Cellular Stress Failure in Ventilator-injured Lungs

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The clinical and experimental literature has unequivocally established that mechanical ventilation with large tidal volumes is injurious to the lung. However, uncertainty about the micromechanics of injured lungs and the numerous degrees of freedom in ventilator settings leave many unanswered questions about the biophysical determinants of lung injury. In this review we focus on experimental evidence for lung cells as injury targets and the relevance of these studies for human ventilator-associated lung injury. *In vitro*, the stress-induced mechanical interactions between matrix and adherent cells are important for cellular remodeling as a means for preventing compromise of cell structure and ultimately cell injury or death. *In vivo*, these same principles apply. Large tidal volume mechanical ventilation results in physical breaks in alveolar epithelial and endothelial plasma membrane integrity and subsequent triggering of proinflammatory signaling cascades resulting in the cytokine milieu and pathologic and physiologic findings of ventilator-associated lung injury. Importantly, though, alveolar cells possess cellular repair and remodeling mechanisms that in addition to protecting the stressed cell provide potential molecular targets for the prevention and treatment of ventilator-associated lung injury in the future.

Keywords: alveolar epithelium; cell injury; cell mechanics; cell repair; mechanical ventilation, plasma membrane tension

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INTRODUCTION

In the United States over 100,000 individuals each year develop Acute Lung Injury (ALI) and Acute Respiratory Distress Syndrome (ARDS), the more severe form of ALI (1). Current estimates of attributable mortality for ALI/ARDS range between 17,000 and 43,000 persons per year. Numerous studies suggest that ALI mortality has improved during the past two decades, but remains high, ranging between 25 and 50% in the most current series (2–6). While mechanical ventilation is integral to the management of patients with injured lungs, a recent ARDS network trial has made it clear that the choice of ventilator settings accounts for as many as one-third of all deaths attributed to ALI (7). This, and a preceding smaller trial (8), represent the successful bench-to-bedside culmination of decades of research that had suggested artificial ventilation can either cause *de novo* lung injury or aggravate preexisting lung injury (reviewed in Reference 9).

The breadth and depth of knowledge regarding mechanical ventilation, ventilator-induced lung injury (VILI), barotrauma, "so-called" biotrauma, and mechanotransduction is far too great to do it justice in a single review. The reader is referred to several outstanding reviews on the pathophysiology and clinical manifestations of VILI (9, 10), on the molecular biology of pulmonary mechanotransduction (11–18), and on the effects of deforming stress on surfactant biology (19–22). In this review we will therefore focus primarily on the structural failure of cells and tissues, a relatively novel and underappreciated area of lung biology, as it is causally related to many of the disease manifestations in ventilator-injured lungs. Widespread endothelial and epithelial cell disruption and plasma membrane blebbing are indeed hallmarks of the entity and account at least in part for the increased microvascular permeability that is readily observed in experimental models of VILI (23–28).

PULMONARY MICROMECHANICS

A review of the cellular pathology of ventilator-injured lungs would be incomplete without some discussion of the current understanding of pulmonary micromechanics in health and disease. It is important to note that there remain major gaps in knowledge because the available imaging tools lack sufficient temporal and/or spatial resolution to quantify stresses and strains on the scale of interest. Nonetheless, an appreciation of the governing principles provides the foundation for the design of physiologically relevant *in vitro* and *in vivo* studies, the physiologic basis for understanding experimental results, and ultimately a basis for their logical application to modes of clinical practice. Throughout this review we will use terms such as stress, strain, elastic modulus, or stiffness, and have listed their definitions in Table 1.

Alveolar Micromechanics of the Normal Lung

For more than 50 years it has been appreciated that the topographical distributions of lung parenchymal stress and strain are

TABLE 1. DEFINITION OF PHYSICAL TERMS

Parameter	Definition	Comment
Strain	Dimensionless parameter describing deformation	Strain can be thought of as an extension ratio, e.g., the fractional length change of a spring under a stress. Ideally, the reference state is the unstressed state of the spring/material. The lung *in situ* is always prestressed, i.e., transpulmonary pressure is not zero. Therefore, unless stated otherwise we reference lung strain to the lung dimension (volume) at end expiration (e.g., lung strain is tidal volume divided by the volume at end-expiration)
Stress	Force per unit area	Note that pressure and stress have identical units and definitions
Stiffness	Quantity that relates stress and strain according to Hook's law	Engineers may refer to stiffness as elastic modulus or shear modulus. It has the units of stress
Yield Stress	The maximal pressure which a substance is capable of supporting without fracturing	Yield Stress is typically 10^{-2} to 10^{-3} times the shear modulus
Plastic Deformation	A deformation of a body caused by an applied stress which remains after the stress is removed	Think mashed potato! All biologic materials including cells undergo plastic deformations, i.e., they do not behave like ideal springs

nonuniform, and the biophysical determinants of this nonuniformity are generally understood (29–32). Accordingly, the lungs and the boundary structures to which they must conform (ribcage, diaphragm abdomen, heart, and mediastinum) are considered gravitationally deformed elastic solids. The shape matching of lung and boundary structures imposes a nonuniform stress and strain field. At least in quadrupeds, the weight of the lung is only a minor determinant of nonuniform transpulmonary pressures and alveolar volumes (30). The gravitational deformations of heart and diaphragm/abdomen turn out to be much more important determinants of regional volume and ventilation than the actions of gravity on the lungs themselves (33, 34). Furthermore, with increasing precision of methods for measuring regional lung function it is now apparent that there is considerable small-scale heterogeneity in lung parenchymal strain that cannot be explained by any gravitational mechanism (35, 36).

Measurements of lung strain in recumbent dogs suggested that the linear dimensions of the lung increase by as much as 40% during an inflation from functional residual capacity (FRC) to total lung capacity (TLC) (37). However, this value is a gross overestimate of the elastic deformation experienced by individual cells and tissue matrix. The lung parenchyma is a connective tissue network that is distorted by surface tension (38). Embedded in this network are airways and blood vessels, which resist deformation to a greater extent than the surrounding parenchyma. This difference in mechanical properties is an important source of interdependence and explains why in cases of barotrauma extraalveolar air generally tracks along bronchovascular bundles or why edema fluid accumulates in perivascular cuffs (39). Models of parenchymal micromechanics that are based on morphometric analyses of perfusion-fixed tissue specimens consider the helical network of elastin and collagen fibers that form the alveolar ducts as the primary stress-bearing structures (40–45). The alveolar walls in turn are largely supported by surface tension, and are thought to simply unfold as lung volume increases (46). This explains why macroscopic strains computed from lung regions that are more than 1 cm^3 may grossly overestimate the stretch experienced by lung cells during breathing. Aware of this limitation, Tschumperlin and Margulies traced the lengths of alveolar basement membranes in electron microscopic images of alveolar walls and estimated their area change with transpulmonary pressure and volume (47). Accordingly, the basement membrane area increased by approximately 35% during an inspiratory capacity maneuver, which corresponds to a linear strain and hence cell stretch estimate of about 15% (47). These observations were in keeping with earlier work suggesting that in the normal tidal breathing range alveolar septae simply unfold as opposed to being stretched (38). Because most fixatives affect tissue hydration and surface tension and thereby distort lung architecture relative to its *in vivo* state, the current models of alveolar micromechanics await morphometric confirmation on living, unfixed specimens (48). Microscopic imaging of canine subpleural alveoli through a pleural window suggested that alveolar volume changes little during normal breathing (49) and that the acinus expands nonuniformly (43). Because the mechanics of subpleural alveoli may be dominated by their coupling to a relatively inelastic pleural membrane, which in these experiments had to be immobilized to generate a focused image, the amplitude of alveolar volume change during quiet breathing is likely to remain a topic of active investigation.

