Forum Review Article

Cross-Talk Between Pulmonary Injury, Oxidant Stress, and Gap Junctional Communication

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Abstract

Gap junction channels interconnect several different types of cells in the lung, ranging from the alveolar epithelium to the pulmonary vasculature, each of which expresses a unique subset of gap junction proteins (connexins). Major lung functions regulated by gap junctional communication include coordination of ciliary beat frequency and inflammation. Gap junctions help enable the alveolus to regulate surfactant secretion as an integrated system, in which type I cells act as mechanical sensors that transmit calcium transients to type II cells. Thus, disruption of epithelial gap junctional communication, particularly during acute lung injury, can interfere with these processes and increase the severity of injury. Consistent with this, connexin expression is altered during lung injury, and connexin-deficiency has a negative impact on the injury response and lung-growth control. It has recently been shown that alcohol abuse is a significant risk factor associated with acute respiratory distress syndrome. Oxidant stress and hormone-signaling cascades in the lung induced by prolonged alcohol ingestion are discussed, as well as the effects of these pathways on connexin expression and function. *Antioxid. Redox Signal.* 11, 355—367.

Introduction

IT HAS LONG been appreciated that gap junctions are ubiquitous in most mammalian tissues, and the lung is no exception (16, 60). Gap junctions are composed of proteins known as connexins, which form channels that enable neighboring cells to be interconnected (69, 107, 149). Gap junction channels serve multiple functions by enabling the diffusion of signaling molecules and metabolites throughout interconnected cells. This, in turn, enables cells in a tissue to function in a coordinated manner. The ability to share metabolites and antioxidant molecules through gap junctions enables the tissue to have a robust response to stress and injury.

A functional gap junction channel is composed of two connexin hexamers (or hemichannels) in two adjacent cells, which dock to form a complete channel (107, 149). Gap junction channels are typically arranged in semicrystalline arrays, known as plaques, at sites of cell–cell contact where intercellular communication occurs. However, free connexin hemichannels dispersed throughout the plasma membrane can also act as *bona fide* plasma membrane channels, enabling the exchange of aqueous molecules between the cytoplasm to the extracellular environment (59, 150).

Connexin Expression in Lung

Of the 20 mammalian connexins (161), several are differentially expressed throughout the lung. The pattern of expression depends on cell phenotype, which influences connexin transcription (Table 1). In normal lung, most epithelial cells express Cx32 and Cx43, whereas endothelial cells express predominantly Cx37, Cx40, and Cx43. The cells that line terminal airspaces, type I and type II alveolar epithelial cells, have been studied in considerable detail (2, 3, 12, 66, 80, 81, 92, 111, 165). The major connexins expressed by alveolar epithelial cells are Cx26, Cx32, Cx43, and Cx46. Others are expressed at low levels, such as Cx30.3 and Cx40. Expression of Cx37 by alveolar epithelial cells *in situ* is also low, but is consistently detectable by immunohistochemistry. Considerably more Cx37 is expressed by bronchiolar epithelium, but this is still less than the level observed for pulmonary endothelial cells (165).

Cx43 is fairly ubiquitous and is the major connexin functionally interconnecting type II and type I cells (3). By contrast, Cx32 is expressed exclusively by type II alveolar epithelial cells in normal adult rat lung. Interestingly, type I cells cannot form functional gap junctions with cells expressing only Cx32 (3). Type II cells form primarily hetero-

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TABLE 1. CONNEXIN EXPRESSION IN THE LUNG

Cell Type	Connexin expression	References
Airway epithelium	Cx26, Cx30, Cx31, Cx32, Cx37, Cx43	23, 75, 97, 147, 165, 186
Trachea	Cx26, Cx43, Cx46	25, 31, 78
Alveolar epithelium		2, 3, 12, 66, 80, 81, 92, 111, 165
Type II	Cx26, Cx32, Cx37, Cx43, Cx46	
Type I	Cx26, Cx37, Cx40, Cx43, Cx46	
Pulmonary endothelium	Cx37, Cx40, Cx43	34, 134, 145, 165, 190
Smooth muscle	Cx37, Cx40, Cx43	22, 92, 130
Lung Fibroblasts	Cx43, Cx45	15, 92, 166, 194

cellular junctions in the normal lung (*e.g.*, with type I cells). Thus, the role for Cx32 in alveolar epithelial physiology is not clear, because it is not likely to be participating in type I–type II cell gap junctions (3), and few type II–type II cell junctions exist. However, a hemichannel role for Cx32 expressed by type II cells is plausible (44). Studies from Cx32-deficient mice suggest a role for Cx32 in alveolar epithelial cell growth control, because these mice are more susceptible to benzene-induced lung toxicity and have a higher incidence of lung tumors (94, 95, 192). This is not unique to Cx32, though, because female Cx43-deficient mice are roughly twice as sensitive to urethane-induced tumor formation as are controls expressing normal Cx43 levels (13).

