a central role. In this chapter, we will review the contribution of invasive mechanical ventilation to the pathogenesis of BPD. First, we will describe how mechanical ventilation promotes lung inflammation and injury. We will then review the biomechanical determinants and mechanotransduction in VILI. Next, we will review the signaling pathways modulating the critical stage of alveolar development and disruption by mechanical ventilation. Finally, we will go over animal models of invasive mechanical ventilation.

L.M. Ramos, MD • T. Najrana, PhD • J. Sanchez-Esteban, MD (✉)
Department of Pediatrics, Neonatal-Perinatal Medicine, Women and Infants Hospital of
Rhode Island, The Warren Alpert Medical School of Brown University,
101 Dudley Street, Providence, RI 02905, USA
e-mail: LauRamos@wihri.org; TNajrana@wihri.org; jsanchezesteban@wihri.org

Role of Mechanical Ventilation in Causing Lung Inflammation and Injury

The pathogenesis of BPD is multifactorial. However, inflammation secondary to mechanical injury plays a central role. Numerous studies have demonstrated that invasive mechanical ventilation induces lung injury [5–7]. Overdistension of the lung damages resident cells and disrupts the alveolar–capillary barrier with subsequent release of proinflammatory cytokines, increase in permeability, and influx of neutrophils and macrophages to the lung [8]. In the next section, we will review how positive pressure ventilation promotes inflammation and lung injury (VILI). It is difficult to completely tease out the contribution of mechanical distention alone to the pathogenesis of lung injury, given that the majority of premature infants exposed to mechanical ventilation also receive moderate amount of oxygen. Moreover, many premature infants might have been exposed in utero to chorioamnionitis and therefore are more susceptible to lung injury after birth [9].

Mechanical Injury of Lung Resident Cells

In order to understand the effects of invasive mechanical ventilation on the generation of inflammation in the lung, it is important to review the contribution of individual cells to lung injury using in vitro model systems.

Alveolar Type II Epithelial Cells (AECII) Type II epithelial cells are key components of the alveolar structure. Physiological mechanical stimulation of alveolar type II cells is important for surfactant secretion [10] and surfactant protein gene expression [11]. However, alveolar type II epithelial cells can also be exposed to overstretch, and therefore to injury secondary to mechanical ventilation. Previous in vitro studies in adult rat type II epithelial cells have demonstrated that excessive stretch can induce apoptosis [12, 13] and cell membrane stress failure [14] resulting in cell death [15]. In addition, cyclic stretch of adult type II cells can also promote inflammation by stimulating release of interleukin-8 (IL-8) [16, 17] and monocyte chemoattractant protein-1 (MCP-1) [18]. Studies from our laboratory have showed that excessive stretch of rat fetal type II cells isolated on embryonic day 19 (E19) of gestation (transition from canalicular to saccular stages of lung development) induces necrosis, apoptosis, proliferation, and inflammation [19]. Moreover, other investigations have also shown that type II epithelial cells are an important source of chemokines that orchestrate leukocyte migration to the peripheral lung during stimulation with lipopolysaccharide (LPS) [20]. These studies support a role for type II cells in the pathogenesis of BPD by releasing proinflammatory cytokines after injury induced by mechanical ventilation.

Alveolar Type I Epithelial Cells (AEC1) AEC1 appear in the late canalicular period and increase in number during the saccular and alveolar stages of lung development. Given that they cover much of the distal epithelium of the lung, AEC1 are at risk for injury mediated by mechanical ventilation. However, the contribution of type

I cells to the pathogenesis of BPD is not clearly defined, probably because of the difficulty in isolating AEC1 cells in vitro [21]. Nevertheless, recent studies have found that these cells produce tumor necrosis factor- α (TNF- α), IL-1 β (IL-1 β), and IL-6 after exposure to LPS [22]. In fact, some authors believe that AEC1 are a more important source of proinflammatory cytokines than AEC2 [23]. Moreover, the receptor for advanced glycation endproducts (RAGE), found only on AEC1 in the lung, may also participate in inflammation and alveolar simplification. AEC1 are important to maintain adequate fluid balance in the alveolus via the tight junctions [24]. These junctions could be affected by mechanical injury leading to pulmonary edema [25].

Fibroblasts Fibroblasts are critical for lung development and repair [26]. Fibroblasts can also secrete cytokines and trigger an inflammatory response [27]. Given that interstitial cells are directly exposed to mechanical injury, we investigated whether lung fibroblasts participate in lung injury secondary to mechanical stretch. Fetal mouse lung fibroblasts isolated during the saccular stage of lung development were exposed to 20 % cyclic stretch to simulate mechanical injury. Our data showed that mechanical stretch increased necrosis and apoptosis in these cells. In addition, there was increased release of proinflammatory cytokines and chemokines IL-1 β , MCP-1, regulated on activation, normal T-cell expressed and secreted (RANTES), IL-6, keratinocyte chemoattractant (KC), and TNF- α [28]. These studies suggest that mesenchymal cells could be an important source of proinflammatory cytokines and chemokines after exposure to mechanical ventilation.

Endothelial Cells Similar to alveolar epithelium, vascular endothelial cells can sense mechanical signals during mechanical ventilation. It has been demonstrated that endothelial cells exposed to 15–20 % distention increase production of the proinflammatory cytokines IL-8 and MCP-1 [29]. Furthermore, excessive mechanical stretch also alters endothelial cell barrier properties contributing to increase in alveolar–capillary permeability and pulmonary edema [30].

Macrophages Lung macrophages exposed to 12 % cyclic strain, to mimic mechanical ventilation, released inflammatory mediators such as TNF- α , IL-8, IL-6, matrix metalloproteinase-9 (MMP-9), and nuclear factor-kappa light-chain-enhancer of activated B cells (NF- κ B). Synergistic proinflammatory effects of mechanical stretch and bacterial endotoxin were observed, supporting the concept that mechanical ventilation is particularly deleterious in infected lungs [31].

