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Regulation and Repair of the Alveolar-Capillary Barrier in Acute Lung Injury

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Abstract

Considerable progress has been made in understanding the basic mechanisms that regulate fluid and protein exchange across the endothelial and epithelial barriers of the lung under both normal and pathological conditions. Clinically relevant lung injury occurs most commonly from severe viral and bacterial infections, aspiration syndromes, and severe shock. The mechanisms of lung injury have been identified in both experimental and clinical studies. Recovery from lung injury requires the reestablishment of an intact endothelial barrier and a functional alveolar epithelial barrier capable of secreting surfactant and removing alveolar edema fluid. Repair mechanisms include the participation of endogenous progenitor cells in strategically located niches in the lung. Novel treatment strategies include the possibility of cell-based therapy that may reduce the severity of lung injury and enhance lung repair.

INTRODUCTION

Acute lung injury and acute respiratory distress syndrome (ALI/ARDS) is a clinical syndrome of noncardiogenic pulmonary edema caused primarily by increased permeability to proteins across the endothelial and epithelial barriers of the lung (1, 2). Patients usually develop acute respiratory failure rapidly because of arterial hypoxemia as well as impaired carbon dioxide excretion and elevated work of breathing (3). Most patients require positive pressure ventilation with positive end-expiratory pressure to treat the arterial hypoxemia. ALI/ARDS is common and has been associated with several clinical disorders. Such disorders include sepsis; pneumonia; aspiration of gastric contents, saltwater, or freshwater; major trauma; transfusion of blood products; acute pancreatitis; and drug reactions (1). In the past ten years, survival has increased due to improved supportive treatment with lung-protective ventilation and a fluid conservative strategy (4, 5). Much has been learned regarding the basic mechanisms responsible for the development and resolution of ALI/ARDS, although these insights have not yet been translated into a specific pharmacological therapy that can diminish injury or hasten the resolution of lung injury (2).

This article focuses on the mechanisms that regulate the barrier properties of the endothelial and epithelial barriers of the lung. We first discuss the mechanisms that regulate lung fluid balance under normal and pathological conditions. Second, we discuss mechanisms of lung injury under pathological conditions that are relevant to clinical lung injury. Such mechanisms include infectious causes, aspiration syndromes, blood transfusion, and mechanical forces that exacerbate lung injury. The fact that the majority of patients with ALI/ARDS survive represents strong evidence that, despite the severe pathology of lung injury, repair mechanisms can reestablish normal endothelial and epithelial barriers and normalize the structure and function of the injured alveoli. Hence, this review also focuses on what has been learned regarding repair mechanisms, including the contributions of focal adhesions, integrins, vesicles, cellular antioxidants, platelets, neutrophils, lymphocytes, and monocytes. We also discuss new work on the potential role of endogenous progenitor cells in the lung to enhance repair. Finally, we briefly review some of the potential clinical approaches to lung repair that are being tested; such approaches include extracorporeal membrane oxygenation and cell-based therapy.

REGULATION OF THE PROPERTIES OF THE ALVEOLAR-CAPILLARY BARRIER

Lung Microvascular Filtration

The lung provides immune defense against inhaled pathogens. Two fluid systems are involved in this defense: (*a*) the lung lymph flow, which provides a conduit for immune communication between alveoli and lymph nodes, and (*b*) the alveolar wall liquid (AWL), which forms the aqueous subphase for the protective layer of surfactant phospholipids and proteins that lines the alveolar epithelium (6). Microvascular filtration, the regulated transvascular flow of water, proteins, and small solutes, is the source of both fluid streams. The filtrate flows in part into lymphatic capillaries, forming lymph, and in part across the alveolar wall by active chloride transport to form the AWL (7). These physiological phenomena can be directly viewed by live fluorescence microscopy of the lung microvascular network (**Figure 1**).

Filtration is the consequence of opposing forces, usually referred to as Starling forces, acting across the microvascular membrane. The microvascular pressure is the major profiltration force. Filtration is also determined by the fluid conductance properties (hydraulic conductivity) of the endothelial barrier. Selective protein hindrance by the endothelial barrier, a process termed protein

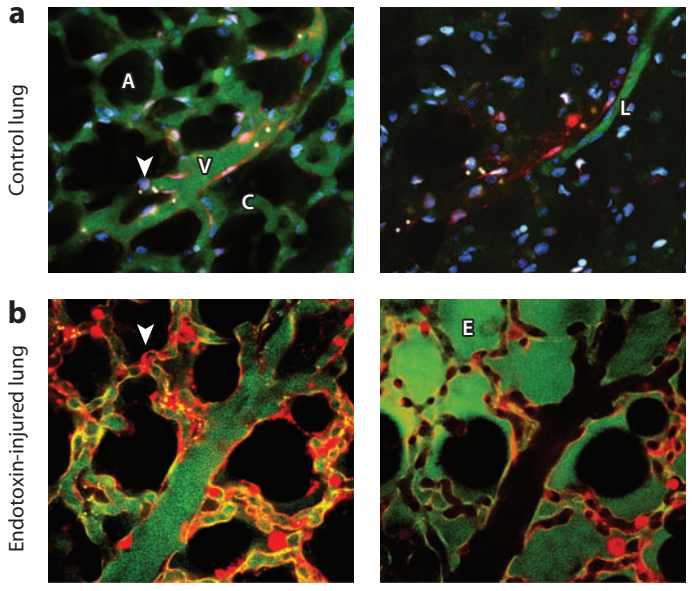

Figure 1

Live imaging of lung microvascular networks. Images obtained by live confocal microscopy show microvascular networks in (*a*) a control lung and (*b*) an endotoxin-injured lung. The vessels (*green*) were infused with FITC-dextran of molecular weight 70 kDa. The images were taken during dextran infusion (*left images*) and 30 min after buffer infusion to clear vascular fluorescence (*right images*). (*a*) The noninjured (*control*) lung contains a venule (V) and alveolar septal capillaries (C). The nonfluorescent spaces are air-filled alveoli (A). Endothelial nuclei are stained with Hoechst 33342 (*arrowhead*). The buffer wash removed all vascular fluorescence. The FITC fluorescence in the lymph (L) indicates that vascular dye crossed the microvascular barrier by filtration and was removed by lymphatic clearance. (*b*) In the endotoxin-injured lung, the vascular dye entered alveolar spaces as edema fluid (E). The numerous red spots (*arrowhead*) are leukocytes (presumably neutrophils) that marginated in microvessels.