Connexins have a relatively rapid half-life of 1–5 h, suggesting that gap junction turnover is a constant process (107). Moreover, cells that express multiple connexins, including cells in the lung, have the potential to form heteromeric or mixed gap junction channels (38, 103). Whether connexins form heteromeric channels is determined by their biochemical compatibility. For instance, Cx32 and Cx43 are incompatible and cannot form heteromeric channels. However, cells also regulate the formation of mixed gap junctions by compatible connexins. For instance, two of the compatible connexins expressed by alveolar epithelial cells, Cx43 and Cx46, form heteromeric channels when expressed by type I alveolar epithelial cells, yet type II cells prevent Cx43 and Cx46 from interacting (2, 42, 82). Other examples of cells that regulate connexin assembly include endothelial cells that restrict formation of mixed gap junctions containing Cx37 and Cx40/Cx43 (79).

The mechanisms that regulate connexin hetero-oligomerization remain unknown. However, it is clear that by regulating connexin interactions, cells have the ability to form discrete functional zones of communication within the pulmonary system (100).

Intercellular Communication in the Lung

Gap junctions serve several functional roles in the lung (7, 34, 100, 102, 142) (Fig. 1). By extension, any condition that disrupts gap junctional coupling can have a deleterious effect on lung function. In the airways, gap junctional coupling can contribute to calcium signaling between ciliated epithelial cells to coordinate ciliary beating (151). Coordinated ciliary movement is needed to ensure the directional flow of mucus out of lungs to clear environmental toxicants and microorganisms. Mechanical stimulation of primary airway ep-

ithelial cells in culture induces an intercellular calcium wave (23, 80), which is transmitted from one cell to another by inositol 1,4,5-triphosphate (IP3) diffusing through gap junctions (24). However, in addition to the gap junction–mediated pathway, intercellular calcium transients between airway cells can be generated by extracellular nucleotide release and paracrine stimulation of purinergic receptors (73). In this case, connexins may help regulate ciliary beat frequency by acting as plasma membrane hemichannels to promote ATP secretion.

Gap junctions also play an important role in regulating secretion of pulmonary surfactant produced by type II alveolar epithelial cells. Pulmonary surfactant is a mixture of lipid and protein that performs the dual purposes of decreasing alveolar surface tension and regulating host defense in the lung (20, 183, 189), described in greater detail later. It is known that direct mechanical stimulation of type II cells can stimulate surfactant secretion through the calcium-dependent fusion of lamellar bodies to the plasma membrane (68, 185). Although this would imply that cell stretch induced by a deep breath could directly stimulate type II cell secretion, type II cells are localized to areas of the lung where they are shielded from direct mechanical stress, as compared with type I cells (136). This helps protect the alveolus, because type II cells are significantly more sensitive to mechanical stress than are type I cells (167).

Instead, the alveolus regulates surfactant secretion as an integrated system in which type I cells act as mechanical sensors that transmit calcium transients to type II cells *via* gap junctions. Strong evidence for this pathway comes from *in situ* fluorescence microscopy analysis of the intact lung (104). These calcium signals can be induced by inflation (10) or by changes in pulmonary vascular pressure (177) and require gap junctional communication between type I and type II cells (3, 66). Gap junctions also enable intercellular signals, which can propagate from one alveolus to the next (76). Again, it is important to note that intercellular calcium transients are not exclusively transmitted through gap junctions because alveolar calcium waves generated by extracellular ATP release and paracrine stimulation of purinergic receptors also contribute to mechanically regulated surfactant secretion (Fig. 1) (80, 82, 135, 146). However, given the ability of gap junction blockers to inhibit calcium waves *in situ*, whether the paracrine pathway can fully compensate for a loss of connexin function is not known.

Functions for gap junctional communication in the vasculature have been covered elsewhere in detail (51, 172). Of rel-