Alveolar Micromechanics in Injury States

There is a relative paucity of detailed morphometric data on injured lungs, and their interpretation is controversial (50–55). The long-held view that the heavy injured lung collapses under its own weight has been challenged (56–58). The challenge rests on the assertion that fluid accumulates in small airways and distal airspaces, which prevents as opposed to promotes the collapse of dependent lung tissue. The effects on gas exchange, i.e., shunt and low $\dot{V}/\dot{Q}$, are similar, regardless of whether one views the dependent lung as airless and collapsed (tissue dimensions are decreased) or occluded by liquid plugs and expanded by edema (tissue dimensions are normal or increased). However, the stresses to which airway and alveolar lining cells are exposed during breathing could be quite different.

Injured lungs possess two attributes that explain why they are at increased risk for additional deformation injury. The first attribute is that the number of alveoli capable of expanding during inspiration is decreased. The injured lung contains normally aerated, poorly aerated, and nonaerated respiratory units (59). A smaller number of less injured units are preferentially recruited (referred to by Gattinoni as "baby lung" [60]), and thus receive a large proportion of the delivered tidal volume. This explains the increased risk of injury from regional overexpansion. The second attribute is that the local impedance to lung expansion is heterogeneous and a result of the distribution of liquid and surface tension in distal airspaces. This heterogeneity in lung impedances results in shear stress being generated between neighboring, interdependent units that operate at different volumes, as was first detailed by Mead and colleagues (61). They pointed out that forces carried by elements of a uniformly

expanded network structure must be uniformly distributed, but that departures from uniformity do generate large stress concentrations. One of the reasons for such stress concentrations, they argued, was that a change in dimension of one element (e.g., a collapsed alveolus) mandates that insertion forces of neighboring elements (the surrounding parenchyma) act over a smaller area. Consequently, local stress, defined as force per unit area, has to increase. Even though Mead's numeric analyses focused on the reduction in alveolar surface area as principle source of heterogeneous stress and ignored strain gradients in the surrounding parenchyma, the resulting insights have proven fundamental to understanding the mechanics of injured lungs.

In addition to stress concentrations resulting from interdependence, there is also injury to small airways and alveolar ducts caused by their repeated opening and closure and by energy dissipation during liquid bridge fracture or stress that is imposed on lining cells by the movement of air–liquid interfaces with respiration (62–64). Modeling approaches to bubble and liquid flow in tubes, while constrained by simplifying assumptions (e.g., rigid tube of uniform diameter, smooth surface), are beginning to shed some light on more quantitative aspects of this problem (65, 66). The relative contribution of these distinct injury mechanisms in different syndromes and disease models is simply not known. Inferences from animal experiments with short-term physiologic endpoints are at best hypothesis generating, but have yet to demonstrate the circumstance under which any one of these injury mechanisms prevails.

THE BLOOD–GAS BARRIER OF VENTILATOR-INJURED LUNGS

The first experimental study of VILI appeared in 1964 and demonstrated that mechanical ventilation with high volumes and pressures altered the surface properties of canine lung extracts (67). In 1974 Webb and Tierney reported that mechanical ventilation with large tidal volumes caused hemorrhagic pulmonary edema in rats (68). The findings established that deforming stresses associated with mechanical ventilation could alter lung barrier function and moreover impair the integrity of the blood–gas barrier. The clinical relevance of these findings was not appreciated until Egan, and later Parker and coworkers, began to explore the effects of lung volume and mechanical ventilation on pulmonary vascular barrier properties (69–73). The critical care community took note of this work only after Dreyfuss and colleagues confirmed Webb and Tierney's observations and demonstrated that tidal volume was a more appropriate determinant of deforming and potentially injurious stress than was peak airway pressure (reviewed in References 9 and 24).

Morphology

Dreyfuss and colleagues were the first to characterize in detail the morphology of the blood–gas barrier of ventilator-injured rat lungs (24), extending earlier observations by John and colleagues in mechanically ventilated rabbit lungs (27). Electron micrographs of rat lungs taken after 5 minutes of injurious ventilation showed interstitial edema and endothelial lesions consisting of plasma-membrane blebs and loss of cell contact with the basement membrane. More prolonged exposure to injurious stress produced alveolar epithelial pathology ranging in spectrum from inter- and intracellular gap formations (Figure 1A) with denuded basement membranes to extensive cell destruction (9, 24, 27). These changes in cellular ultrastructure may be viewed as evidence for deformation related cell remodeling and/or yielding of the cells' stress-bearing elements. Interestingly, type II alveolar epithelial cells appeared relatively spared, suggesting that they had experienced a smaller deformation on account of their location in alveolar corners.

In a series of studies motivated by interest in high-altitude physiology, the group of West (reviewed in Reference 74) studied the consequences of capillary pressure on the blood–gas barrier (Figure 1B). The blood–gas barrier of rabbit lungs exposed to high capillary pressures revealed not only transcellular epithelial gaps and endothelial lesions, but also basement membrane breaks (75, 76). However, frank cell necrosis and large alveolar wounds were not observed. On the basis of these findings West's group suggested that under certain conditions capillary pressures could exceed the structural limit of the basement membrane, which is the primary stress-bearing element of the blood–gas barrier. For the most part it is composed of a network of type IV collagen fibers, which can withstand considerable tensile stress (74). Physiologic and pathologic conditions in which pulmonary capillary stress failure has been observed or strongly suspected include high-altitude pulmonary edema (77), congestive heart failure, mitral stenosis, and Goodpasture's Syndrome, which is characterized by an immune mediated weakening of the collagen IV lattice (78), as well as high intensity exercise in race horses (75) and elite athletes (79). West's group also emphasized important mechanical interactions between lung volume, capillary pressure, and the probability of capillary stress failure, which is in keeping with experimental observations on isolated perfused and mechanically ventilated rabbit lungs and a case report of a patient with ARDS (75, 80).

Although structural failure of capillary basement membranes has not to date been demonstrated in ventilator-injured lungs, the presence of pulmonary hemorrhage in rat and canine VILI models, which is readily apparent to the naked eye, would be hard to explain by any other mechanism (24, 68, 81). At the same time, not all hydrostatic pulmonary edema results in capillary stress failure (53, 54). That is because the transmural pressures at which intraalveolar capillaries experience yield stress is quite high and was estimated by West and colleagues to approximate 40 mm Hg (75). Capillary pressures associated with ultrastuctural changes in adherent endothelial and epithelial cells tend to be considerably lower (54).

Majno and coworkers recognized as early as 1969 that vascular permeability was at least in part controlled by contractile endothelial cell proteins (82). Majno and colleagues postulated that inflammatory mediators caused active endothelial contraction with formation of intercellular gaps and subsequent extravasation of plasma. Since then, a great deal has been learned about the endothelial regulation of pulmonary vascular barrier properties and about the role of adhesion receptors and cytoskeletal proteins in this process (reviewed in References 83–87). Moreover, a body of work including the pioneering studies by Neal and Michel on frog mesenteric vessels (Figure 1C) has established that certain endothelial agonists and high vascular pressures cause gaps not only between adjacent endothelial cells but also within or through individual endothelial cells (88–94). These gaps close rapidly upon removal of the deforming stress, restoring normal vascular permeability. These observations are in keeping with the observed plasticity of the blood–gas barrier in transient pulmonary venous hypertension (93) and intermittent hyperinflation (94).