sieving, allows unhindered transvascular transport of small molecules (e.g., insulin) but restricts passage of large molecules such as plasma proteins of size equal to or greater than that of albumin. The resulting higher protein concentration in plasma versus that in tissue establishes the plasma protein osmotic pressure that opposes filtration and that thereby promotes water retention in the blood and therefore maintenance of blood volume.

The prevalent view that filtration occurs at similar rates in all segments of the pulmonary microvascular bed has been challenged. The lung's unique hemodynamic properties are attributable to the so-called sheet structure of alveolar capillaries (8). According to this view, blood flow occurs not through tubular capillaries, as in systemic beds, but through two apposed sheet-like endothelial layers. This unique structural feature may account for the major drop in vascular pressure that occurs across the alveolar capillary bed (9–11). As a consequence, in much of the capillary bed, microvascular pressure is lower than the filtration-opposing plasma protein osmotic pressure, raising the question of whether filtration occurs in the alveolar septum. Whether the lung's postcapillary venules filter or absorb fluid was addressed by the split-drop approach, by which the transvascular fluid flux is determined in micrometer-scale segments of the microvascular bed (12). Split-drop determinations confirm that filtration does occur across postcapillary venules and that these vessels are intrinsically leaky (12, 13).

If the liquid formed by filtration in the postcapillary venules tracked proximally in the alveolar septum, the increase in septal fluid could impair oxygen diffusion. Under physiological conditions, liquid does not accumulate in the septum, because the liquid is cleared by the lung's interstitial pressure gradient, which is due to the fact that interstitial pressure is higher at the septum than in the peri-lymphatic interstitium (10, 14). The gradient directs the fluid formed at the venules toward the beginning lymphatics, thereby promoting interstitial fluid clearance (15).

The question of whether microvascular filtration occurs in the alveolar septum remains open because of the lack of direct evidence. Although intravenously injected fluorescent albumin appears in the lung interstitium (16), the site of albumin leakage remains undetermined. These issues have also been addressed through assays of the lung's microvascular filtration coefficient (*Kf*). The *Kf* reports the lung's total filtration rate as the rate of lung weight gain induced by step increases in lung vascular pressure (17, 18). *Kf* measurements made following compression of alveolar capillaries by high inflation pressure, a maneuver expected to block filtration in the alveolar capillary segment, indicate that more than half the total filtration occurs across vessels outside the alveolar septum, especially in the venous segment (19, 20).

Given all the findings, the emerging picture suggests that, although the greater part of the lung's vascular surface area resides in the alveolar capillaries, the lung's microvascular pressure drop inhibits fluid production along much of this gas-exchanging vascular segment. Vessels are leaky in the extra-alveolar segment, particularly in the first generation of venules downstream from alveolar capillaries. By this arrangement, fluid content of the alveolar septum is kept at a minimum, whereas sufficient fluid production occurs in the extra-alveolar vascular segments to account for lymph and AWL formation.

Vascular Role in Lung Immunity

The lung's innate immune response provides systemic protection against potentially inhaled pathogens. The response involves neutrophil, monocyte, lymphocyte, and platelet recruitment to pulmonary vessels and subsequent migration of the recruited leukocytes to the alveolar space. Injury to the microvascular and alveolar barriers occurs through paracrine effects of peroxide release from leukocytes or by the propermeability effects of thrombin formed as a result of induced procoagulant mechanisms. Platelets evidently play a role in the injury because they deposit procoagulant proteins on the endothelial surface (21) and their removal can protect against ALI (22). Other contributors to the injury are mechanical stresses, such as vascular stretch resulting from lung overexpansion (23) or increased vascular pressure (24, 25), and peroxide release by hypoxic red cells (26).

Endothelial cells at branch points of venular capillaries lying immediately distal to the alveolar capillary bed have come under increasing scrutiny as initiation sites of the lung's innate immune response. The branch-point endothelia in these venules richly express the proinflammatory receptor TNFR1 (27). The endothelial cytosol is enriched with Weibel-Palade bodies containing the leukocyte adhesion receptor P-selectin (25). Continuous Ca^{2+} exchange between the endoplasmic reticulum and the cytosol by the sequence involving inositol trisphosphate (IP_3)-induced Ca^{2+} release and SERCA-induced Ca^{2+} uptake causes cytosolic Ca^{2+} oscillations in the resting steady state (28). These findings suggest that endothelia of the venular capillaries are phenotypically primed to initiate immune responses.

Challenged by the mechanical stress of venous hypertension (29) or by exposure to soluble tumor necrosis factor (TNF)-α, a cytokine that increases in blood during sepsis and contributes to ALI (30), venular endothelia respond by doubling the amplitude of cytosolic Ca^{2+} oscillations. As a result, Ca^{2+} oscillations increase in the mitochondrial matrix, causing sequential activations

of Ca^{2+}-dependent dehydrogenases, mitochondrial electron transport, and mitochondrial superoxide production (27). Mitochondrial superoxide dismutase converts the superoxide to H_2O_2, which diffuses out of the mitochondria to activate cytosolic targets such as Weibel-Palade bodies, causing endothelial expression of P-selectin, and the nuclear factor κB (NFκB) pathway, causing proinflammatory gene transcription and hence endothelial expression of the leukocyte adhesion receptor E-selectin (27).