In summary, these in vitro studies indicate that pulmonary resident cells may play a critical role in the initiation of the inflammatory cascade by sensing mechanical signals mediated by mechanical ventilation and triggering the release of proinflammatory cytokines (Fig. 2.1).

Alterations in Alveolar–Capillary Permeability

The increase in alveolar–capillary permeability is present during the early stages of mechanical injury [8]. Protein leakage into the lung of preterm infants has been observed soon after the initiation of mechanical ventilation [32]. There are

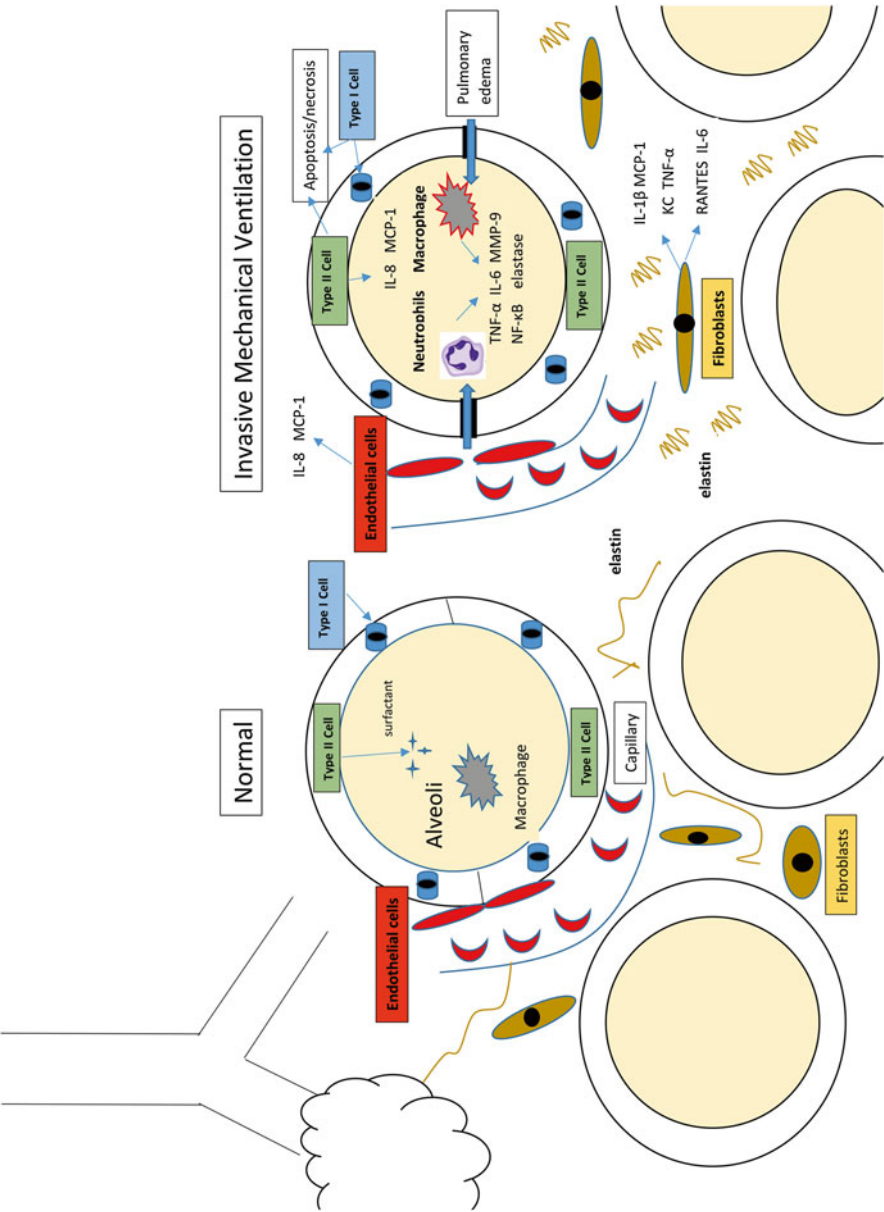


Fig. 2.1 Invasive mechanical ventilation can damage lung resident cells, such as type II and type I epithelial cells, fibroblasts, endothelial cells, and macrophages, with subsequent release of proinflammatory cytokines. In addition, overdistension of the lung can disrupt the alveolar–capillary barrier causing an increase in permeability, followed by pulmonary edema, and further recruitment of neutrophils and macrophages to the lung. *IL* interleukin, *MCP-1* monocyte chemoattractant protein-1, *TNF- α* tumor necrosis factor alpha, *MMP-9* matrix metalloproteinase-9, *NF- κ B* nuclear factor kappa-light-chain-enhancer of activated B cells, *KC* keratinocyte chemoattractant, *RANTES* regulated on activation, normal T-cell expressed and secreted

experimental evidences to support damage to the alveolar–capillary barrier induced by mechanical injury [7]. Major alterations in pulmonary epithelial and endothelial permeability have been reported in isolated lungs and intact animals exposed to mechanical ventilation. For example, increased vascular permeability and subsequent alveolar edema occurred with a peak inspiratory pressure as low as 13 cm H₂O in isolated perfused rat lungs [33]. Likewise, microvascular permeability injury was also demonstrated in intact animals [34]. Positive pressure ventilation in lambs resulted in altered alveolar permeability to small solutes using clearance of aerosolized ^{99m}Tc-DTPA as a marker [35]. Electron microscope studies provided clear evidence for the major abnormalities in the epithelial and endothelial cells that resulted in increased permeability during VILI. Damage of alveolar type I epithelial cells was observed in rabbits ventilated with a peak inspiratory pressure of 20 cm H₂O for 6 h [36]. In these studies, some endothelial cells were detached from their basement membrane, resulting in the formation of intracapillary blebs. There were also occasional breaks in endothelial cells. More prolonged exposure to injurious stress produced alveolar epithelial pathology ranging from inter and intracellular gap formations with denuded basement membranes to extensive cell destruction [37]. The blood–gas barrier of rabbit lungs exposed to high capillary pressures revealed not only epithelial gaps and endothelial lesions but also basement membrane breaks. Although increase in microvascular transmural pressure may contribute to pulmonary edema, these electron microscopy findings strongly support that changes in the permeability of the alveolar–capillary barrier are a main determinant of ventilator-induced pulmonary edema [7].