Cellular Stress Failure in Injured Lungs

To test if plasma membrane injury and repair are phenomena in ventilator-injured lungs, Gajic and coworkers perfused *ex vivo* mechanically ventilated rat lungs with solutions containing the membrane-impermeant label propidium iodide (PI) (95). When PI enters a cell through a plasma membrane defect, it interchelates with DNA and emits red fluorescence upon excitation

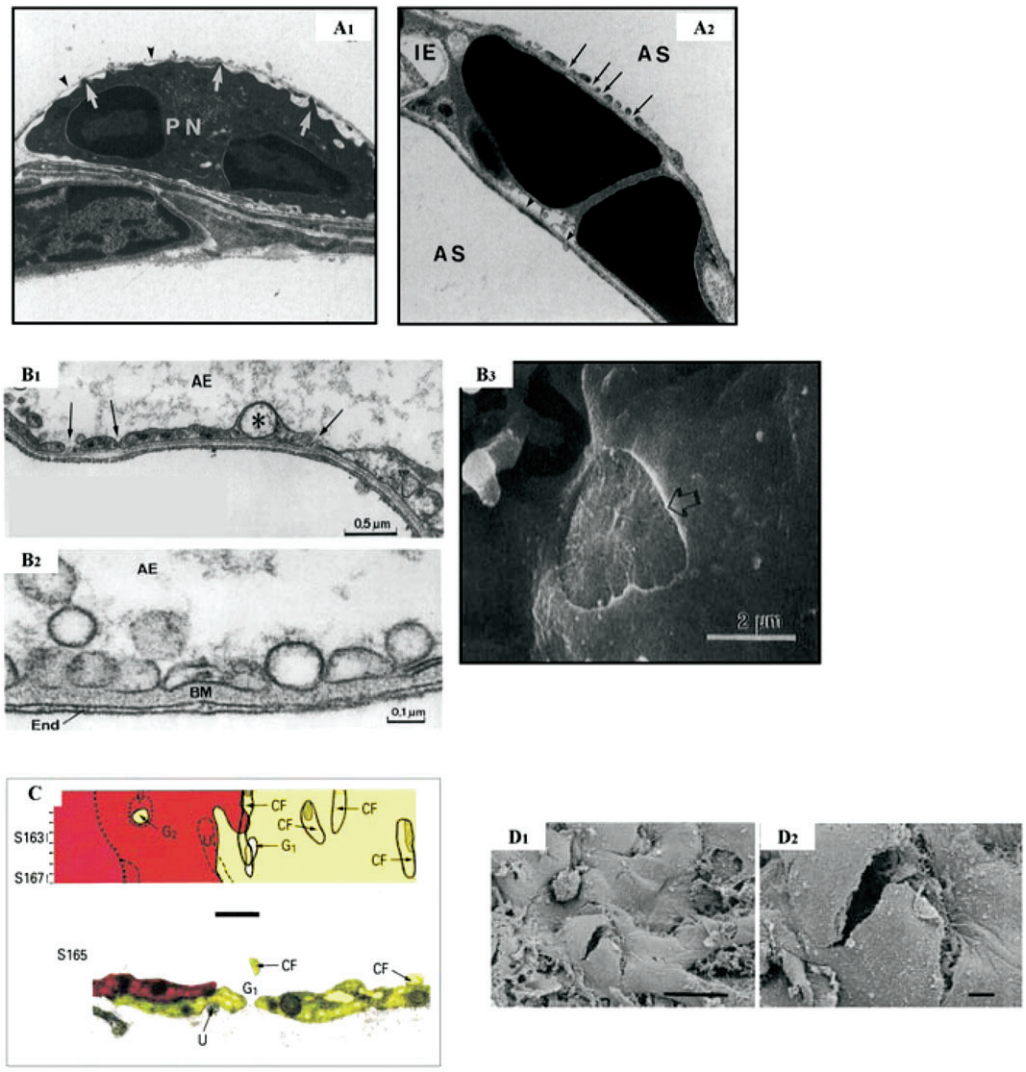

Figure 1. Examples of vascular lesions resulting from deforming stress. (*A*) Images of the blood–gas barrier (i.e., intraalveolar capillaries) of rats exposed to injurious mechanical ventilation. Note endothelial (A1) and epithelial (A2) blebbing and gaps that are marked by *arrows*. AS = alveolar space; IE = interstitial edema; PN = polymorphonuclear neutrophil. (Reproduced with permission from Dreyfuss D, et al. *Principles and Practices of Mechanical Ventilation.* New York: McGraw-Hill, 1994. pp. 793–811.) (*B*) Images of the blood–gas barrier of rabbits with hydrostatic pulmonary edema. Note blebbing and vesicle formation (B1and B2, *thin arrows* and *asterisks*) as well as the large alveolar fenestration with denuded/exposed basement membrane (B3, *wide arrow*). AE = alveolar edema; BM = basement membrane; End = endothelium. (B1 and B2 reproduced with permission from Reference 54; B3 reproduced with permission from Reference 25.) (C) Images of two adherent endothelial cells (*red* and *yellow*) from a frog mesenteric capillary that is exposed to high vascular pressures. *Upper panel* = en-face view; *lower panel* = cross-section. Note the intracellular gap formation (G1) and the preserved intercellular tight junction. (Reproduced with permission from Reference 89.) (*D*) Scanning electron-micrograph of an intraalveolar pulmonary capillary from a mechanically ventilated patient with acute respiratory distress syndrome (D1). Note that the capillary/basement membrane fracture (D2 is magnified view). (Reproduced with permission from Reference 80.)

with blue light. PI-positive cells can therefore be identified in optical sections of subpleural airspaces obtained with laser confocal microscopy (Figure 2). In a series of validation experiments, Gajic and colleagues showed that the number of subpleural cells with membrane defects increases with increasing tidal volumes and duration of stress exposure, and that cell injury correlates reasonably well with other physiologic and histologic injury markers. More importantly, by comparing preparations that had been labeled during ventilation at injurious settings with those labeled after removal of the injurious stress, Gajic and associates inferred that over 60% of injured cells repair plasma membrane defects.

In aggregate experimental studies confirm that injurious mechanical ventilation produces stress failure of capillary basement membranes as well as of adherent cells. It is not clear if epithelial and endothelial cells are the only lung cells predisposed to stress failure, what role the subcortical cytoskeleton plays in strain-related plasma membrane breaks, or if stress failure of the basement membrane invariably leads to stress failure of cells. It is also not clear if intercellular and/or intracellular gap formation in adherent cells is an active remodeling response to adapt to large substratum strains. In frog mesenteric microvessel experiments relevant to lung deformation, the pressure at which plasma extravasates through intracellular gaps was shown to be temperature-sensitive (96). Because low temperature had no measurable effect on the vessels' compliance, the investigators attributed gap formation to inhibition of active deformation-induced cell remodeling as opposed to structural failure consequent to a basement membrane break (i.e., the capillary is more leaky but not more fragile). Alternatively, recent experiments using rat pulmonary microvascular endothelial cells suggest that vessel leakiness resulting from mechanical cell wounding may be a result of weakened cell–cell adhesion resulting from decreases in the expression of the cell juctional protein, β-catenin (97).