Given these findings, endothelia of these venular capillaries appear to rely on Ca^{2+}-induced activation of mitochondrial electron transport, and hence on ATP production and reactive oxygen species (ROS) production in mitochondria, to induce the proinflammatory phenotype. This ROS production is not injurious, and its defensive effects are different than those due to the pathologically induced excessive ROS production that causes mitochondrial dysfunction and proapoptotic responses such as mitochondrial release of cytochrome *c*.

The mitochondrial ROS induced by Ca^{2+} oscillations are protective against injury. Thus, the mitochondrial H_2O_2 release resulting from TNF-α-induced Ca^{2+} oscillations activates the metalloproteinase TACE (tumor necrosis factor–converting enzyme) on the luminal endothelial membrane (27). This activation causes shedding of TNFR1, increasing plasma levels of the receptor (31) and limiting the proinjury effects of TNF-α. In venular capillaries containing mitochondria with reduced levels of the Rieske iron-sulfur protein, a factor critical for mitochondrial electron transport, H_2O_2 production decreases (27), reducing TNFR1 shedding and thereby increasing injury. These findings suggest that the intrinsic protective effects of mitochondria-induced TNFR1 shedding may be lost when mitochondria become dysfunctional in lung endothelium.

The Lung Endothelial Barrier

The vascular endothelium is the first barrier to be encountered by fluid or inflammatory cells tracking from the vasculature to the alveoli. Loss of endothelial barrier properties causes the major vascular fluid leak underlying edema formation in ALI. Effective lung repair in ALI requires correction of two critical abnormalities of fluid regulation, namely hyperfiltration across the endothelial barrier and edema accumulation in the air spaces (see discussion of alveolar edema formation below). In ALI, the failure of endothelia to sieve proteins, a barrier property that prevents large proteins from crossing the barrier (see discussion on protein sieving above), causes the vascular hyperpermeability and hyperfiltration underlying pulmonary edema. Under conditions in which sieving fails and large proteins easily cross the barrier, intravenous protein delivery for colloidal volume resuscitation may not be a successful therapeutic strategy (32), because proteins can diffuse across the nonrestrictive endothelial barrier to equalize transvascular protein concentrations and thus abolish the filtration-opposing effect of plasma proteins (33) or even increase microvascular permeability (34).

The endothelial paracellular pathway, the major filtration route, contains protein assemblies that form tight junctions and adherens junctions (AJs). Barrier repair may involve AJs because these junctions can assemble rapidly (35). AJs are formed by the transmembrane cadherins: VE-cadherin in human and mouse lung endothelium (36–38) and E-cadherin in rat lung endothelium (39, 40). In AJs, cadherin is stabilized by homotypic *trans* interactions through ectodomains that establish cell-cell adhesion and by *cis* interactions that are required for cadherin packing in the cell membrane (41–43). The cytoplasmic domain of cadherin binds β-catenin (44), which in turn binds α-catenin (41, 45). The cadherin link to f-actin may be formed by α-catenin (46), although this scenario is not universally accepted (47). Some recent evidence indicates that the f-actin-bundling protein α-actinin-1 may form the link (48).

Evidence for the role of AJs in endothelial barrier regulation comes from studies in which endothelial monolayers were exposed to antibodies against the cadherin ectodomains (49) or mutant cadherins lacking the ectodomains or the β-catenin binding site were expressed (50). These AJ-inhibiting protocols induced endothelial hyperpermeability. Activation of TACE induced endothelial hyperpermeability by cadherin degradation (51). These findings suggest that weakening or loss of cadherin interactions at AJs or loss of cadherin from the endothelial junction accounts for the essential molecular defects underlying endothelial barrier failure.

The mechanisms regulating barrier properties in the pulmonary vascular bed remain inadequately understood, despite long-standing interest in this area. In some systemic beds, as, for example, in skeletal muscle (52) and the trachea (53), hyperpermeability is attributed to the formation of micrometer-scale endothelial gaps in postcapillary venules. However, the role of such gap formation in the formation of pulmonary edema remains unclear. As predicted by the fourth power law of fluid flux across channels, the formation of large gaps that remodel the endothelial cleft from the normal nanometer-scale geometry to micrometer-scale widths should increase the transendothelial fluid flux (J_v) by orders of magnitude. However, at edema-forming concentrations, permeability agonists increase the J_v of pulmonary venules by only 2–3 times (54–56), suggesting that micrometer-scale gaps do not usually form in pulmonary venules.

As recently reviewed (57), the theory that lung microvascular hyperpermeability results from the formation of micrometer-scale endothelial gaps comes largely from studies in cultured cells. On the basis of these studies, a contractile mechanism similar to that of smooth muscle may exist in endothelial cells (58). A central component of this hypothesis is myosin light-chain kinase (MLCK), phosphorylation of which causes actomyosin-based cell contraction, micrometer-scale gap formation, and hence barrier hyperpermeability. To determine whether the MLCK mechanism applies to endothelial cells in vivo, Yu et al. (59) selectively deleted MLCK in endothelial cells. Lipopolysaccharide (LPS)-induced lung injury was not attenuated in these MLCK-deleted mice, indicating that MLCK-induced endothelial contraction and micrometer-scale gap formation are probably not critical for lung hyperpermeability in ALI.