Influx of Neutrophils and Macrophages to the Lung and Their Contribution to the Inflammatory Response

Increase in pulmonary vascular permeability and subsequent influx of neutrophils and macrophages into the lung occur soon after initiation of mechanical ventilation [32, 38]. As discussed before, the initial influx of inflammatory cells is probably initiated by lung resident cells, as demonstrated in isolated and perfused rat lung exposed to mechanical injury [39]. However, neutrophils and macrophages recruited to the lung play a critical role in pulmonary inflammation by producing inflammatory mediators, recruiting more inflammatory cells, and therefore amplifying the inflammatory response (Fig. 2.1). There are many studies demonstrating the influx of leukocytes into the lung in response to mechanical ventilation [40, 41]. Woo and Hedley-Whyte [42] observed that overinflation of lungs in open-chest dog models produced edema and accumulation of leukocytes and macrophages in the alveoli. Similar findings were observed in piglets ventilated at high tidal volumes [43] and in premature monkeys exposed to mechanical ventilation. However, neutrophil influx occurs earlier during the inflammatory response followed by macrophages [41]. These data are consistent with bronchoalveolar lavage studies in premature infants exposed to mechanical ventilation where neutrophils were detected at 3–4 days, whereas macrophages were predominantly observed after 10 days [44].

Migration to the lung and activation of neutrophils and macrophages are mediated largely via proinflammatory cytokines. Tracheal aspirates of premature infants with BPD show an increase of chemoattractant factors such as IL-8, IL-6, TNF α , IL-1, IL-16, complement component 5a (C5a), lipoxygenase products, leukotriene B₄, elastin fragments, fibronectin, MCP, macrophage inflammatory protein (MIP), etc. [38, 45]. Among them, IL-8 and perhaps IL-6 seem to have important roles in the initial recruitment of inflammatory cells to the lung, given that elevation of these cytokines was found to precede the influx of neutrophils in tracheal aspirates from preterm infants who developed BPD [38, 46]. It has been shown experimentally that ventilation strategies with high or low volumes have an impact on the release of proinflammatory cytokines [39, 47]. Moreover, several animal models of BPD have demonstrated that invasive mechanical ventilation increases the level of proinflammatory cytokines [40, 48–51]. On the other hand, low levels of anti-inflammatory cytokines, such as IL-10, were observed in the bronchoalveolar lavage of patients with BPD [52]. In vitro studies from our laboratory have shown that mechanical injury of fetal epithelial cells and fibroblasts increases the release of proinflammatory cytokines and decreases the release of IL-10. Preincubation of these cells with IL-10 prior to stretch reduced apoptosis and the release of proinflammatory cytokines [19, 28]. These studies indicate that an imbalance between proinflammatory and anti-inflammatory cytokines, favoring proinflammatory cytokines, could be critical to promote lung injury [38].

In addition to releasing proinflammatory cytokines, neutrophils and macrophages produce potent proteases, especially elastase, which is believed to play an important role in the damage of the extracellular matrix (ECM) and impairment of alveolarization observed in patients with BPD [53]. Transforming growth factor-beta (TGF- β) is induced in the lung after injury to limit the inflammatory response and to regulate tissue repair [54]. However, in the presence of persistent injury, this process could be abnormal, resulting in severe pulmonary fibrosis [55]. It has been demonstrated that overexpression of TGF- β resulted in significant fibrosis with extensive deposition of ECM proteins such as collagen, fibronectin, and elastin [56]. TGF- β is produced mainly by macrophages, and elevated levels of TGF- β in the bronchoalveolar lavage of premature infants are predictive and associated with BPD [57, 58].

Biomechanical Determinants of VILI

Under normal conditions, the lung is exposed to a variety of mechanical forces such as mechanical strain and shear stress. *Mechanical strain* occurs when a force is applied to an elastic tissue causing stretch or distortion. These forces are generated to overcome the elastic recoil of the lung during normal breathing. During lung injury, the magnitude of these forces is increased. A second form of mechanical distortion is *shear stress*, generated when fluids such as blood or air move across a cell surface, generating a force parallel to the plasma membrane that produces a tangential distortion of the cell. Such shearing forces occur in the conducting airways, due to airflow, and in the vascular systems as a consequence of blood flow [59].

VILI is the result of complex interactions among various forces acting on lung structures during mechanical ventilation [60]. In a broad sense, the concept of VILI can be defined at the whole organ, cellular, or molecular levels. There are various forms of VILI, including volutrauma (caused by overdistension of the lung), atelectrauma (by repeated opening/closing of the lung), and biotrauma (by release of inflammatory mediators). Studies by Dreyfuss and Saumon in the late 1990s [7] clearly showed that lungs are injured if they are inflated to volumes that exceed total lung capacity (*volutrauma*) [5]. This concept has been tested in preterm lambs, where as few as six manual inflations of 35–40 mL/kg compromised the subsequent response to surfactant administration [61]. Likewise, preterm lambs ventilated with a tidal volume of 20 mL/kg before surfactant treatment had more acute lung injury than lambs ventilated with 5 or 10 mL/kg [62]. Similarly, lung injury can occur if lungs are ventilated below a normal functional residual capacity (FRC) (*atelectrauma*) resulting in cyclic opening and closing of the lung and injury. During positive-pressure ventilation, the distal lung can undergo rapid and cyclic transitions from being small and flooded at the end of expiration to being large and air-filled at the end of inspiration. This transition can generate significant shear stress forces and lung injury at the alveolar surface as the edema fluid redistributes and the alveolar volume changes. In the surfactant-deficient lung, ventilation at low lung volumes caused release of cytokines *in vivo* and in isolated lungs [47] and during intermittent mandatory ventilation; use of inadequate PEEP to maintain an appropriate FRC also increased lung injury [63]. On the other hand, recruitment of lung volumes to increase FRC protected against VILI and reduced the need for high levels of inspired oxygen [64]. Therefore, these studies clearly show that in premature infants on mechanical ventilation, the use of optimum tidal volume in combination with “ideal” PEEP would minimize lung injury.