CELL RESPONSES AND CONSEQUENCES OF DEFORMING STRESS

The response of cells to deforming forces is a result of the cell's ability to "sense" and transduce these stimuli. Experiments

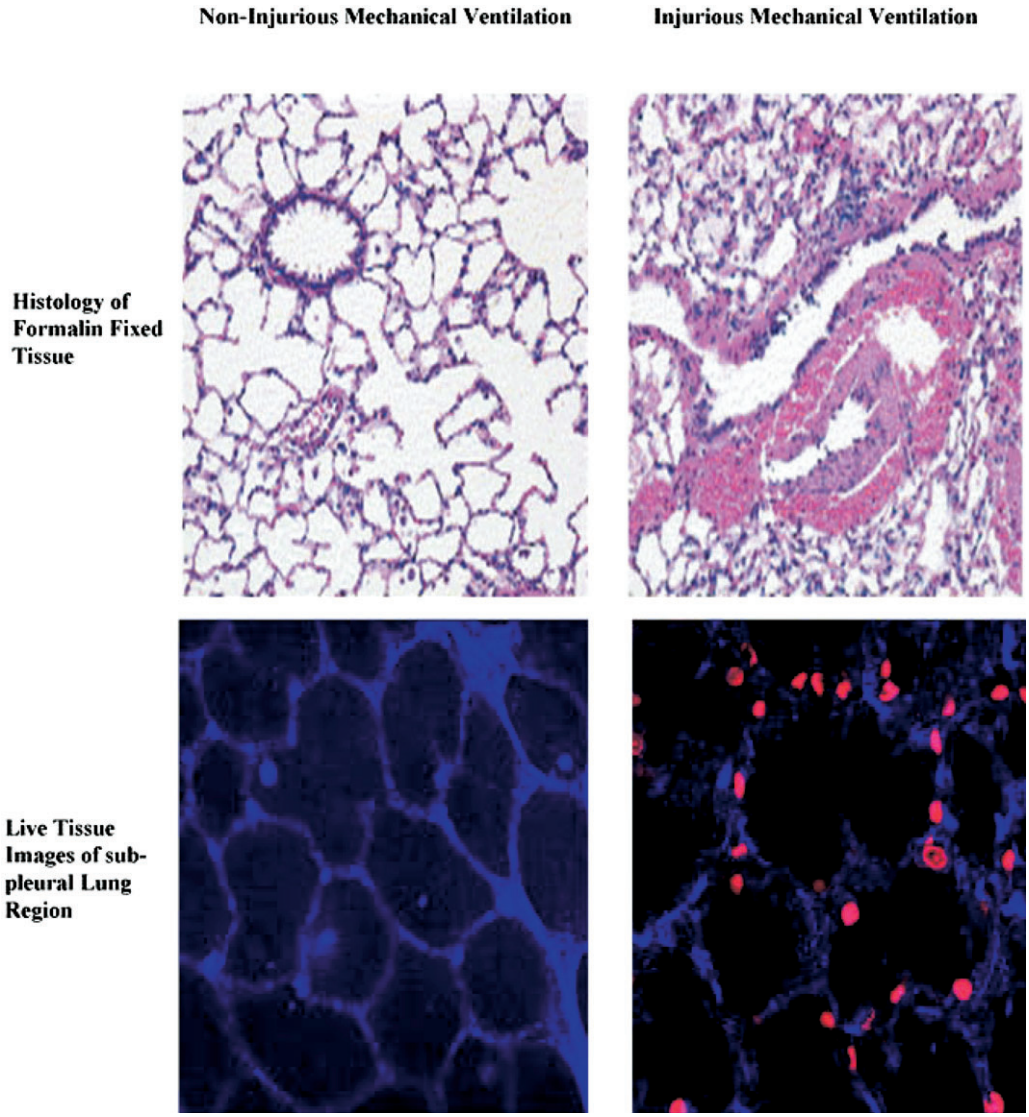

Figure 2. Light microscopic (*upper panel*) and live tissue images (*lower panel*) of isolated perfused rat lungs after mechanical ventilation at noninjurious (tidal volume 6 ml/kg) or injurious (tidal volume 40 ml/kg) settings. The perfusate contained propidium iodide, a membrane-impermeant molecule, which on entering the cell emits a red fluorescence when it is interchelated with RNA or DNA. Note the increased cellularity, the perivascular hemorrhage, and the damage to small airway lining cells in the histologic section of the injured lung. Note the prominent red nuclei of transiently or permanently wounded subpleural cells in the live tissue images obtained with laser confocal microscopy. (Reproduced with permission from Reference 95.)

studying the molecular mechanisms of mechanotransduction have implicated numerous candidates. However, the nature of their interrelatedness, cooperativity, and cell and tissue specificity remain areas of continued rigorous investigation. The reader is referred to a number of excellent reviews addressing cellular mechanotransduction (98–100). In this section we will discuss the general principles of cellular microrheology as a platform for highlighting the cellular consequences and adaptive and reparative responses to cell deformation that mimics the injurious effect of mechanical ventilation *in vivo*.

Microrheology of Living Cells

The principle stress-bearing elements of the lung, which account for its tendency to recoil, are elastin and collagen fiber networks and surface tension. Indeed, the lung can be viewed as a tissue network that is distorted by surface tension (46). While the resistance of cells to deformation contributes little to overall lung stiffness, lung cells must nevertheless adapt to deformations of the scaffolding to which they adhere. Cells interact with their surroundings through adhesion receptors such as integrins, which provide dynamic bidirectional links between the cytoskeleton and the extracellular matrix (101–103). An increase in basement membrane surface area that accompanies a large tidal breath thus imposes a shape change on adherent alveolar epithelial and microvascular endothelial cells. Both epithelial and endothelial cells are subjected to deforming stress during breathing as a result of the interdependent effects of lung volume, transpulmonary pressure, surface tension, and vascular pressure on the blood–gas barrier (75). The resulting cellular shape change mandates that cell surface to volume ratio increase, and this is generally accompanied by a reorganization of the cell's stress-bearing elements (i.e., the cytoskeleton).

The cytoskeleton is an interconnected network of biopolymers that exert centripetal forces on the surrounding matrix (104, 105). It is covered by the plasma membrane, a lipid bilayer, the molecular constituents of which are organized in specific outer and inner leaflet domains (106). Compared with cytoskeletal proteins, the plasma membrane carries little stress under physiologic conditions (107). Nevertheless, it may experience lytic tensions when a large shape change is externally imposed. This can be readily documented in epithelial monolayers that are grown on malleable membranes and subjected to large deformations (108–112). Interestingly, plasma membrane defects resulting from large substratum strains tend to be transient and rapidly repaired (112).