Proteins of the connexin (Cx) family, which form gap junctional channels (GJCs) between cells (60), have been implicated in lung endothelial barrier function. Cx43, Cx40, and Cx37 form GJCs in lung endothelium, allowing cell-cell flux of solutes smaller than 1 kDa. Traditionally, the vascular role of GJCs is ascribed to the communication of intercellular Ca^{2+} signals that coordinate vascular contractility (61). However, recent findings indicate that endothelia of noncontractile lung microvessels express Cx43 and that GJCs formed by Cx43 subserve the spatial spread of Ca^{2+} in the capillary network (62). Thus, endothelium-specific deletion of Cx43 blocked the thrombin-induced hyperpermeability response of the lung microvascular barrier, pointing to Cx43 as responsible for endothelial barrier regulation in the lung. Ca^{2+} increases in a single endothelial cell spread across the capillary network to induce expression of the leukocyte adhesion receptor P-selectin far from the activated cell. Such GJC-mediated disseminated Ca^{2+} responses may be the mechanistic basis for the spread of lung microvascular injury in ALI (63).

The Palade Transcytosis Hypothesis

A long-standing debate in lung microvascular fluid transport concerns the Palade hypothesis. According to this hypothesis, proteins cross the endothelial barrier by transcytosis, a process in which endothelial plasmalemmal vesicles, or caveolae, shuttle proteins from plasma to the interstitium (64). Thus, the Palade hypothesis invokes active transport rather than passive paracellular transport. A major challenge to the Palade hypothesis comes from studies in which tissue cooling, which blocks active cellular processes, did not block transvascular albumin transport, suggesting

that the transport occurs by passive processes and not by active processes (65). Also, the Palade hypothesis has not been well supported by studies in mice lacking caveolin-1, the caveolar coat protein. Although these mice do not develop caveolae, they appear to have no abnormalities of transvascular albumin transport (66), indicating that passive mechanisms regulate transvascular albumin transport.

To dynamically test the Palade hypothesis, Oh et al. (67) carried out live optical imaging studies of lung microvessels injected with a fluorescent antibody against the specific caveolar target aminopeptidase P (APP). The caveolae-targeted antibody, but not a control antibody against a noncaveolar endothelial epitope, rapidly crossed the microvascular barrier, as indicated by an increase of interstitial fluorescence. Knockdown of caveolin-1, a procedure that eliminates endothelial caveolae, blocked the fluorescence increase. These interesting findings led Oh et al. to conclude that, in support of the Palade hypothesis, the APP antibody crossed the microvascular barrier by caveolar transcytosis. Although these findings imply that the bound APP antibody dissociated from its caveolar epitope on the endothelial abluminal membrane, the mechanism of this dissociation remains unclear. One possibility that argues against Oh et al.'s caveolar interpretation is that the caveolae-targeted antibody was internalized and degraded in lysosomes. As a result, free fluorophore rather than the intact antibody may have been released to the interstitium, accounting for the increase in interstitial fluorescence.

Siddiqui et al. (68) tested the Palade hypothesis in wild-type and caveolin-1 knockout mice by quantifying the decrease in lung weight induced by a step increase in plasma albumin concentration. The expectation here was that, because albumin transcytosis is inhibited in knockout mice, the induced increase in plasma albumin is better sustained in knockout mice than in wild-type mice, causing greater fluid withdrawal (reverse filtration) from the lung's extravascular space. A fair interpretation of this result is possible if all components of the fluid transport system are similar for the wild-type mice and the knockout mice. However, this is not the case. Caveolin-1-deficient mice undergo severe lung remodeling, fibrosis, and alveolar septal thickening (66), which likely impact the paracellular transport mechanism because of major differences in surface area of filtration and in junctional morphology in these two groups of mice (69). These lung abnormalities in the caveolin-1 knockout mice muddy interpretations of the lung weight data as well as mechanistic analyses of the relative roles of the caveolar and paracellular fluid pathways.

The Palade hypothesis has been tested in the context of ALI. A prediction of the hypothesis is that, to the extent that caveoli subserve transvascular protein and fluid transport, the absence of caveoli should inhibit fluid transport, decreasing the risk of pulmonary edema. This prediction was realized: Caveolin-1-deficient mice were protected from ALI following exposure to endotoxin (70) or to hyperoxia (71). However, a caveat is that neutrophils are dysfunctional in caveolin-1-deficient mice (72). Because neutrophil activation plays an important role in ALI, the protection of caveolin-1-deficient mice against ALI may be due to the absence of the neutrophil-induced component of the injury rather than to decreased caveolar fluid transport. The extent to which the Palade hypothesis accounts for lung hyperpermeability in ALI continues to be unclear, and further research is needed.

The Alveolar Epithelial Barrier

The normal alveolar epithelial barrier is composed of alveolar epithelial type I (AT1) cells and alveolar epithelial type II (AT2) cells. These cells form a very tight barrier that normally is largely impermeable to proteins and solutes (73). The alveolar liquid subphase that exists between the apical surface of epithelial cells and the normal layer of surfactant is maintained by active ion transport. Direct experimental data are lacking to support this scenario, except for evidence that

the pH is 6.92, which is consistent with H^+ or HCO_3^- transport across the alveolar epithelium (74). Several studies in different animal models demonstrated that protein molecules cross the normal alveolar epithelial barrier very slowly either from the interstitial-to-alveolar direction or from the alveolar-to-interstitial direction (75). Although there is some debate, most of the evidence indicates that the movement of proteins across the normal alveolar epithelial barrier is determined largely by molecular weight and Stokes radius (76). Thus, the rate of alveolar absorption in vivo is size dependent for most proteins, with an inverse relationship between permeability and the corresponding molecular weight (76). Evidence from clinical studies of the resolution of hydrostatic pulmonary edema in patients also indicates that size selectivity of protein clearance in humans occurs during the resolution phase of pulmonary edema in patients in whom the alveolar epithelium is uninjured (76). There are some exceptions to this pattern. For example, human growth hormone (with a molecular weight of 22,000 kDa) demonstrates a more rapid clearance across the respiratory epithelium than would be expected for albumin (75). Thus, there may be a specific receptor or transport mechanism for human growth hormone.