FIG. 1. **Intercellular communication in the lung.** The lung consists of several distinct functional compartments. Shown in the inset are the terminal airspaces, alveoli, and bronchioles. (**a**) In the airways, including bronchioles, diffusion of IP3 through gap junctions enables the propagation of calcium waves, which help synchronize ciliary beating to allow directional transport of mucus. (**b**) The alveolar epithelium is a heterogeneous monolayer consisting of type II cells and type I cells. The alveolus acts as an integrated system in which type I cells respond to mechanical stimulation with an increase in intracellular calcium, which, in turn, is transmitted to type II cells *via* gap junctions to induce lamellar body fusion and secretion of pulmonary surfactant. Also shown is the alternative pathway, mediated by ATP secretion and paracrine stimulation *via* purinergic receptors. (**c**) In lung capillaries, transmission of calcium waves through pulmonary endothelial cell gap junctions upregulates the transport of P-selectin to the plasma membrane, thus transmitting a proinflammatory stimulus.
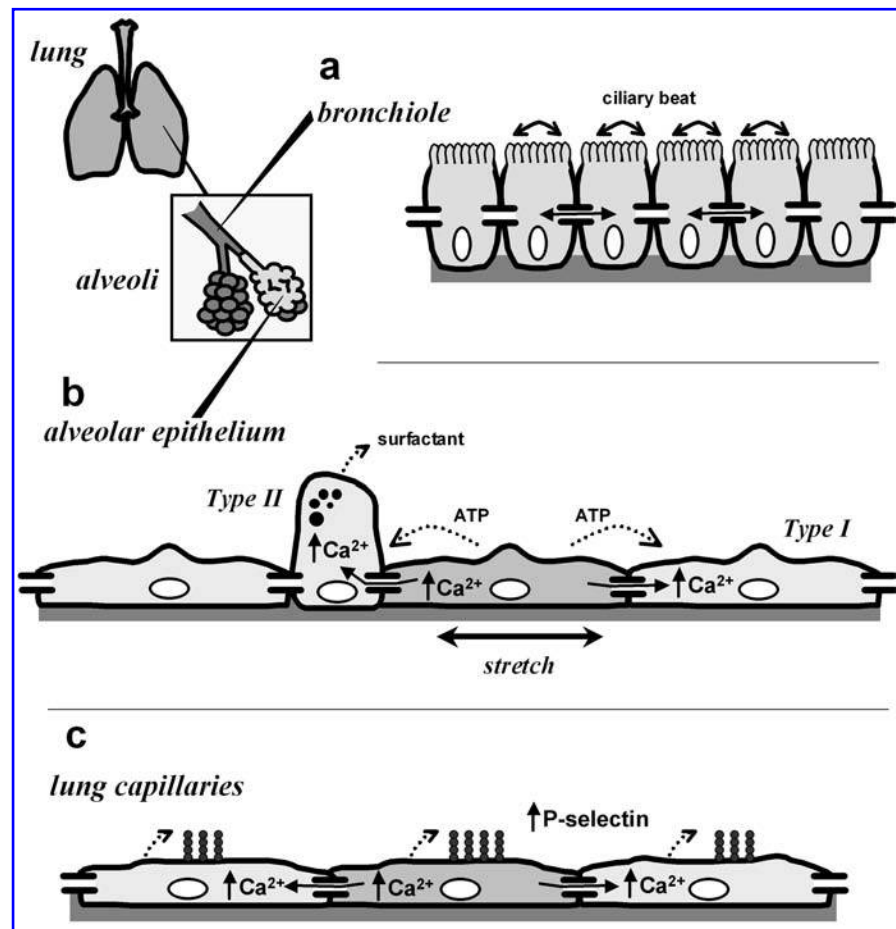
evance to the pulmonary circulation, calcium waves that propagate along pulmonary vessels through gap junctions have been imaged in the intact perfused lung (134, 191). The requirement for Cx43 in pulmonary endothelial calcium waves was confirmed by using an endothelium-specific Cx43-deficient mouse model in which these waves were no longer present (134). One surprising result from *in situ* imaging was the discovery of spontaneous calcium signals generated from a subset of endothelial cells, referred to as "pacemaker cells," localized to pulmonary branch points (191). Pacemaker cells are significantly more sensitive to mechanical stress, which results in a calcium wave of increased amplitude as compared with that of nonpacemaker cells. However, the frequency of the calcium oscillations in mechanically stressed vessels still matches the frequency in unstimulated vessels, underscoring the role of pacemaker cells in establishing signal-oscillation frequency (106).

Calcium waves induced by mechanical stimulation have been shown to increase pulmonary endothelial P-selection expression at the cell surface, suggesting a link to the inflammatory response (105, 134). This potentially injurious effect of endothelial Cx43 contrasts with pulmonary epithelium, where Cx43 is beneficial. Interestingly, the proinflammatory role for endothelial Cx43 is counterbalanced by an antiinflammatory role for Cx37 expressed by circulating monocytes (187). In this case, Cx37 inhibits inflammation by forming hemichannels that mediate ATP release and reduce monocyte adhesion to endothelial cells, as demonstrated in

a mouse model of atherosclerosis. Although endothelial Cx37 had no effect in this system, whether Cx37 plays a role in modulating other pulmonary inflammatory responses remains to be determined. Pulmonary Cx40 also may have an antiinflammatory function in preventing lung injury and fibrosis (34).