The mechanical properties of solid materials can be described

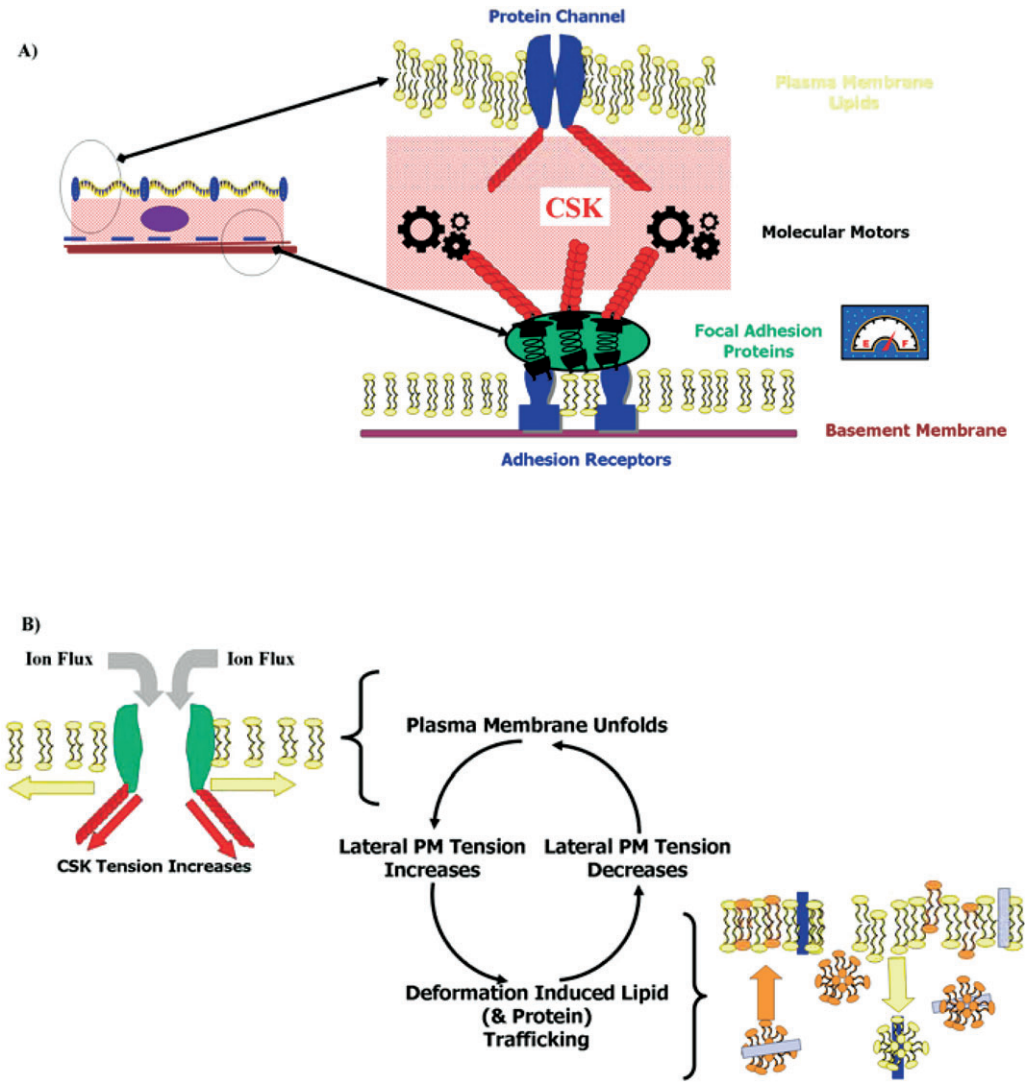

Figure 3. Cartoon of putative cellular mechanosensing structures (*A*) and their response to deforming stress (*B*). (*A*) Deformation (strain) of the matrix (basement membrane) generates a force, which is transmitted via adhesion receptors (e.g., integrins) to the cell. To date, over 50 different focal adhesion proteins have been identified that link adhesion receptors to the tension bearing elements of the cytoskeleton (CSK). They are thought to be a major locus of mechanosensing, i.e., they respond to forces that are either generated by the cells (via molecular motors) and are carried via the CSK or which are externally imposed (e.g., in the lung during breathing) and transmitted to the CSK. Moreover, tension-bearing elements of the CSK can connect directly to protein channels (shown in *blue*), thereby mechanically gating ion flux through them. (*B*) An externally imposed shape change is associated with the unfolding of excess plasma membrane. As lateral tension of the unfolded plasma membrane increases channel proteins (e.g., mechanosensitive cation channels), that are suspended by hydrophobic matching in the lipid bilayer, undergo a conformational change and ion flux (e.g., Ca^{2+}) increases. The increase in plasma membrane tension triggers a vigorous lipid (and protein) trafficking response (*brown* and *yellow vesicles*) that results in a net growth of plasma membrane surface area.

by elastic constants that characterize the material's resistance to changes in volume and shape (31). Within this framework, yield-stress and lytic stress are quantities denoting the material's susceptibility to plastic deformation and structural failure. Material properties have been estimated for cell constituents such as cytoskeletal proteins, cytoskeletal networks, lipid bilayers, and plasma membranes, as well as whole cells including alveolar and bronchial epithelial cells (113, 114). In the context of a discussion on cell injury two characteristics of biomaterials deserve comment: (*1*) the distinct rheologic properties of network structures (115–117) and (*2*) the importance of active remodeling in determining cell plasticity (118–120).

Because cells and specifically their cytoskeleton are network structures, the mechanical properties of individual stress-bearing elements (e.g., single actin fibers) are only secondary determinants of a cell's deformation resistance. Cells are prestressed networks of tension-bearing microfilaments that are coupled to compression-resistant microtubules and extracellular matrix molecules (117). As such, their resistance to deformation is critically dependent on the interconnectedness of the network structure and on the rate at which molecular contacts between stress-bearing elements can be broken, degraded, and reformed. Living cells display a great deal of plasticity, that is, the network of stress-bearing elements remodels readily when it is subjected to a deforming stress (121–123) Deformation induced remodeling involves active, energy-dependent processes that may well provide a safeguard against the development of structural failure (112, 124, 125).

Cellular Remodeling: Prevention of Plasma Membrane Wounding

Matrix- and cytoskeleton-dependent mechano-sensing and remodeling. Proteins that link the extracellular matrix with stress bearing cytoskeletal biopolymers play a pivotal role in mechanosensing and transduction (101, 126–129). They are assembled in so-called focal adhesion sites, plaques, or complexes and can be thought of as mini-strain gauges that monitor local force (Figure 3). Focal adhesions are highly plastic structures and remodel in a force-dependent manner. This allows adherent cells to probe the regional impedance of the surrounding scaffolding and in turn provides cues for directional control of locomotion and cell shape (101, 105). The most extensively studied adhesion receptors involved in mechanotransduction are the integrins. After ligand-induced activation, integrins transduce matrix-dependent intracellular biochemical signals (130) through structural associations with adaptor proteins, which include GTPases (131), receptor

and nonreceptor tyrosine kinases (132–136), and phosphoinositides (137, 138). Other adhesion molecules such as cadherins are also increasingly recognized as playing important roles in mechanotransduction (133) and as molecular targets of deformation induced impairments of epithelial and endothelial barrier function (97, 139, 140).

Through numerous distinct but interrelated biochemical signals, matrix molecules undoubtedly play a major role in lung remodeling induced by stress (141–143). For example, bronchial epithelial cells exposed to a compressive stress *in vitro* elicit a profibrotic response in unstressed fibroblasts (144). Compressive stress was shown to shrink the lateral intercellular space surrounding epithelial cells, and thereby triggered signaling via autocrine binding of epidermal growth factor family ligands to the epidermal growth factor receptor (145). Remodeling responses are also initiated through paracrine signaling involving fibronectin, collagen, and matrix metalloproteinases (MMP). In a rat model of VILI, expression of the extracellular matrix metalloproteinase inducer (EMMPRIN), gelatinase A and B, MT1-MP were induced in lung endothelium (146, 147). In the rabbit, mechanical ventilation with high positive end-expiratory pressure (PEEP) was associated with increased mRNA expression of extracellular matrix proteins such as $\alpha 2$(IV) procollagen and fibronectin (141). These effects were measured in the absence of alveolar cell breaks, as quantified by electron microscopy, and were accompanied by an increase in mRNA of the mitogenic growth factors TGF-β_1 and basic fibroblast growth factor. The authors attributed the findings to wall stress–induced vascular remodeling.