AT1 and AT2 cells contain vesicles that are capable of endocytosis. Also, albumin may undergo receptor-mediated uptake through binding to gp60 on alveolar epithelial cells. However, studies of intact animals in which vesicular transport was pharmacologically blocked indicate that this pathway is probably a minor contributor to the transport of albumin and other serum proteins across the alveolar epithelial barrier. For example, monensin and nocodazole, pharmacological agents that decrease transcytosis by different mechanisms, were evaluated in rabbit studies to quantify the removal of albumin and IgG (77). In these studies carried out with the late Jo Rae Wright, the inhibitors were also studied for their effects on clearance of the surfactant apoprotein A (SP-A) from the alveolar space. SP-A was selected because it was a positive control for endocytosis inhibition; AT2 cells remove SP-A from the airspaces by receptor-mediated endocytosis. Monensin increased the quantity of SP-A associated with lung tissue after 2 h. Also, both monensin and nocodazole reduced uptake of FITC-labeled albumin in cultured alveolar epithelial cells, as well as in in vivo studies. However, the results of the in vivo studies showed that neither drug inhibited the removal of radiolabeled albumin or IgG instilled into the lung, supporting the conclusion that most of the protein removal occurred across paracellular pathways. The exact anatomic location or locations by which protein molecules can cross the alveolar epithelium or the distal airway epithelium have not been clearly established. The removal of insoluble proteins in the presence of lung injury requires clearance by macrophages (78, 79).

The expression and regulation of transmembrane tight junction proteins appear to be the major determinants of paracellular permeability to solutes, proteins, and perhaps ions (80–83). The most important of these tight junction proteins appear to be in the claudin family (84, 85). Claudin-4 is expressed at high levels in both AT1 and AT2 cells (86). There is some circumstantial evidence that claudin-4 levels may be associated with intact alveolar fluid clearance in human lungs (87). There is much to be learned about the role of claudins in regulating paracellular permeability to ions, solutes, and proteins. Selective knockouts of specific claudins should provide more insight into their function in the near future.

In addition to the tight barrier properties of the alveolar epithelium, the other critical property of the alveolar epithelial barrier for maintaining lung fluid balance is the capacity to remove alveolar fluid by vectorial ion transport. Although there is some uncertainty regarding the exact locations at which airspace fluid is removed from the distal airspaces (alveolar epithelium versus distal airway epithelium) (7), the predominant evidence favors sodium-dependent transport as the critical driving mechanism that regulates the removal of excess alveolar fluid under normal conditions (e.g., the perinatal lung) and pathological conditions (e.g., cardiogenic and noncardiogenic pulmonary edema) (88, 89). The rate of alveolar fluid clearance can be accelerated by cAMP agonists, including

elevated endogenous levels of epinephrine or the exogenous administration of β-2 adrenergic agonists (90). Several catecholamine-independent pathways, including glucocorticoids and thyroid hormone, increase the rate of alveolar fluid clearance. The details of this active ion transport system have been reviewed (88, 90). Because AT2 cells can be readily isolated from the lung and studied in vitro, our understanding of their contribution is more complete than for AT1 cells. Sodium uptake occurs on the apical surface, partly through amiloride-sensitive and amiloride-insensitive channels. Subsequently, sodium is pumped actively from the basolateral surface into the lung interstitium by Na,K-ATPase. An epithelial sodium channel (ENaC) that is crucial to sodium movement across the apical cell membrane was cloned and well characterized in 1994 (91, 92). Subsequent evidence indicates that CFTR (cystic fibrosis transmembrane regulator) is expressed in AT2 cells and plays a role in cAMP-mediated fluid transport (93–95). New information has been obtained regarding the role of AT1 cells, which also contribute to vectorial fluid transport (96, 97). AT1 cells express aquaporin 5 (98), Na,K-ATPase (97), and ENaC (97, 99). Human alveolar epithelial type II cells secrete and absorb liquid in response to local nucleotide signaling (100). Thus, the ability of the alveolar epithelium to preserve the essential function of the lung to absorb oxygen and excrete carbon dioxide is subserved by its tight barrier properties as well as by its capacity for active removal of excess alveolar fluid.

Surfactant in the alveolar compartment may also have a significant effect on maintaining normal lung fluid balance. Depletion of surfactant in experimental models leads to pulmonary edema, including flooding of the airspaces, although the pathways for alveolar flooding may include several mechanisms such as epithelial cell injury and increased transvascular hydrostatic forces. For example, increased alveolar surface tension due to surfactant deficiency may result in a negative pressure surrounding pulmonary capillaries that could promote fluid filtration. Measurement of alveolar liquid pressure by micropuncture in isolated lungs in mature and immature fetal rabbits, with and without surfactant replacement, indicated that alveolar liquid pressure was lower in immature than in mature lungs at comparable airway pressures. Surfactant replacement in immature lungs resulted in pressure-volume curves and alveolar liquid pressures similar to those in mature lungs (101). Thus, surfactant depletion may predispose immature or mature lungs to the development of pulmonary edema.

In addition to the role of surfactant in maintaining alveolar stability and its contribution to lung fluid balance, elucidated largely by John Clements and the investigators who worked with him, mature surfactant also contains surfactant proteins, which Wright (102) discovered to have an important role in host defense.