There are several factors, from the biomechanical point of view, to explain injury of the lung. First, the lung of premature infants is not uniformly ventilated; there are areas of normal ventilation alternating with poorly aerated or even completely collapsed lung units. Therefore, the less injured areas of the lung are preferentially recruited during positive-pressure ventilation with the subsequent risk of injury from overexpansion. In addition, the resistance to lung expansion is not uniform. This heterogeneity in lung impedance results in shear stress being generated between neighboring, interdependent units that operate at different volumes [65]. Moreover, there is also injury to small airways and distal lung caused by the repeated opening and closure and by stress on the lining cells by the movement of air–liquid interfaces with respiration [66].

How does the lung respond to prevent/minimize lung injury? One of the mechanisms is by the *reorganization of the cell's stress-bearing elements* formed by elastin and collagen network of the ECM, integrins, and cytoskeleton. During mechanical ventilation, epithelial cells, basement membrane, and endothelial cells are exposed to mechanical deformation, and as a result, there is an increase in basement membrane surface area and a change in the shape of epithelial and endothelial cells. The cellular change is generally accompanied by the reorganization of the cell's stress-bearing elements. Therefore, this deformation-induced remodeling of the stress-bearing elements is critical to prevent cellular stress failure. Another important

mechanism is by *vesicular lipid trafficking* to and from the plasma membrane. This lipid trafficking serves to regulate cell surface area and plasma membrane tension and ultimately helps to prevent plasma membrane stress failure [67–69].

Despite all these preventive mechanisms, excessive mechanical forces can injure the plasma membrane [37, 70]. It has been calculated that “a typical plasma membrane can sustain strains of only 1–3 % before it breaks” [71]. Several studies have examined the biophysical determinants of plasma membrane stress failure in cultured alveolar epithelial cells and found a correlation with strain amplitude and strain rate [15, 68, 72]. The ability to restore membrane integrity after cell wounding is essential for cell survival. One of the mechanisms is by “self-sealing” whereby hydrophobic interactions between phospholipids and water would drive lipid flow toward the free edges of a defect [73, 74]. Another mechanism is mediated by calcium. Small disruptions trigger calcium-dependent exocytosis of vesicles near the wound site, lower plasma membrane tension, and thereby facilitate wound closure [75]. For larger defects, vesicular endomembranes present in the lysosomes are transported and fused to the membrane defect via a rise in cytosolic Ca^{2+} [76–78].

Alveolar Epithelial Barrier Function The epithelial barrier is composed of tight junctions connected to the actin cytoskeleton via occludin or zonula occludens. It has been shown that mechanical strain of alveolar epithelial cells, mimicking mechanical ventilation with high tidal volumes, resulted in actin-mediated cell contraction with subsequent increased paracellular permeability [79]. This process is regulated by the family of Rho GTPases RhoA, Rac-1, and Cdc42 and mediated by Ca^{2+} /calmodulin-dependent phosphorylation of myosin light chain (MLC) kinase with consequent contraction of the actin–myosin complex and breakdown of intercellular junctions [80, 81]. In addition to maintaining the integrity of the epithelial barrier by the tight junctions, the cells need mechanisms to reabsorb the fluids present in the interstitium and alveolar spaces after lung injury mediated by mechanical ventilation [82]. This process is mediated by active transport of Na^+ through amiloride-sensitive cation channels (ENaC) present in the apical cell membranes and the Na^+/K^+ -ATPases localized mainly in the basolateral cell membrane [83–86]. It has been shown that mechanical ventilation with higher tidal volumes leads to decreased alveolar fluid reabsorption [87], probably due to a decrease in Na^+/K^+ -ATPase activity [88].

Alveolar Epithelial Plasma Membrane Stress Failure As discussed before, cells experience plasma membrane stress failure when the matrix to which they adhere undergoes large deformations and this is a frequent mechanism for necrotic cell death [89]. Data from animal models clearly demonstrate that ventilation with high distending pressures causes tissue destruction [7, 90]. This was tested in ex vivo mechanically ventilated rat lungs. They found that the number of subpleural cells with membrane defects rises with increasing tidal volumes and duration of ventilation. In addition, they found that over 60 % of injured cells were able to repair the plasma membrane defects [70]. In vitro studies from our laboratory also found that

20 % stretch of fetal epithelial cells increased release of lactate dehydrogenase (LDH) by 50 % when compared to control, unstretched cells [91] supporting the concept that overdistension of the membrane can cause cell death by necrosis.

Stress Failure of Endothelial and Epithelial Barriers Not only can the plasma membrane break, but also the contact between cells [14, 92]. If such a disruption happens to the pulmonary endothelial and epithelial barrier, this could cause a loss of compartmentalization. As a result, local proinflammatory mediators can spread in the systemic circulation [93–95]. In addition, loss of the barriers will also allow white blood cells to enter the lungs and promote inflammation. Therefore, exposure to mechanical force produces alveolar epithelial pathology ranging from inter and intracellular gap formations, denudation of the basement membrane, and cell destruction [7, 36, 96]. The current hypothesis is that disruption of the integrity of the endothelial cellular barrier occurs via cytoskeletal rearrangement and generation of tensile forces within the cell [97]. These changes result in cellular contraction with subsequent interruption of intercellular adhesion complexes and the creation of gaps between neighboring cells, or through cells, that allow the exudation of fluid, macromolecules, and leukocytes into the interstitium and ultimately into alveolar spaces [98–101].