It is not our intent to provide a comprehensive review or even a complete list of the large number of publications dealing with injured lungs and matrix and adhesion molecules. We simply underscore that these molecules are integral to cell and tissue remodeling regardless of whether their expression is triggered by the structural failure of a stress-bearing element or initiated by some other mechanotransduction event.

Regulation of cell surface area and plasma membrane tension. The hypothesis that deformation induced remodeling is vital in the prevention of cellular stress failure applies not only to remodeling responses involving matrix and cytoskeletal proteins, but also to vesicular lipid trafficking to and from the plasma membrane (Figure 3). This lipid trafficking serves as a means of regulating cell surface area and plasma membrane tension and ultimately helps to prevent plasma membrane stress failure (112, 124, 125, 148). To the extent to which plasma membrane stress failure is one of the triggers of proinflammatory signaling and/or cell death in ventilator-injured lungs, it becomes important to understand how cells regulate plasma membrane surface area and tension.

In a thought-provoking essay, Morris discusses the evolutionary question of how apparent regulatory feedback loops for cell volume and surface area came into being (149). She concludes that size regulation in the earliest protocells would have been governed by liposome physics and develops the argument that monitoring and regulation of lipid bilayer tension ultimately determines a cell-size set point. According to that theory, changes in bilayer tension could have altered membrane conductivity for osmolytes and consequently effected a cell volume change. Modern cells have evolved more elaborate control mechanisms linked to protein-regulated expenditures of energy and fluxes of materials. Cells sense a multitude of flux rates such as the rates of endocytosis, exocytosis, protein channel or pump activities, and the polymerization rates of cytoskeletal networks. Volume, surface area, and shape are simply consequences of the weighted distributions of the respective rate constants, but are not the sensed quantities themselves. Plasma membrane tension is central to many of these control loops. As detailed below, changes in plasma membrane tension not only effect membrane ion and water conductivities but also the rates of membrane addition (exocytosis) and retrieval (endocytosis) (150). For example, increases in plasma membrane tension, in response to a hypoosmolar challenge, produce a net increase in plasma membrane, whereas a fall in tension results in membrane retrieval (151, 152).

Deformation-induced lipid trafficking. Changes in the dimension of the connective tissue matrix during breathing impose a shape change in adherent cells which ought to intermittently raise plasma membrane tension. Therefore, Vlahakis and colleagues reasoned that to prevent lytic membrane tensions, alveolar epithelial cells would demonstrate a net exocytic lipid trafficking response during stretch in culture (125). Not only did they demonstrate active lipid vesicle trafficking to the plasma membrane that was temperature- and energy-dependent, but also that the response could be inhibited by both cytoskeleton active agents and by cholesterol depletion of the plasma membrane (112). Recent observations have confirmed the importance of deformation-induce lipid trafficking over plasma membrane unfolding in the surface area regulation of tonically stretched alveolar epithelial cells (148). It must be emphasized that the mechanisms underlying this trafficking response cannot be equated with those governing deformation-induced surfactant secretion by type II alveolar epithelial cells (153, 154).

Deforming stresses can also trigger endocytic responses, as was demonstrated in umbrella cells of pressurized porcine bladders and in radially strained alveolar epithelial cells (155, 156). That stretch would trigger endocytosis of surface membrane lipids might seem counterintuitive when considered in the context of plasma membrane tension regulation. However, the experimental evidence is undeniable and suggests that exocytic and endocytic trafficking responses are intrinsically linked (157). While exocytosis and endocytosis may be temporarily dissociated, their relative rates probably do vary with plasma membrane tension. Furthermore, preliminary evidence from alveolar epithelial cell lines suggests that stretch-induced internalization of lipids proceeds via distinct molecular pathways (156).

Several observations on normal and injured lungs raise interest in the molecule and pathway specificity of deformation triggered vesicular trafficking. When lungs suffer relatively mild forms of interstitial pulmonary edema, the lipid microdomains of lung cell surface membranes undergo a substantial reorganization (158, 159). The functional consequence of membrane remodeling, which is almost certainly accompanied by changes in surface protein expression, remains to be explored. *In vitro*, such changes are associated with changes in cell phenotype and by inference, changes in the cells' susceptibility to mechanical injury (109, 160, 161). For example, membrane remodeling by loading alveolar epithelial cell membranes with lipids such as cholesterol results in the formation of specialized microdomains (*see below*), which in turn accelerates transdifferentiation from the Type II to Type I phenotype, which possess different mechanical properties (162, 163). This may also be relevant insofar as the alveolar exudate of injured lungs contains cell debris and is cholesterol rich (164). As the progenitor of the type I cell, during alveolar wound healing, type II cells must divide and differentiate in a cholesterol-rich environment. If and how excess cholesterol effects the differentiation of ATIIs to the ATI phenotype *in vivo*, and the consequences of this differentiation on deformation-induced lipid trafficking and mechanotransduction, are not known.

The plasma membrane of eukaryotic cells is enriched in cholesterol and phosphatidylcholine and also contains high levels of sphingolipids (165, 166). Lipids and proteins of the plasma membrane are hydrophobically matched to maintain a low membrane-energy state. Changes in protein and lipid composition