Gap Junctions and Lung Injury

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) can develop in response to stresses as diverse as sepsis, trauma, gastric aspiration, and pneumonia (179). Hallmarks of ARDS include increased oxidant stress, lung inflammation, surfactant dysfunction, and disruption of the alveolar and endothelial barriers (39, 65, 115, 137). Because optimal lung function requires functional cell–cell contacts, even partial disruption of an epithelial monolayer can be a significant contributor to the severity of ARDS.

Significantly, >85% of patients with ARDS have at least a partial defect in lung-fluid clearance, which contributes to a high degree of patient morbidity and mortality (21, 193). Compromised barrier function is a particular concern, because patients with impaired lung fluid balance are 3 times more likely to die of ARDS than are patients with a maximal ability to clear lung fluid (164, 180). Alveolar flooding directly compromises gas exchange; unfortunately, the requirement for mechanical ventilation to improve tissue oxy-

genation causes direct alveolar cell injury, which further exacerbates lung function (114, 160, 168, 175).

Cross talk between connexins and tight junctions

Lung barrier function is controlled by tight junctions (101, 122, 155, 169, 171). Although gap junctions are not a direct structural component of tight junctions, they are frequently seen adjacent to tight-junction strands with freeze-fracture electron microscopy (154). However, connexins do not necessarily colocalize with tight junctions (61), and the gap junction content of some tight junctions is low (154).

Functionally, treatment of a rat lung endothelial cell line (RLE) or primary porcine brain endothelial cells with the gap junction inhibitors glycyrrhetinic acid or oleamide decreased barrier function by ~50 to 75% as compared with controls, based on transendothelial resistance and small-molecule flux measurements (129). Cx40 and Cx43 are biochemically associated with several tight-junction proteins, including occludin, claudin-5, and ZO-1, based on co-immunoprecipitation analysis. Because RLE cells are claudin deficient, experiments were done with cells in which claudin expression was reconstituted. Nonetheless, results with the RLE cells were consistent with primary brain endothelium, expressing endogenous claudins.

As an example from epithelia, expression of transfected Cx32 induced a ~25% increase in the barrier function of immortalized hepatocytes derived from Cx32-deficient mice (98). In this system, Cx32 expression enhanced localization of ZO-1 and JAM-A to the plasma membrane, suggesting an increase in tight-junction formation. Interestingly, Cx26-transfected hepatocytes show a reduction in the ability of ouabain to decrease barrier function; however, Cx26 had no effect on baseline transepithelial resistance (TER) (56). This underscores the notion of different connexins having specific roles for regulating tight junctions.

This specificity is due in part to differences in the ability of connexins to interact with different junction scaffold proteins (55). Because Cx40 and Cx43 interact with ZO-1, a protein that also directly interacts with claudins and occludin (45), any role for Cx40 or Cx43 in regulating tight junctions is likely to involve ZO-1, either as a cross-linking scaffold protein, *via* ZO-1 recruitment to cell junctions or *via* an effect on the distribution of the ZO-1 pool between gap and tight junctions. In contrast, whereas Cx32 can also co-immunoprecipitate with tight-junction proteins (96), it does not directly bind to ZO-1. Instead, interactions between Cx32 and tight junctions may be mediated by another scaffold protein, such as discs large homologue 1 (Dlgh1), which directly interacts with Cx32 (47). As another distinct mode of interaction, Cx26 binds directly to the hydrophilic surface of the coiled-coil C-terminal domain of occludin, which does not interact with Cx32 (131).

What role can gap junctions have in regulating other classes of cell junctions? One possibility is that gap junctions transmit intercellular signals to coordinate junction assembly. A related possibility is that gap junctions serve to balance the concentration of metabolites between cells (such as calcium or GTP), so that junction-regulatory proteins are exposed to comparable microenvironments. Alternatively, connexins may structurally regulate junctions through a direct interaction with other tight-junction proteins. Given the heterogeneity of gap junction and tight junction protein expression and assembly, the need for further work in this area is clear.

Connexins in lung injury

Connexin expression in the lung changes during the injury response (100, 102). In the injured lung, type II cell hyperplasia increases the frequency of type II cells in direct contact with other type II cells (116), both of which express Cx32 (4, 6, 81). Because type I–type II cell communication is mediated through Cx43-compatible connexins and is not mediated by Cx32 (3), this has the potential to provide type II cells with an independent pathway for communication that does not involve type I cells and may be used to regulate the injury response.