Mechanotransduction in Ventilator-Induced Lung Injury (Fig. 2.2)

Mechanotransduction refers to the processes through which cells sense and respond to mechanical stimuli by converting them to biochemical signals that elicit specific cellular responses [102]. The degree and magnitude of mechanical deformation observed with injurious forms of ventilation probably do not occur normally in nature. Therefore, it is unlikely that evolutionary mechanisms have been specifically developed to deal with the overdistension that can occur with mechanical ventilation. Consequently, the inflammatory response generated by mechanical ventilation might “borrow” mechanoreceptors and signal transduction pathways from other more established signaling systems [60, 103]. An example is the epidermal growth factor receptor (EGFR). Mechanical signals through this receptor are important for lung development [104]. However, EGFR also participates in mechanical ventilation-induced lung injury [105].

Mechanoreceptors

1. Stretch-activated Ion channels
2. Integrins–focal adhesions–cytoskeleton

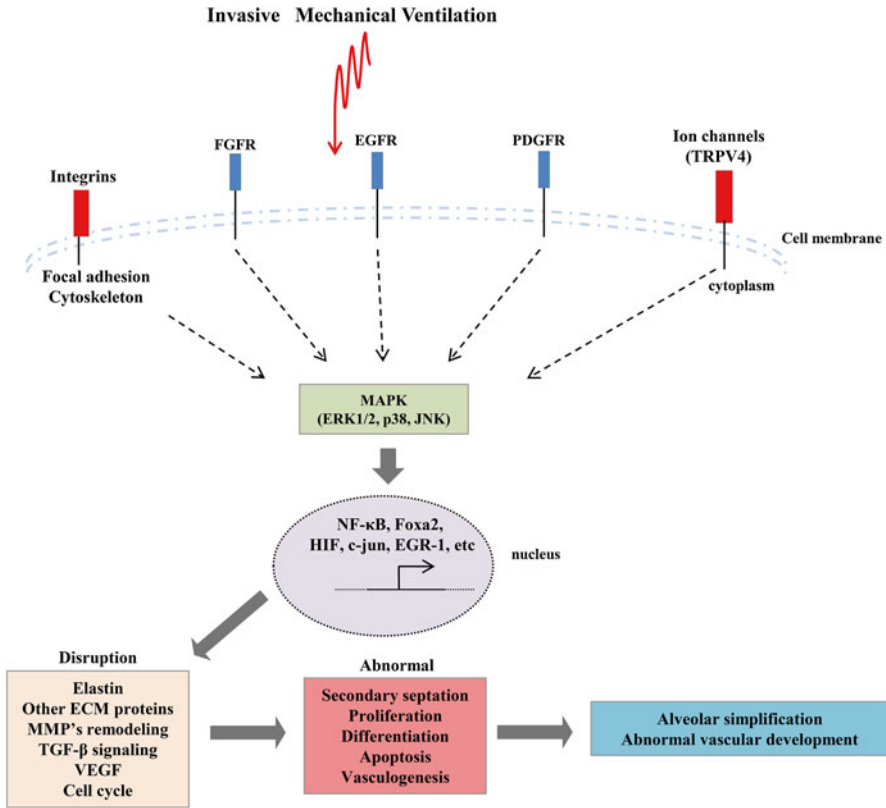


Fig. 2.2 Mechanoreceptors and signaling pathways activated by invasive mechanical ventilation that lead to abnormal alveolar development. Abbreviations: *FGFR* fibroblast growth factor receptor, *EGFR* epidermal growth factor receptor, *PDGFR* platelet-derived growth factor receptor, *TRPV* transient receptor potential vanilloids, *MAPK* mitogen-activated protein kinases, *ERK* extra-cellular signal-regulated kinases, *JNK* c-Jun N-terminal kinases, *NF-κB* nuclear factor kappa-light-chain-enhancer of activated B cells, *Foxa2* forkhead box A2, *EGR* early growth response, *HIF* hypoxia-inducible factors, *ECM* extracellular matrix, *MMP* matrix metalloproteinase, *TGFβ* transforming growth factor beta, *VEGF* vascular endothelial growth factor

Stretch-Activated Ion Channels

It has been demonstrated that physical forces applied to the plasma membrane of the cells can increase the permeability for ions [106, 107]. Ion channels can be activated either by direct opening of the channel by strain or as a consequence of forces transmitted to the channel via membrane-associated proteins [108]. Previous studies in alveolar epithelial cells have shown that Na^+ pump activity increases in proportion to the magnitude of stretch [60]. Moreover, cation channels seem to mediate the inflammatory response generated in the lung after mechanical stretch. Parker et al. [109] found that the increased microvascular permeability by mechanical injury was abolished by gadolinium (a stretch-activated nonselective cation channels

inhibitor), suggesting that stretch-activated cation channels may initiate the increase in permeability induced by mechanical ventilation. Waters et al. [110] also showed that $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity was significantly increased in murine lung epithelial cells exposed to mechanical stretch. These studies suggest that stretch-activated cation channels may also regulate $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity, a factor that may play an important role in lung edema clearance.

Calcium channels are also important mechanoreceptors in lung injury. Boitano et al. demonstrated that mechanical stretch of airway epithelial cells opened the voltage-gated Ca^{2+} channels [111]. Transient receptor potential vanilloids (TRPV) channels have gained recent attention as key modulators of lung injury. TRPV4 in particular is a Ca^{2+} -permeable cation channel known to play an important role in osmotic and mechanical sensing [112]. In adult lungs, TRPV4 is expressed in the capillaries of septal walls and in alveolar type I cells. TRPV4 agonists produced blebs or breaks in the endothelial and epithelial layers of the alveolar wall and increased lung endothelial permeability [113]. In addition to regulating the integrity of the lung alveolar capillary endothelium [114–116] and maintenance of the airway epithelial barrier function [117], TRPV4 has been recently implied as a key mediator of inflammation. TRPV4 inhibitors have potent anti-inflammatory effects by limiting neutrophil and macrophage infiltration, and blunting proinflammatory cytokine and chemokine production [118]. Moreover, recent studies from our laboratory have shown that TRPV4 is expressed in the fetal lung and may play an important role in the transduction of mechanical signals in the distal epithelium by modulating inflammation via p38 and extracellular signal regulated kinases (ERK) pathways [119]. All together, these data suggest that TRPV4 blockade may represent a therapeutic approach to decrease pulmonary edema and inflammation in lungs exposed to mechanical ventilation.