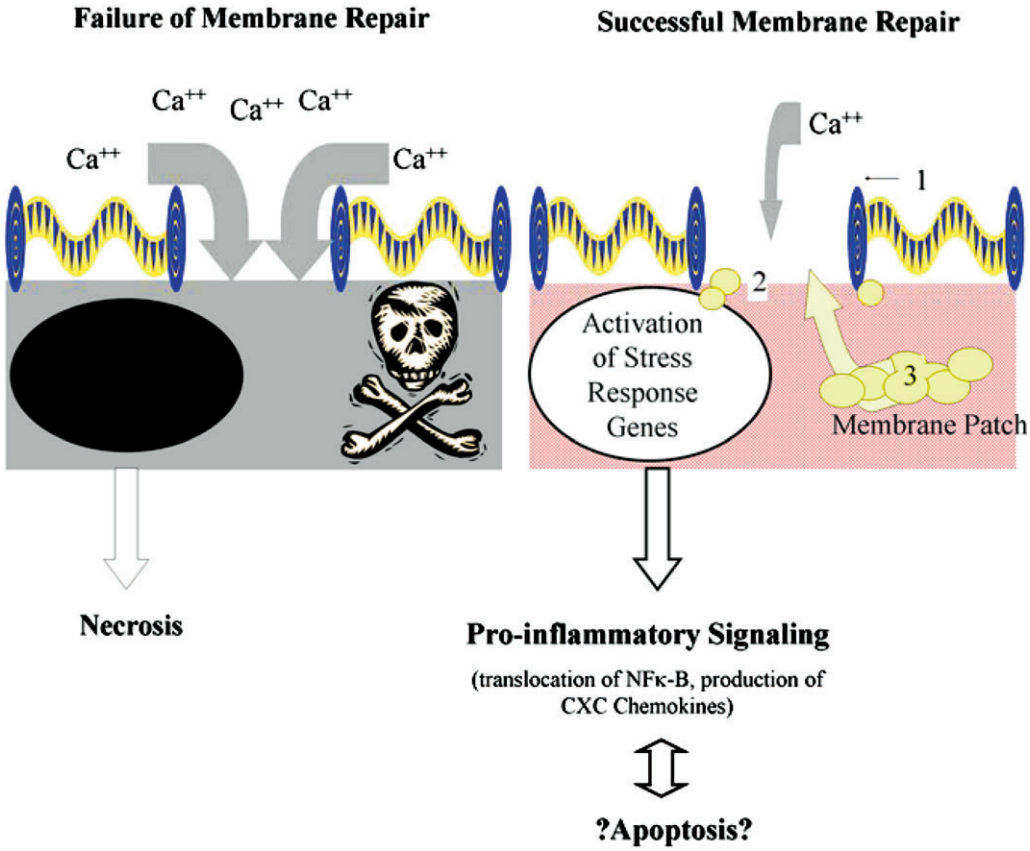

Figure 4. Schematic of the cellular response to membrane stress failure. Calcium enters the cell through a plasma membrane defect. Sustained large elevations in intracellular Ca^{2+} produce necrosis. Smaller transients in intracellular Ca^{2+} initiate cell repair responses. Cells repair membrane defects but several mechanisms (*right-hand side*). Mechanism 1 involves lateral flow plasma membrane lipids driven the free energy (analogous to surface tension) at the wound edge. This mechanisms is thought to play a role in the healing of small defects. Mechanism 2 is the fusion of early endosomes with the plasma membrane. Mechanism 3 involves the coalescence of vesicular organelles (usually lysosomes), which form a patch and plugs the wound by Ca^{2+}-induced, site-directed exocytosis. Wounding and repair trigger also the translocation of nuclear transcription factors like NFκ-B, leading to the induction of early stress response genes and thereby initiate proinflammatory signaling cascades.

alter the membrane energy state and thereby influence cell function (167). Sphingolipids play important roles in a wide variety of cell functions, including mechanotransduction (157). Their concentration in cell membranes is tightly regulated in close association with cholesterol, with which they form membrane microdomains (168, 169). In endothelial cells, fibroblasts, and some epithelial cells, sphingolipids, cholesterol, and GPI-anchored proteins appear to have a preferential association with 50- to 100-nm pits called caveolae as defined by the marker protein caveolin (170). These structures play an important role in non–clathrin-dependent endocytosis and in contrast to surfactant secreting type II cells can be readily identified in type I alveolar epithelium (171–174). To the extent to which caveolae are plasma membrane invaginations that may unfold when laterally stressed, they might not only be important for the mechanotransduction of deformation-induced lipid trafficking, but also central for the maintenance of sublytic membrane tension.

Plasma Membrane Wounding

Plasma membrane wounding is a common event in exercising muscles and it plays a central role in the pathogenesis of progressive muscle failure in some forms of muscular dystrophy (175, 176). Cell wounding is the reason why patients with increased myocardial stress often have elevated serum levels of "cardiac enzymes," and it probably occurs in the lining cells of the gastrointestinal tract on a regular basis (177, 178). Increasingly, it is being recognized that excessive mechanical forces in the lung result in tissue damage that is characterized by lung cell injury and plasma membrane wounding (24, 95).

Normally, the plasma membrane carries tensile stress that is at least one order of magnitude lower than that born by filamentous actin (104, 150, 179, 180). The tension at which the plasma membrane fractures is estimated to range between 1 and 25 mN/m, corresponding to membrane strains of only 1 to 3% (181, 182). Lytic tensions vary with the composition and organization of the lipid bilayer as well as with the timeframe for breakage (180, 183). At least in model membranes, lytic tensions are loading rate–dependent, implying a kinetic process that begins with nucleation of a molecular-scale defect, which either resolves spontaneously or grows to become an unsustainable hole.

Several studies have examined the molecular as well as biophysical determinants of plasma membrane stress failure in cultured alveolar epithelial cells (108–112). Results of these studies may be summarized as follows: The probability of stress failure varies with strain amplitude and strain rate. For example, minimal wounding occurs in A549 cells, a human adenocarcinoma cell line, when strain rates are kept at or below 3%/second at a normally injurious strain amplitude (112). The susceptibility for deformation-related stress failure varies considerably between cells and cell culture systems, as does the probability of subsequent membrane repair. Interventions that effect cytoskeletal assembly or vesicular trafficking increase the susceptibility of cells to wounding presumably by impairing their ability to remodel stress-bearing structures (112).

Plasma Membrane Repair

The ability to restore membrane integrity after cell wounding is essential for cell survival and virtually all cells possess the means to do so (Figures 3 and 4). Until recently the prevailing view held that injured plasma membranes repaired primarily by "self-sealing" whereby hydrophobic interactions between phospholipids and water would drive lipid flow toward the free edges of a defect (184, 185). Indeed, this mechanism is readily observed in model membranes and red blood cells (186). However, by

itself it is insufficient for repair of large wounds and does not account for resealing in nucleated cells (186).

In 1994, Steinhardt and coworkers described wounding responses in sea urchin eggs and provided the first clues that repair was governed by Ca^{2+}-dependent membrane trafficking and fusion events (187). During the subsequent decade several groups of investigators have extended these observations to mammalian cells and have added considerable detail to our understanding of the responsible molecular mechanisms (reviewed in Reference 175). Small disruptions on the order of 1 μm evoke a calcium-dependent exocytosis of vesicles near the wound site, lower plasma membrane tension, and thereby facilitate wound closure (188). The generation and trafficking of vesicles involves nonmuscle myosin and is sensitive to disruptions of the actin cytoskeleton (189, 190). A rise in cytosolic Ca^{2+} consequent to the loss of plasma membrane integrity also promotes the coalescence of vesicular endomembranes (190–194). These are transported as a "patch" to the site of larger defects and fuse there with the plasma membrane. Lysosomes appear to be a ubiquitous source of endomembrane patches in wounded cells and the release of lysosomal contents after membrane injury may well be a primitive defense mechanism against invading pathogens (195–200). Additional novel classes of vesicular organelles have been implicated in cell repair, but their specific roles and functions remain to be defined (201, 202).

Cells also possess adaptive mechanisms to protect their plasma membranes against repeated mechanical insults. In 3T3 fibroblasts, repeated membrane wounding results in long-term potentiation of Ca^{2+}-regulated vesicular exocytosis in turn generating faster membrane resealing rates (189, 195, 203). This adaptive response requires cAMP-dependent protein kinase A over the short term (minutes) and cAMP response element-binding protein over the long term (days). Vesicular fusion reactions are catalyzed by diverse proteins, which mediate the initial recognition of the membranes that are destined for fusion (196). They pull the membranes close to each other to destabilize the lipid/water interface and to initiate mixing of the lipids. For example, synaptotagmins function as Ca^{2+} sensors in membrane fusion and play a prominent role in lysosomal exocytosis (204–206). Synaptotagmin VII–deficient mice show defects in cell resealing and develop a form of autoimmune myositis (207). This suggests that defective membrane repair and the consequent release of intracellular contents overwhelms immune tolerance to self antigens. Although lung morphology and function of synaptotagmin VII–deficient mice have not been characterized to date, it is of note that another syndrome with impaired lysosomal exocytosis, the Hermansky-Pudlak syndrome, is associated with lung pathology (208).

EFFECTS OF DEFORMING STRESS ON GENE EXPRESSION AND CELL SURVIVAL

Since the landmark paper by Tremblay and colleagues, which demonstrated a relationship between ventilator settings and inflammatory signaling, the immune and inflammatory responses of lungs to mechanical stress have been extensively studied (209; reviewed in References 13 and 15). Notwithstanding some debate about model, cell, and timing specific differences in the expression of inflammatory mediators, in aggregate the evidence leaves little doubt that inflammation is integral to the pathobiology of the syndrome (210–213). The concomitant impairment in lung barrier function contributes to the loss of compartmentalization and may account for many of the systemic manifestations of ventilator-associated lung injury (214–220). The inflammatory effect of mechanical deformation on uninjured lungs ("one hit") compared with preinjured lungs ("two hits") remains an area of important and continued investigation (221).