MECHANISMS OF INJURY

Injury to the lung endothelium is the primary cause of increased-permeability pulmonary edema in ALI. Hyperfiltration of plasma across the lung vascular bed results in the accumulation of protein-rich edema in the interstitium and the airspaces of the lung, even if lung vascular pressure is normal (**Figure 1**) (103). Injury to the lung endothelium may occur by several mechanisms, although neutrophil-dependent lung injury has been well established as a major pathway in experimental models of sepsis, pneumonia, aspiration of hydrochloric acid, ischemia-reperfusion, trauma and shock, and transfusion-associated lung injury (104). In both infectious and noninfectious lung injury, neutrophils accumulate in the microcirculation of the lung; become activated; and subsequently degranulate and release several toxic mediators, including ROS, proteases, proinflammatory cytokines, and procoagulant molecules. These mediators can increase lung vascular permeability (104). Injury by neutrophils and their intracellular products may increase vascular permeability by altering focal adhesions, transmembrane integrins, and the cytoskeleton of the

endothelial cells. Endothelial cell apoptosis may occur in the presence of some injuries, which could lead to further loss of epithelial barrier integrity.

Platelets can enhance or deteriorate lung endothelial barrier function (105). There is recent evidence that platelets may play a role in neutrophil-mediated lung injury. Platelet depletion markedly reduced both acid-induced lung injury and blood transfusion–induced lung injury in mouse models (106). In these models, platelet sequestration in the lung was neutrophil dependent, although neutrophil sequestration was not platelet dependent. The exact molecular pathways that link platelet-mediated injury and neutrophil-mediated injury are not completely understood. Neutrophil recruitment in the microcirculation of the lung is mediated by step-by-step interactions with the endothelium, usually referred to as rolling adhesion, a process that is often followed by extravasation of the neutrophils into the interstitial and alveolar compartments.

In addition to the effects of neutrophils (or monocytes) on the lung endothelium, some inflammatory mediators act directly on the lung capillaries, resulting in increased expression of chemokines and cell surface molecules that are important for leukocyte adhesion (104, 107–110). Consequently, leukocyte adhesion to the endothelium and the accumulation of neutrophils in the microcirculation increase. These processes in turn can lead to inflammatory lung injury and to further accumulation of other leukocytes in the lung. Thus, pathogenesis in lung injury depends in part on endothelial activation by several pathways. Recent data from an influenza model of lung injury suggest that lung endothelium may orchestrate a major part of lung injury that occurs in virus-induced lung failure (111). Also, injury to the endothelial barrier may be mediated by products of bacteria or viruses, independently of the effects of activated leukocytes. For example, toxins produced by *Pseudomonas aeruginosa* and *Staphylococcus aureus* break down the endothelial barrier as well as the epithelial barrier (112, 113).

Finally, the injured lung is particularly susceptible to mechanical injury that can result from elevated tidal volume or elevated airway pressures (114, 115). Pathways of injury include greater activation of macrophages and neutrophils, with an enhanced proinflammatory response, direct injury to the lung endothelial and epithelial barriers, and translocation of bacteria and their products into the systemic circulation that results in shock syndrome that may further injure the pulmonary circulation (116).

Mechanisms of alveolar epithelial injury are not as well known as mechanisms of injury to the pulmonary circulation. Neutrophils and their products appear to play an important role in some models of lung injury by increasing paracellular permeability (117). Transepithelial migration of neutrophils in the distal airspaces involves adhesion, migration, and effects that may occur after migration into the alveoli. However, the movement of neutrophils into the distal airspaces of the lung is not always associated with lung injury. Instillation of neutrophil chemotactic agents into the distal airspaces of the human lung can attract large numbers of neutrophils without altering barrier permeability to protein (118). With greater activation, neutrophil movement across the epithelium can directly damage the alveolar epithelium, perhaps by the release of toxic intracellular molecules that induce the dissolution of tight junctions and induce apoptosis and necrosis of AT1 and AT2 epithelial cells. Several neutrophil-derived mediators, including proteases, ROS, and matrix metalloproteinases, cause increased epithelial permeability and injury (119, 120). Small-animal models also indicate that angiotensin II may mediate alveolar epithelial cell injury (121, 122).

ALI causes surfactant dysfunction by releasing factors that interfere with the physical properties of the alveolar air-liquid interface. The efficacy of a pulmonary surfactant depends on its packing at the interface and on its phase-transition temperature, that is, the temperature at which the lipid transitions from a gel to a fluid, or the so-called liquid crystalline phase (123). For the main lipid component of alveolar surfactant, the transition temperature is close to body temperature. Thus, alveolar surfactant retains phase equilibrium at the sol-gel interface and is able to perform as a

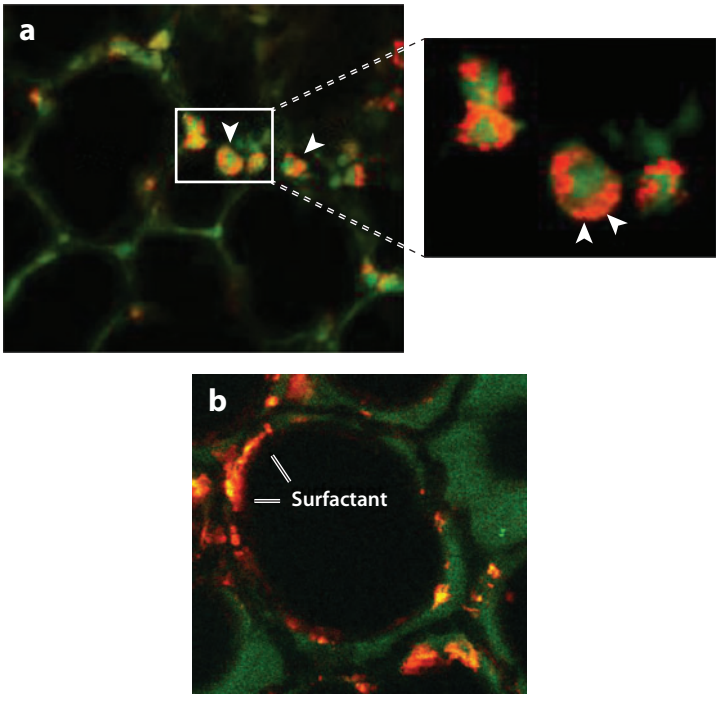

Figure 2

Live imaging of alveolar surfactant. (*a*) The left image shows a field of alveoli. Type 1 epithelial cells are demarcated by the green intracellular fluorescence of calcein green. Type 2 epithelial cells (*arrowheads*) contain red fluorescence of surfactant protein B in lamellar bodies. High magnification (*right*) of the region in the rectangle shows lamellar bodies (*arrowheads*) at the cell periphery that are ready for surfactant secretion. (*b*) The fluorescence of the lipid dye FM1-43 indicates the manner in which surfactant distributes in the alveolus. Surfactant spreads throughout the alveolus in a continuous film, occasionally forming aggregates; these aggregates may provide a reservoir for replenishing surfactant at the air-liquid interface.