Integrins–Focal Adhesions–Cytoskeleton

Integrins, focal adhesion plaques, and the cytoskeleton are the probable key structures in sensing mechanical signals [120]. Integrins belong to a family of transmembrane receptors that cluster in focal adhesion plaques [121]. Their main role is to anchor cells to the basement membrane and ECM [122]. The cytoplasmic portion of integrins is in close contact with proteins such as vinculin, paxillin, talin, Src kinase, and focal adhesion kinase, which serve as adapter molecules for anchoring the cytoskeleton microfilaments and microtubules to the plasma membrane [122, 123]. Conformational changes of integrins, such as those induced by mechanical strain, lead to the activation of focal adhesion kinase and the mitogen-activated protein kinase (MAPK) pathway [124–126]. Previous studies from our laboratory in isolated fetal epithelial cells showed that mechanical strain induces conformational changes of integrin $\alpha 6 \beta 1$ and releases EGFR ligands via ADAM 17 [127].

Other models propose that the cytoskeleton itself senses cellular deformation. Stretch-induced deformation of the actin filaments and microtubules can activate intracellular signaling pathways similar to those induced by stimulation of focal adhesion kinase [128, 129].

Evidence also indicates that cell matrix and adhesion molecules may be important in mediating the inflammatory response characteristic of VILI. It has been shown that magnetic twisting applied to beads coated with collagen, but not to control beads, increased transcription of inflammatory genes such as TNF- α and TGF- β , suggesting that both the magnitude and specific cell–matrix connections are important determinants of the airway epithelial response to mechanical forces [60, 108].

Mechanotransducers and Signaling Pathways Related to VILI

Growth Factor Receptors There is evidence to suggest that receptor tyrosine kinases can serve as mechanotransducers [130]. It has been demonstrated that mechanical forces can activate platelet-derived growth factor (PDGF) receptor in vascular smooth muscle cells [131] and fetal rat lung cells [132]. Moreover, compressive stress of airway epithelial cells [133] or mechanical strain of fetal epithelial cells [127, 134] activates EGFR via release of EGFR ligands by an autocrine/paracrine mechanism.

Intracellular Signaling Pathways A growing body of evidence indicates that MAPK plays an important role in transducing mechanical signals to the nucleus [120]. MAPK signaling pathways regulate essential cellular functions, such as cell proliferation, inflammation [135], and programmed cell death [136]. Activation of ERK, c-Jun N-terminal kinases (JNK), and p38 has been observed in various cell types exposed to mechanical stretch [126, 137–140]. Uhlig et al. [141] showed that ERK and JNK are phosphorylated in rat lungs following injurious mechanical ventilation. Protein kinase A, a cAMP-dependent kinase, has been shown to be increased in mechanically ventilated animals [142] and in cultured fetal type II epithelial cells exposed to stretch [143].

The adherens junction and gap junction seem to be also important in mechanotransduction [144]. Ko and coworkers demonstrated that mechanical forces applied to adherens junctions in fibroblasts activate stretch-sensitive calcium channels and increase actin polymerization [135]. In addition, mechanical stretch-induced intercellular Ca²⁺ signaling in airway epithelial cells [145, 146]. The intercellular calcium propagation was mediated via gap junctions [147]. Considering the importance of integrity of alveolar epithelium in maintaining the barrier function in the lung, cell–cell interaction may play an important role in mechanotransduction, especially during mechanical ventilation [107].

Transcription Factors One of the most important mediators of inflammation is NF- κ B, which plays a critical role in the generation of proinflammatory cytokines in response to mechanical injury. NF- κ B contains a DNA “shear-stress” response element in the promoter region and binds to IL-6, IL-8, IL-1 β , and TNF- α promoter sequences [60, 148]. A number of in vitro and ex vivo studies have shown that NF- κ B is upregulated in response to stretch [149–151]. NF- κ B activation was observed in the tracheal secretions of premature infants exposed to mechanical ven-

tilation. Furthermore, stimulation of NF- κ B was associated with elevated levels of TNF α , IL-8, prolonged mechanical ventilation, and BPD [152, 153].

Gene Expression Several studies have addressed gene expression profiling in VILI. Copland et al. found upregulation of transcription factors (early growth response-1 or EGR-1 and c-Jun), stress proteins (heat shock protein-70 or HSP-70), and inflammatory mediators (IL-1 β) after 30 min of high tidal volume ventilation in rats [154]. Microarray analysis of isolated perfused mouse lungs ventilated with high tidal volumes revealed upregulation of genes related to growth factors, cellular communication, cytoskeletal function, proinflammatory cytokines and genes for immune/defense response, signal transduction pathways, and apoptosis [155–157]. Transcription analyses of lung samples from ventilated premature baboons, mouse, and rat models of BPD identified several highly conserved genes in response to mechanical ventilation. These included elastin (ELN), gastrin-releasing polypeptide (GRP), and connective tissue growth factor (CTGF) [158].