The signal transduction pathways that link deformation to some gene response are being characterized in ever-increasing detail (14, 18, 222). Nevertheless, the importance of plasma membrane wounding in initiating a widespread proinflammatory gene response in ventilator-injured lungs is difficult to discern (213, 223). It is clear that not all molecular responses to mechanical ventilation are associated with lung edema or micron-scale plasma membrane lesions (224). Indeed, observations on macrophages and epithelial cells in culture suggest that deforming stress can trigger the release of proinflammatory cytokines in the absence of gross cell injury (211, 212, 225). At the time these observations first appeared, there was some debate if and how an alveolar macrophage might be deformed during mechanical ventilation, and if macrophages or epithelial cells were the predominant source of inflammatory mediators in ventilator-injured lungs. In the interim, the role of epithelial cells as active participants in pulmonary immune responses has been largely acknowledged (226). However, there are virtually no data on epithelial cell strain *in situ*, let alone data on how an epithelial deformation is transmitted to an adherent macrophage. Be this as it may, many whole animal models of VILI in which inflammatory mechanisms have been characterized have employed ventilation strategies known to produce cell and plasma membrane stress failure (24, 68, 95). When plasma membrane lesions are produced in cell culture, they invariably cause the translocation of nuclear factor-κB followed by the induction of early stress response genes (Figure 4) (227). It is reasonable to think that similar events occur in intact mechanically ventilated lungs. Moreover, the resulting induction of CXC chemokines could be amplified and transmitted to uninjured cells by cell contact– as well as non–cell contact–dependent pathways (213, 228–231). In that scenario, plasma membrane wounding becomes the critical mechanosensing event that initiates and propagates the inflammatory response in the whole organ. Understanding the gene profile of cells that wound, compared with those that are able to prevent or repair wounding, may provide potential protein candidates for further study and chemotherapeutic targeting.

Cell wounding not only results in gene expression, but can ultimately lead to cell death via cell necrosis or apoptosis. Apoptosis or programmed cell death is an integral mechanism of inflammation, tissue remodeling, and repair. Not surprisingly, therefore, the role of apoptotic mechanisms in lung injury have generated considerable interest, and several reports that injured lungs contain apoptotic cells have appeared (232–234). While type II cell hyperplasia and cell necrosis dominate the acute phase of VILI, tissue specimens from patients with resolving ARDS have revealed type II cell apoptosis (235). The paucity of apoptotic cells in acutely injured lungs of hyperventilated animals contrasts with the abundance of apoptotic cells in the kidney and GI tract (220). Although it is established that deforming stress can trigger apoptotic responses in lung and muscle cells, it is not known if plasma membrane injury or nuclear deformation are contributing or even necessary priming events (236–242). In addition, the signals that lead a cell to necrosis or apoptosis remain to be elucidated.

In the preceding paragraphs, we have highlighted just a few examples of the effects of deforming forces on important cellular responses such as gene expression and resultant protein production, cell proliferation, and survival. Several important issues remain to be explained. First, what is the downstream effect of mechanical forces resulting in cell wounding compared with wounding and subsequent repair? What are the determining structural, physiologic, and biological characteristics of cells that fall into each category? Finally, what if any difference is there

in this cellular response between the cells of the lung (epithelium versus endothelium versus [lipo-]fibroblast)?

CONCLUSIONS

Motivated by our interest in ventilator-induced and ventilator-associated acute lung injury, we have reviewed the determinants and consequences of the mechanical failure of lung structures. We have focused on tissue elements that are not ordinarily considered important for stress bearing, namely cells and their plasma membranes. Nevertheless, they are susceptible to deformation injury and serve both as sensors and effectors of the innate immune responses that are triggered by physical stress. We have deliberately sought to assimilate observations from nonpulmonary fields to broaden our and the reader's perspective on a problem that should be of interest to practicing intensivists and pulmonary scientists alike.

Central to our review is the premise that many manifestations of VILI, be they edema, inflammation, or tissue remodeling and fibrosis, can be traced to stress failure of cell membranes. Although injury is not the only trigger of deformation-related cell signaling, one must never forget that it is cells and not alveoli, airways, blood vessels, or connective tissue that sense and transduce local stress. Two interventions, which have received close attention in the critical care literature, namely low tidal volume and high PEEP, clearly reduce cellular stress failure in experimental lung injury models (95, 62). Therefore, one may consider gas exchange, lung aeration, or respiratory mechanics to be surrogates for the real therapeutic objective, namely to prevent cellular stress failure and to enhance airway and alveolar wound healing by physical means. Because wound healing requires cell migration, proliferation, and epithelial transdifferentiation, it is important to understand if and how the aeration of a previously flooded or "closed" airspace influences these critical cell biologic functions. Specifically, there remain fundamental questions about mechanisms through which interfacial forces, cell strain, and gas tension interact to effect epithelial wound repair.

Intensivists, who interpret gas exchange and mechanics in a cell biological context, may develop a different perspective on rationale and efficacy of certain treatment approaches. For example, some believe that bilevel pressure ventilation obviates the need for limiting tidal volume in mechanically ventilated patients with injured lungs (243). This opinion is largely grounded in the observation that preserving diaphragm activity prevents the atelectasis of diaphragm apposed dependent lung. In other words, the loss of regional aeration is equated with injury. However, the prognostic relevance of the surrogate endpoint, atelectasis, remains unclear. Cells are injured because the matrix to which they adhere undergoes large deformations or because their apical membranes are "abraded" by the cyclic movement of air–liquid interfaces and foam across them (64, 112). None of the observed effects of bilevel pressure ventilation on regional lung aeration directly address these cell injury mechanisms. Moreover, the sometimes overlooked work by Mascheroni and colleagues argues that hyperventilation-induced lung injury need not be restricted to positive pressure breathing (244). This is because large oscillations in alveolar volume and surface area, be they generated by machines or the respiratory muscles, over time impair the physicochemical properties of surfactant (245–247). The point of this example is not to refute claims of efficacy of bilevel pressure ventilation, but only to raise caution against the ready acceptance of a surrogate physiologic endpoint as proof of benefit.

The focus on cellular stress failure as central to VILI also suggests new treatment targets. These include molecules involved in the regulation of deformation-induced cytoskeletal remodeling, lipid trafficking, and membrane repair. Much preclinical work will need to be done before related approaches can be tested at the bedside. On the other hand, some "old drugs" that are currently in use for different indications may already point in this direction. For example, it has been known for some time that β-adrenergic receptor agonists preserve barrier properties of ventilator-injured lungs (248). Among the many putative mechanisms of benefit is a cyclic adenosine monophosphate (cAMP)-mediated effect on the endothelial cell cytoskeleton. To think that a change in the resistance of endothelial cells to deforming stress could alter their susceptibility for stress failure would take but a small leap of faith and is an intriguing and testable hypothesis. The current approach to mechanical ventilation is built on the foundations of classic cardiopulmonary physiology and respiratory system mechanics. In dealing with VILI, the critical care community has in the past decade discovered an important connection between mechanics and innate immunity. The term "biotrauma" coined by Arthur Slutsky embraces and underscores this connection (249). Has the time come to add plasma membrane and cytoskeletal biology to the topics an intensivist should know something about? We hope that this review convinces the reader that the answer is "yes."

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