During the acute phase of lung injury, connexin expression in the alveolus is altered, where Cx43 and Cx46 expression is elevated (3, 92). Conversely, Cx40 expression at the whole-lung level decreases during the acute phase of injury (145). Some Cx46-expressing alveolar epithelial cells do not express typical type II cell markers and thus may represent a distinct subtype of cells proliferating in response to injury (58). Cx46 has relatively limited permeability, as compared with Cx32 and Cx43 (118, 123), suggesting a possible role for Cx46 in limiting metabolic depletion or intercellular transmission of toxic agents. Interestingly, lung fibroblasts isolated from patients with idiopathic pulmonary fibrosis have decreased Cx43 expression and function (166). The downstream effects of impaired fibroblast communication and whether epithelial Cx43 expression is decreased in late stages of fibrotic lung disease are not known.

Studies from connexin-deficient mice have also demonstrated a potential role for gap junctions in preventing lung injury. Isakson and co-workers (34) have shown that mice deficient in Cx40 and endothelial Cx43 spontaneously develop symptoms similar to pulmonary fibrosis (34). Knockout mice deficient for expression of either Cx40 alone (52, 158) or endothelial Cx43 alone (117) did not have an obvious pulmonary phenotype. However, as early as 8 weeks after birth, $Cx40^{-/-}$:endothelial $Cx43^{-/-}$ mice showed deficiency in lung-barrier function, disorganized alveoli, and increased extracellular matrix deposition (34). Although the mechanistic basis for this phenotype is not known, these results suggest that intercellular communication between the vasculature and airspaces helps maintain lung morphology and function. Nonetheless, these results are difficult to reconcile with Cx43-mediated proinflammatory signaling in the pulmonary vasculature (134).

Oxidant Stress and Signaling in the Alcoholic Lung

Although alcohol abuse is classically associated with liver disease (144), recent evidence has confirmed that chronic alcohol abuse is also a major risk factor contributing to the severity of ARDS (87, 125, 127). In a study of ICU patients, it was found that after adjusting for smoking and hepatic dysfunction, patients with a history of alcohol abuse were more than twice as susceptible to ARDS than were nonalcoholic patients (127). In large part, the increased susceptibility to ARDS caused by prolonged ethanol ingestion is due to a fundamental defect in lung-barrier function as a result of impaired tight-junction formation between type I alveo-

lar epithelial cells (50, 64). As described earlier, decreased barrier function (*e.g.*, a leaky lung) contributes to the severity of ARDS (179).

Dietary ethanol causes oxidant stress in the lung (126). The lung is particularly sensitive to oxidant stress, which is minimized by the antioxidant glutathione in the alveolar airspaces (85). Thus, one source of oxidant stress is from ethanol metabolism to acetaldehyde, which directly depletes the reduced glutathione pool (30, 126) (Fig. 2). The prominent role for oxidant stress and reactive oxygen species (ROS) in alcoholic lung suggests that antioxidant therapy could be a useful therapeutic approach. In animal models of alcohol ingestion, a diet enriched in the glutathione precursor procystine prevents the alcoholic lung phenotype (29, 62). However, complete reversal of the alcoholic lung phenotype requires several weeks of treatment and is not a suitable approach for an immediate treatment regimen for alcoholic lung.

Three different studies have demonstrated that ethanol treatment of cells *in vitro* inhibits gap junctional communication (1, 26, 181). The ability of ethanol to inhibit gap junctions could be due to direct partitioning into cell membranes, analogous to the inhibitory effect of long-chain alcohols on connexins (86). Ethanol-induced depletion of the glutathione pool and increased oxidant stress can also inhibit gap junctional communication (170). The effect of ethanol on connexin expression is more variable, where ethanol was shown to inhibit Cx43 expression (26, 181), but had little effect on Cx26 or Cx32 (1, 181).

Whether ethanol has a comparable effect on other connexins or cell types remains to be determined. Specific alterations in gap junctional communication in response to oxidant stress may be a mechanism to decrease the intercellular transmission of toxic agents (14, 49), while also maintaining intercellular transfer of antioxidant compounds, including glutathione (57). Alternatively, complete shutdown of gap junctional communication can help preserve the bulk of the tissue at the expense of more extensive damage to isolated individual cells.

Hormone signaling in alcoholic lung

In addition to its metabolic effects on the antioxidant glutathione pool, ethanol also induces cell-signaling pathways that contribute to oxidant stress. In particular, ethanol stimulates angiotensin II activity (19) which, in turn, upregulates NADPH oxidase (Nox) (156). Interestingly, angiotensin II has also been shown to upregulate cardiovascular and epithelial Cx43 expression and function (26, 46, 89, 91) and can antagonize the effect of ethanol on gap junctional communication (26). In contrast, Cx40 appears to be less affected by angiotensin II (46). Given the role for endothelial Cx43 in inflammation, this is consistent with the notion that angiotensin II is proinflammatory as well (27).