Signaling Pathways Modulating Alveolar Development and Disruption by Mechanical Ventilation (Fig. 2.2)

In order to understand the potential impact of mechanical ventilation on lung development, it is important first to review the molecular mechanisms regulating late stages of lung development and, specifically, alveolar formation. Extremely low birth weight (ELBW) infants are born at the transition from the canalicular to the saccular stages of lung development. At this time, the histology of the distal lung is characterized by clusters of widened air space called saccules. The walls of these sacs, the primary septae, are closely associated with the capillary network. At the same time, the cuboidal epithelial cells begin to differentiate into the alveolar epithelial type 1 and type 2 cells (AEC1 and AEC2). During alveolarization, the saccules are subdivided by the ingrowth of ridges or crests known as secondary septae. Both myofibroblast and endothelial cells migrate to these crests, and a scaffold of matrix proteins is deposited, enriched in elastin at the tip [159]. These later stages of lung development require a precise temporal and spatial coordination of multiple signaling pathways, making this process susceptible to disruption by mechanical ventilation [160, 161].

Alveolar Septation

Alveolar septation involves substantial tissue growth and remodeling, including the deposit of elastin and other proteins and ECM remodeling [160]. Elastin deposition at the tip of the primary septa plays a critical role in secondary septation or alveolarization. Myofibroblasts produce elastin from tropoelastin by the enzyme lysyl

oxidase. The essential role for elastin in alveolar development was demonstrated in genetically modified mice. Deletion of the elastin gene impaired distal airway development with fewer and dilated distal air sacs and attenuated tissue septa [162]. Myofibroblasts differentiation and elastin production are regulated by platelet-derived growth factor- α (PDGF- α). Knockout mice lacking PDGF- α show loss of alveolar myofibroblasts and associated elastin fiber deposits, resulting in a significant compromise in alveolar formation [163, 164]. Retinoic acid (RA) and fibroblast growth factor (FGF) are also key controllers of elastin formation. RA enhances the expression of PDGFA/PDGFR α [165] and tropoelastin [166], and increases the total number of alveoli [167, 168]. FGF signaling is also important for alveolarization. Secondary septation is compromised in mice lacking FGFR3 and FGFR4 [169]. Moreover, FGF18 promotes myofibroblast expression of tropoelastin, lysyl oxidase, and microfibril proteins such as fibrillins and fibulins that act as scaffold for elastin assembly [170].

Elastin was found to be increased in ventilated infants who died from BPD [171, 172]. Elastic fibers were described as thickened, tortuous, and irregularly distributed [173]. Moreover, increased elastase activity was observed in guinea pigs exposed to mechanical ventilation [174] and in infants who developed BPD [175]. Therefore, exaggerated elastase activity can result in destruction of mature cross-linked elastic fibers, reduction of proteins that regulate elastic fiber assembly and disordered pulmonary elastic deposition, as described in preterm lambs and newborn mice exposed to mechanical ventilation [176, 177]. Disruption of other ECM proteins such as hyaluronan, laminin, etc. has been also attributed to elastase hyperactivity [178, 179]. Taken together, these studies indicate that elastolytic damage may contribute to reduced secondary septation and alveolarization and explain altered lung elastin deposition and defective septation observed in patients with BPD [180, 181].

In addition to elastin, deposition of other components of the ECM, such as collagen, fibronectin, and proteoglycans is important for alveolar septation [160]. Postmortem studies in newborn infants with BPD found a damaged collagen network with thickened collagenous saccular walls and a wide interstitium with increased quantity and size of collagen fibers [182].

Remodeling of the ECM is also critical for alveolar septation. MMPs play a central role in this process. MMP-1, MMP-2, and MMP-9 are expressed during alveolarization in humans and mice [183, 184]. Mice lacking MMP-2 show abnormal saccular development [185]. However, enhanced proteolytic activity also appears to be detrimental to the developing lung. Overexpression of MMP-2, MMP-8, and MMP-9 has been identified in airway secretions of infants with BPD [186]. A similar pattern was found in the bronchoalveolar lavage fluid in baboon models of BPD [187, 188]. Therefore, a perturbation of a fine balance of ECM remodeling by MMPs induced by mechanical ventilation could have a negative impact on alveolar septation.

TGF- β plays an important role in lung development by regulating cell survival and ECM production and remodeling [189]. It has been shown that transient decrease in TGF- β activity alters cell proliferation and inhibits septation [190]. In

contrast, overexpression of TGF- β 1 in the neonatal mouse lung induces histological changes observed in BPD, such as enlarged alveolar sacs, poor secondary septation, thick and hypercellular septa, and abnormal capillary development [168, 191, 192]. High levels of TGF- β have been detected in airway secretions from preterm infants with BPD [58]. Moreover, TGF- β signaling is disrupted in animal models of BPD. Mechanical ventilation increases the expression of TGF- β in preterm lambs [193], and neutralization of the abnormal TGF- β activity improves alveologenesis and microvascular development in the injured developing lung [194]. The mechanism by which an increase of TGF- β activity alters alveologenesis is not clearly defined although previous studies indicate that it could be mediated in part by enhancing apoptosis and decreasing proliferation of AEC2 cells [195]. Thus, the balance of TGF- β signaling appears to be critical to alveolar development.

Regulation of Cell Proliferation, Differentiation, and Apoptosis

Tissue remodeling during later stages of lung development requires well-coordinated regulation of cell proliferation and apoptosis [168]. Proliferation and differentiation of the distal epithelium play a critical role in alveolar septation. As discussed before, at the transition from canalicular to saccular stages, cuboidal epithelium begins to differentiate into alveolar type II and type I cells. Both cell types are critical in the signaling pathways regulating alveolar formation. For example, deletion of the transcription factor *Foxa2*, required for alveolar type II cell differentiation [196], caused extensive airspace enlargement and altered septation [197]. Moreover, mice null for *T1 α* , an alveolar type I cell marker, showed abnormal distal lung cell proliferation, and narrower and irregular air spaces [198]. These studies support that both type II and type I cells are important for alveologenesis.