surfactant under physiological conditions. In ALI, the incorporation of nonsurfactant lipids, such as cholesterol derived from damaged cells, and plasma proteins that exude into the alveolus disrupt the air-liquid interface, reducing the efficacy of the lung's natural surfactants (124).

Surfactant dysfunction in ALI also results from abnormalities of surfactant synthesis and secretion. AT2 cells synthesize surfactant phospholipids under control of the transcription factor STAT-3 (signal transducer and activator of transcription-3). Lung-specific deletion of STAT-3 decreases surfactant production and exacerbates ALI (125). AT2 cells store surfactant in vesicles termed lamellar bodies (LBs). The incorporation of fluorophores in LBs enables live detection of single AT2 cells in the optically imaged lung (**Figure 2**) (126). Lung hyperinflation, the physiological stimulus for surfactant secretion (127), causes loss of LB fluorescence (126). Time-dependent determinations of the fluorescence loss provide dynamic quantification of the rate of surfactant secretion.

The micromechanics of alveolar expansion play a critical role in the regulation of surfactant secretion. New findings challenge the older view that alveoli expand uniformly (128). Live optical imaging indicates that alveolar expansion causes asymmetric dimensional changes in different segments of the alveolar wall such that some segments undergo greater stretch than others (129). In general, AT2 cells appear to be protected from the stretch of alveolar expansion; the stretch

is taken to a greater extent by AT1 cells that form the major part of the alveolar wall. As a result, Ca^{2+} oscillations increase in the AT1 cytosol. The oscillations are transmitted to AT2 cells through GJCs, causing Ca^{2+}-induced surfactant secretion (126). Thus, AT1 cells appear to be alveolar mechanosensors, responding to the stretch of lung expansion by conveying surfactant-secreting Ca^{2+} signals to the AT2 cell. Because alveolar expansion is the physiological stimulus for surfactant secretion, edema or other factors released in ALI that alter alveolar micromechanics can interfere with the AT1 cell's mechanosensor function, thereby inhibiting surfactant secretion and deteriorating alveolar stability.

Finally, in the presence of ALI, the capacity of the lung to remove alveolar edema is markedly decreased (130, 131). There is evidence that apoptosis and necrosis of alveolar epithelial cells (132, 133) as well as inflammation-induced downregulation of sodium and chloride transporters (134) are major mechanisms. Several studies have also identified hypoxia as downregulating alveolar fluid clearance by reducing ENaC delivery to the apical membrane and Na,K-ATPase delivery to the basolateral membrane (135, 136).

On the basis of in vivo studies, live bacteria can impair alveolar fluid clearance, in part by producing proinflammatory cytokines in the lung airspaces that result in breakdown of the alveolar epithelial barrier (116), as well as specific downregulation of ion transporters (134). Other inflammatory molecules, including leukotriene D4, reduce alveolar fluid clearance (137). Interleukin-1β and transforming growth factor-β can depress alveolar fluid clearance by interfering with vectorial sodium transport (138, 139). Influenza virus infection can alter ion transport by inhibiting amiloride-sensitive sodium current across the mouse tracheal epithelium (140). The inhibitory effect of influenza virus may be mediated by binding the viral hemagglutinin to a cell surface receptor, which then activates phospholipase C and protein kinase C (PKC). PKC can reduce ENaC activity so that influenza infections in the lung inhibit ENaC functions (141). Finally, one study tested how influenza virus can reduce vectorial fluid transport across AT2 cell monolayers and reduce the open probability of single ENaC channels in apical cell–attached patches. Intratracheal administration of the influenza virus rapidly inhibited amiloride-sensitive (i.e., ENaC-dependent) alveolar fluid transport, and thus influenza infection may exacerbate alveolar edema (142). There is also evidence that mycoplasma lung infection decreases alveolar fluid clearance and functional ENaC channels through the production of reactive oxygen-nitrogen intermediates (143).

MECHANISMS OF REPAIR

Resolution and repair depend on removal of the protein-rich alveolar edema fluid, clearance of the neutrophils and all debris, and restoration of the barrier properties of the alveolar epithelium and the lung endothelium (2). As discussed above, removal of alveolar edema occurs by vectorial ion transport–driven edema fluid clearance (88, 90). Resolution of inflammation occurs by several pathways that require macrophages and lymphocytes (144–146).

Endogenous Progenitor Cells

Endogenous stem and progenitor cell populations have been described in several organs, including the bone marrow, brain, and heart. However, for stem and progenitor populations, the location, types, and overall contribution to lung repair within the adult lung have only recently been explored.