Clearly, inflammation and the concomitant infiltration of neutrophils and activation of alveolar macrophages contribute to oxidant stress in response to acute lung injury (36). The intense oxidant load on alcoholic lung provides a condition in which the alveolar epithelium is prone to injury and apoptosis (29). As a response to oxidant stress, alveolar epithelial cells increase expression and secretion of TGF-β, the majority of which is the TGF-β1 isoform (19). In the other-
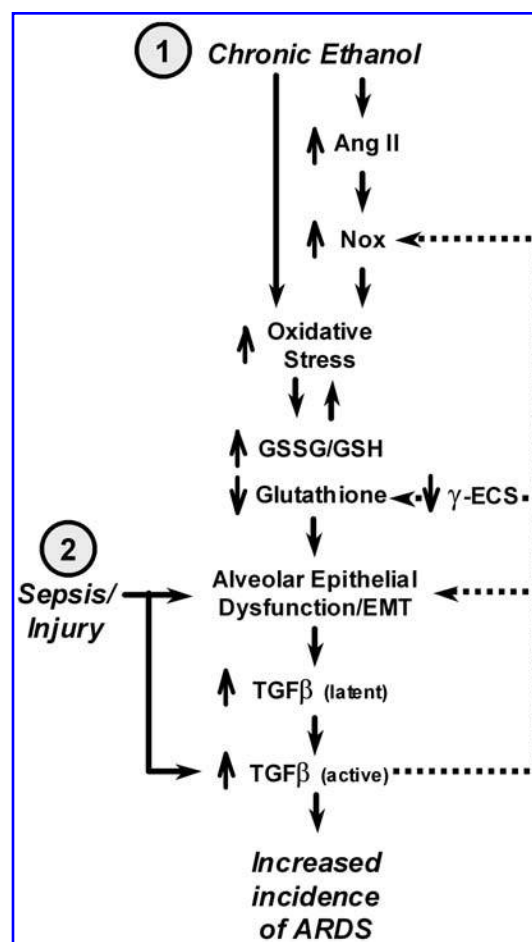

FIG. 2. Oxidant and hormone stress responses in the alcoholic lung. Prolonged ethanol ingestion initiates and exacerbates oxidant stress *via* several pathways. Here is depicted a two-hit model for the role of alcoholic in ARDS. (**1**) Prolonged alcohol abuse causes direct oxidant stress because of the metabolism of ethanol to acetaldehyde. Ethanol also induces angiotensin II, which stimulates both the endothelium and epithelium to upregulate Nox activity. Oxidant stress depletes the alveolar epithelial glutathione pool, which induces cell damage and stimulates the cells to undergo an epithelial-to-mesenchyme transition (EMT) as a compensatory mechanism. Alveolar epithelial cells undergoing EMT increase production and secretion of TGF-β and have impaired alveolar barrier function, which adds further stress to the lung. (**2**) A second hit, such as direct trauma, infection, or sepsis, has an exaggerated effect on the alcoholic lung because of impaired alveolar epithelial function and the presence of a large pool of latent TGF-β, which is readily activated and exaggerates the normal injury response. Note the feedback loops in the diagram (*dashed lines*), indicating the potential for "runaway" activation of a deleterious injury response.

wise healthy alcoholic lung, most of this TGF-β is inactive; however, a significant increase in active TGF-β is also found as compared with normal lung. This has influences on alveolar epithelial function by promoting the cells to undergo an epithelium-to-mesenchyme transition (EMT) (90, 93, 184) and is consistent with models in which TGF-β influences the acute phase of lung injury as well as the chronic phase (128, 138). Further, the large latent pool of TGF-β induced by pro-

longed ethanol ingestion plays an important role in exacerbating the influence of other insults on the lung (87). In essence, the alcoholic lung is primed to have an exaggerated response to the effects of subsequent insults that promote TGF-β activation (8, 88) (Fig. 2).

In addition to impairing the epithelial cell phenotype and compromising alveolar barrier function, EMT has been shown to decrease expression of Cx43 by embryonic carcinoma cells downstream of increased Snail expression and decreased cadherin expression (43). Whether this is the case for alveolar epithelial cell junctions remains to be determined. TGF-β can also increase oxidant stress by decreasing γ-glutamylcysteine synthetase expression (9, 84), thus reducing the antioxidant glutathione reserves of the lung. TGF-β also increases ROS production by increasing Nox expression (74, 163) and H_2O_2 production (176). In addition to ROS, reactive nitrogen species, including peroxynitrite, are generated during acute lung injury (67, 159), which can inhibit gap junctional communication (157).