Several growth factors have been shown to promote proliferation and differentiation of epithelial cells. FGF7 (keratinocyte growth factor) for example, has a potent stimulatory effect on proliferation and maturation of type II cells [199]. FGF7 has also been shown to prevent lung epithelial injury induced by mechanical ventilation [200]. Insulin growth factor (IGF) also promotes alveolar development [201]. Heparin-binding EGF (HB-EGF) has been shown to induce type II cell differentiation and a modulator of cell proliferation [127, 134].

Studies in the premature baboon model of BPD [202] and newborn rats [203] have demonstrated that mechanical ventilation causes cell cycle arrest at the G1–G2 phase by affecting cyclin and Cdk expression. Both mesenchymal and epithelial cells were equally affected. In vitro studies from our laboratory also found that mechanical strain inhibits cell proliferation in fetal rat lung cells [91]. This effect could be mediated via the EGFR since decrease of proliferation mediated by mechanical stretch was absent in fibroblasts isolated from EGFR knockout mice [204]. Given the critical role of cellular proliferation in alveolar formation, these studies support the concept that proliferative arrest secondary to mechanical ventilation may cause a reduction in alveolarization resulting in alveolar simplification.

Apoptosis is also important for normal lung development and specifically for remodeling of the distal lung [205]. However, an imbalance between proliferation and apoptosis can compromise alveolar development. Studies by Bland's group observed a fivefold increase in the number of terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-stained cells with a corresponding increase in active caspase-3 protein in the lungs of pups exposed to mechanical ventilation without a significant effect on cell proliferation, as assessed by proliferating cell nuclear antigen (PCNA) staining of lung sections. These findings, which are similar to those previously reported for 4-day-old pups that received mechanical ventilation with 40 % O₂ for 24 h [180], suggest that cyclic stretch of the lung during development increases apoptosis and causes septal loss leading to defective alveolar formation [177].

Vascular Endothelial Growth Factor

Vascular Endothelial Growth Factor (VEGF) is critical not only for angiogenesis [206] but also for alveolarization [207, 208]. Administration of VEGF inhibitors impaired pulmonary vascular growth and secondary septation [207, 209] VEGF expression is decreased in patients with BPD. Histology of these patients shows rarefied and dilated peripheral vessels, fewer air–blood barriers, and a decreased airspace–parenchyma ratio [210, 211]. VEGF is a downstream target of hypoxia-inducible factors (HIFs). Deletion of HIF-2 reduces VEGF levels and leads to early death due to respiratory failure [212]. Relative hyperoxic conditions associated with preterm birth could potentially lead to decreased expression of HIF [213]. In addition, premature infants exposed to mechanical ventilation also received a moderate amount of oxygen. All of this could inhibit HIF with the subsequent decrease in VEGF expression and disruption of lung vascular growth and alveolar septation [208]. VEGF is decreased in the preterm baboon model of BPD [214]. Mechanical ventilation of newborn mice reduces VEGF expression and results in lung structural abnormalities consistent with BPD [181]. Moreover, overexpression of VEGF in newborn rats increases survival, promotes lung angiogenesis, and prevents hyperoxia-induced alveolar damage [215].

Animal Models of Invasive Mechanical Ventilation

Animal models of invasive mechanical ventilation have provided insights into the mechanisms of VILI [168, 216, 217] Studies in preterm baboon at the saccular stage have shown that mechanical ventilation with moderate degree of hyperoxia induces inflammation, by releasing proinflammatory cytokines and recruiting inflammatory cells to the lung, and impairs alveolar and vascular development [49,

214, 218]. Similar findings were observed in chronically ventilated preterm lambs [40]. Even a short period of mechanical ventilation was sufficient to induce inflammation, demonstrated by an increase of the proinflammatory cytokines such as IL-1 β , IL-6, MCP, etc. [48]. High tidal volume ventilation at the alveolar stage in rats and mice also promoted inflammation [50, 51, 219]. Some limitations of these models are the inclusion of hyperoxia injury in addition to mechanical stretch caused by the ventilators. To specifically address the component of mechanical strain in lung injury, Bland's group [177] exposed 6-day-old mice to mechanical ventilation without hyperoxia and found that stretch alone can inhibit alveolar septation and angiogenesis and increase apoptosis and lung elastin, which was distributed throughout alveolar walls rather than at septal tips. In another model, Harding's group [220] investigated the injurious effects of mechanical ventilation per se in the immature lung using a technique to ventilate the lungs of the ovine fetus with an intact placenta. After 24 h of mechanical ventilation of preterm lungs at the sacular stage, lung parenchyma and bronchioles were severely injured; tissue space and myofibroblast density were increased, collagen and elastin fibers were deformed and secondary crest density was reduced. Bronchioles contained debris and their epithelium was injured and thickened. In this model, mechanical ventilation did not upregulate genes involved in the production of proinflammatory cytokines [220]. In a similar model, injury caused by mechanical ventilation increased EGR-1 and expression of pro- and anti-inflammatory cytokines within 1 h. Mechanical injury induced granulocyte/macrophage colony-stimulating factor and matured monocytes to alveolar macrophages by 24 h [48]. Also, and as previously discussed, mechanical ventilation for 24 h without oxygen in a 7-day rat model led to cell cycle arrest [203]. In summary, these models show that mechanical injury without hyperoxia replicate some of the hallmarks typical of BPD such as inflammation, impairment of alveolarization, altered elastin deposit, and abnormal vasculogenesis.

Conclusions

In conclusion, overdistension of the lung can damage lung resident cells and disrupt the alveolar–capillary barrier with subsequent release of proinflammatory cytokines, increase in permeability, and influx of neutrophils and macrophages to the lung. In addition to inducing an inflammatory response, invasive mechanical ventilation can also have a negative impact on important signaling pathways regulating alveolar development, such as secondary septation, cell proliferation, differentiation, apoptosis, and vasculogenesis. The final consequence is alveolar simplification and abnormal vascular development observed in the histology of patients with BPD. Therefore, aggressive extubation and the use of noninvasive modes of ventilation [221], would be considered beneficial and have been shown to have a positive impact on the incidence of this devastating disease in premature infants.

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