Increased oxidant stress has the added potential to exacerbate alveolar injury and TGF-β expression by creating a positive-feedback loop, particularly if TGF-β expression and activation are driven by a second insult, such as sepsis or direct trauma (63). TGF-β can directly influence gap junctional communication; however, this effect varies depending on cell type. Studies have demonstrated that TGF-β1 increases (35, 141, 173), decreases (32, 108, 148), or has no effect (110) on gap junctional communication. TGF-β1 has also been found to simultaneously upregulate Cx43 and suppress Cx37 expression by endothelial cells (109), suggesting that differential regulation of connexins by TGF-β can provide a mechanism to alter intercellular communication.

Relevant to the lung injury response, we examined the effect of TGF-β on gap junctional communication between type I alveolar epithelial cells (Fig. 3). Primary rat alveolar epithelial cells were isolated and cultured for 6 days to generate a model type I cell monolayer (4, 135). The cells were

FIG. 3. TGF-β1 inhibits gap junctional communication between alveolar epithelial cells. Primary rat type II cells were isolated and cultured for 6 days in minimal essential medium to produce a model type I cell monolayer. The cells were then treated with varying amounts of TGF-β1 for 16 h; then the extent of gap junctional communication was determined by visualizing the intercellular transfer of calcein microinjected into individual cells with fluorescence microscopy. Dye transfer was quantified by counting the number of calcein-labeled cells per microinjection. Data were combined from two independent experiments counting ≥20 microinjections/treatment. Increasing concentrations of TGF-β1 significantly decreased intercellular communication, as determined by t test (*$p < 0.05$).

then treated for 16 h with varying amounts of activated TGF-β1, and the level of intercellular communication was assessed by microinjecting calcein into individual cells and measuring the extent of dye transfer 5 min after microinjection. Consistent with previous reports, control cells were highly coupled and transferred calcein to nearly 20 cells through gap junctions (2). Treatment with increasing levels of TGF-β inhibited gap junctional communication by ~50%. Thus, alveolar epithelial cells decreased intercellular communication in response to TGF-β. This suggests that if a similar phenomenon occurs *in situ*, then one effect of TGF-β would be to dampen the intercellular communication required to regulate surfactant secretion and thus potentially further to promote acute lung injury.

Role of Pulmonary Surfactant in Acute Lung Injury

The alveolus acts as a coordinated system to regulate pulmonary surfactant secretion. Pulmonary surfactant is a mixture of lipids and proteins synthesized by type II cells, which lines the alveolar airspace to reduce surface tension at the air/liquid interface. The majority of surfactant lipid is phosphatidylcholine (PC), with phosphatidylglycerol (PG) and phosphatidylethanolamine (PE) as the other major phospholipids present (174). By and large, most mammalian surfactant lipid is saturated [*e.g.*, dipalmitoyl-phosphatidylcholine (DPPC)], which can form a barrier to inhaled oxidants, such as ozone (37). Conversely, unsaturated lipids present in surfactant are reactive and can act as an antioxidant sink (162). Extensive lipid oxidation, including damage to cell-membrane lipids is deleterious (140). Oxidant damage to type II cells alters their ability to synthesize surfactant lipids, which further compromises the surface activity (40, 124). Consistent with this, overexpression of the antioxidant enzyme peroxiredoxin 6 is protective, because it can reduce phospholipid hydroperoxides in a glutathione-dependent reaction (53, 119, 178). Pulmonary surfactant also contains significant catalase and superoxide dismutase activity, which contributes to its ability to quench oxidant stress (121). In addition, surfactant lipids can have a direct role to help attenuate oxidant stress associated with inflammation by inserting into neutrophil membranes, which, in turn, inhibits Nox activity (33).

The other major components of pulmonary surfactant are four surfactant proteins, SP-A, SP-B, SP-C, and SP-D. SP-B and SP-C are hydrophobic and directly contribute to the biophysical properties of surfactant (20, 183). By contrast, SP-A and SP-D are members of the collectin protein family and are largely hydrophilic (41, 72). Although these proteins can bind to surfactant lipids and help organize them into higher-level structures, such as tubular myelin, they are largely dispensable from the standpoint of surfactant biophysics (28, 77, 99). Conversely, SP-A enhances surfactant lipid turnover, by enabling uptake of DPPC by type II cells (17). SP-D also helps regulate the surfactant lipid pool size, because surfactant degradation by macrophages and type II cells is impaired in SP-D–deficient mice (28), although the mechanistic basis for regulation of surfactant metabolism by SP-D remains unknown.

SP-A and SP-D play key roles in regulating lung inflammation, which can have downstream effects on oxidant stress and alveolar damage (188). Consistent with an immunoreg-
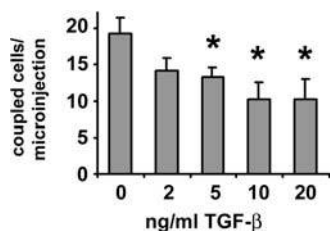
ulatory role for SP-A and SP-D, these proteins are required for efficient clearance of bacterial infections (54). Both SP-A and SP-D have carbohydrate-recognition domains that recognize bacterial polysaccharides, whereas the collagenous stalk region of the proteins binds to neutrophils, macrophages, and type II cells (70, 132, 152). SP-A and SP-D also bind viruses (71, 112). Thus, the collectin surfactant proteins act as co-receptors, or opsonizing agents, by coating pathogens and enabling them to be recognized and destroyed by the innate immune system in the lung.

A hyperreactive immune response and resultant oxidant stress is deleterious. One way that SP-A and SP-D can help keep this in check is to increase the efficiency of bactericidal activity so that the net inflammatory response is minimized (182). However, a comparison of SP-A– and SP-D–deficient mice indicates different roles for these proteins in regulating the macrophage respiratory burst (113). Alveolar macrophages from SP-A–deficient mice showed a dampened oxidative burst in response to phorbol esters, whereas macrophages from SP-D–deficient mice were hyperreactive (113). Note that macrophages from the SP-D–deficient mice are loaded with surfactant, which may shift them to a state more susceptible to activation.

Importantly, several examples show that the stressed and/or inflamed alveolus upregulates SP-A and SP-D while simultaneously decreasing SP-B and SP-C expression (11, 83). This has the dual effect of both increasing inflammation and depressing surfactant surface activity, which can further compromise lung function (139, 189). Consistent with a general role for surfactant in enhancing the antioxidant capacity of the lung, SP-D–knockout mice (in which the surfactant pool size is increased) resist hyperoxia (83). This, and the success of surfactant therapy for premature infants (5), has led to the notion that natural and pulmonary surfactants can be used as part of a treatment regimen for ARDS (115). However, treatment of adults with exogenous surfactant has had limited success. In part, this stems from a focus on using surfactant to recover the biophysical function of surfactant in adults, who have a large mature airspace as opposed to infants. Conversely, exploiting the immunomodulatory function of surfactant may ultimately be a more fruitful approach to treating adults with ARDS (115).

A net decrease in gap junctional communication will have a negative impact on surfactant and lamellar body secretion (76, 134). However, evidence suggests that type II cells secrete the majority of SP-A and SP-D through a distinct vesicular pathway from the hydrophobic surfactant proteins and lipids packaged into lamellar bodies (120, 133). Consistent with this, the SP-A content of lamellar bodies is low, and SP-D is largely undetectable (153). Although a complete lack of surfactant production and secretion is clearly deleterious, an imbalance in the regulation of surfactant secretion, which increases the relative level of SP-A and SP-D in the airspace at the expense of surface-active and antioxidant components of pulmonary surfactant can also compromise lung function. Whether this is due to decreased or altered intercellular communication in the alveolus during injury or infection remains to be determined.

Summary and Future Directions

The lung consists of several distinct functional zones, each of which is interconnected through gap junction channels.

Gap junctions play several functional roles in the lung, and disruption of intercellular communication can have pathologic consequences. Although some tantalizing links exist between oxidant stress, lung injury, and gap junctional communication, more-direct evidence would help support this notion. It also is not clear whether gap junctions interconnect different pulmonary subcompartments, such as the alveolar epithelium and pulmonary circulation. Although several lines of evidence suggest that this is the case, the extent to which this occurs and which connexins mediate this type of cross-talk remains to be determined.

Although many different connexins clearly help assure proper pulmonary intercellular communication, Cx43 appears to play a central role in the regulation of alveolar function. However, this remains to be tested in transgenic mouse models, because Cx43-deficient mice have a neonatal lethal phenotype (143). Intriguingly, $Cx43^{-/-}$ newborn mice are cyanotic, suggesting pulmonary edema. Although this is due in large part to a defect in the cardiac outflow tract, compromised alveolar epithelial function could also contribute to the neonatal lethal phenotype. Lung-targeted Cx43 knockouts *via* the cre-lox system, analogous to endothelial targeted Cx43-deficient mice (117, 134), or dominant-negative connexins expressed in mice (18, 48), are expected to provide useful insights into Cx43 function in the lung.

Acknowledgments

Abbreviations

ALI, acute lung injury; ARDS, acute respiratory distress syndrome; ATP, adenosine triphosphate; Cx, connexin; Dlgh1, discs large homologue 1; DPPC, dipalmitoyl-phosphatidylcholine; EMT, epithelial-to-mesenchyme transition; ICU, intensive care unit; IP3, inositol 1,4,5-triphosphate; JAM, junction adhesion molecule; Nox, nicotinamide adenine dinucleotide phosphate oxidase; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; RLE, rat lung endothelial; ROS, reactive oxygen species; TER, transepithelial resistance; TGF, transforming growth factor.

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