Prior studies suggested that new alveolar epithelial cells are generated from AT2 cells. The prevailing theory has been that AT2 cells are the only cells that can repopulate denuded alveolar

epithelium and form new AT1 and AT2 cells. However, an important recent study in mice identified a cell population in the distal lung that express α6β4 (147). These cells do not express surfactant protein C (SPC), and they can proliferate and differentiate into either AT1 cells or AT2 cells both in vitro and in vivo. When cultured under a renal capsule in mice, these cells form alveoli with positive staining for AT2 cells (SP-C), with evidence of blood flow. Furthermore, these implanted cells self-organize into distinct airway-like Clara cells, with secretory protein ($CC10^+$) and SPC^+ sacular structures. In a bleomycin model of lung injury with an SPC-driven inducible Cre to fate map alveolar epithelial cells, the majority of AT2 cells in areas of fibrosis were not derived from preexisting AT2 cells, demonstrating that SPC^- progenitor cells replenish AT2 cells during repair. Thus, these mouse experiments support the hypothesis that there is a stable alveolar epithelial cell progenitor population in the adult lung, with evidence of alveolar epithelial cell progenitor cell differentiation after lung injury. In addition, other investigators recently reported isolation from whole-lung suspension of stem-like cells that express α6β4 (148). In terms of airway repair, the regenerative capacity after lung injury has been attributed in part to a subset of $Scgb1a1^+$ cells that are located near the bronchoalveolar junction and repopulated airway epithelium following lung injury induced by naphthalene, a toxin that selectively depletes Clara cells. Moreover, these investigators described bronchoalveolar stem cells that are located at the bronchoalveolar junction and that express Scgb1a1 and SPC (149). These cells proliferate following naphthalene- or bleomycin-induced lung injury, thus indicating self-renewal and multipotency in clonal assays. However, other investigators carried out in vivo lineage-tracing experiments using labels driven by the Scgb1a1 promoter and reported that these cells regenerate airway epithelium following naphthalene-induced lung injury. These investigators also showed that following adult hyperoxia these cells did not appear to contribute to alveolar repair (150). In contrast, a recent report indicated that by using an inducible Cre knock-in mouse lineage tag $Scgb1a1^+$ cells, the $Scgb1a1^+$ cells that were labeled prior to bleomycin injury did contribute to alveolar epithelium during repair (151). Also, following virus-induced lung injury with influenza, p63-expressing cell populations in the bronchiolar epithelium proliferate and form pods; these pods can expand to alveolar-like structures expressing alveolar-specific genes and regions of the lung that subsequently regenerate into normal lung tissue (152). Finally, one research group reported that there may be a small population of $c\text{-}KIT^+$ cells that can produce all components of the human lung (153).

Thus, recent research has identified progenitor cell niches within the lung that can contribute to lung repair after injury. Most of this work has been done in mouse lungs, and progenitor cells in the human lung need to be identified.

Potential Clinical Approaches to Lung Repair

Because lung-protective ventilation is effective in reducing mortality and increasing ventilator-free days in patients with ALI (4), considerable research has demonstrated that lower tidal volume and reduced airway pressure facilitate resolution of lung injury (114). Thus, adjunctive approaches to rest the lung while supporting gas exchange may facilitate lung repair, even in the setting of the most severe lung injury. Therefore, there is a rationale for extracorporeal membrane oxygenation (ECMO) in the treatment of very severe lung injury that is refractory to other therapies. ECMO can be applied rapidly and safely to very severely ill lung injury patients (154). With the support of extracorporeal removal of carbon dioxide and extracorporeal oxygenation, the injured lung can be maintained at a moderate level of positive end-expiratory pressure and ventilated with very low tidal volumes to facilitate lung repair. Although ECMO has not been proven to be superior to other therapies, it has been used as a rescue therapy effectively in many patients with very severe

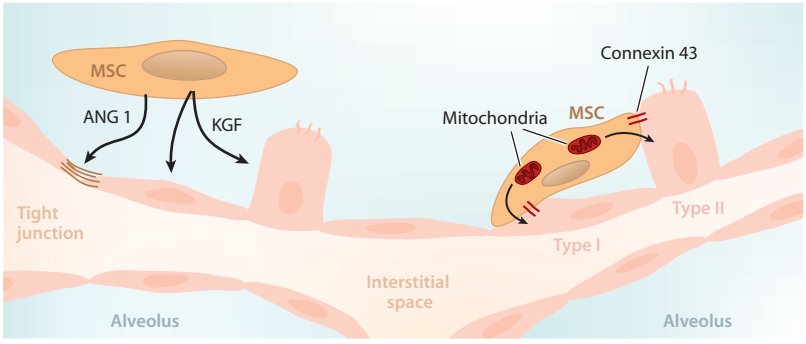

Figure 3

Schematic diagram to illustrate cell contact–independent and –dependent mechanisms from treatment of acute lung injury with airspace delivery of bone marrow–derived mesenchymal stem cells (MSC). (*Left*) MSC release angiopoietin-1 (ANG 1) and keratinocyte growth factor (KGF), which have beneficial effects as paracrine proteins on the repair of injured alveolar epithelium. (*Right*) MSC attach to alveolar epithelial type I and II cells by connexin 43 molecules. The arrows indicate the impending transfer of the mitochondria from the MSC to the alveolar epithelium. Such transfer can rescue injured alveolar epithelium by restoring more normal levels of ATP.

lung injury. In such cases, lung repair may be facilitated because the mechanical stress on the lung is relieved, thus enhancing resolution.

In the future, it may be possible to bioengineer replacement lungs in patients who have refractory lung injury. The novel technology of induced pluripotent stem (iPS) cells could be used to autologously generate new lung tissue from patients. This strategy was made possible by the landmark 2006 discovery by Takahashi & Yamanaka (155) that differentiated fibroblasts could be transcriptionally preprogrammed into iPS cells. After considerable research, four transcription factors were found to be required for this reprogramming. Intact mice can now be generated from iPS cells, thus establishing these cells as true stem cells. In addition, the remarkable progress in generating human lung from a decellularized lung scaffold in mice and rats also raises the possibility that replacement of the entire lung could be possible in the future (156).

Finally, a more immediate potential pathway to treating severe clinical lung injury could involve allogeneic human bone marrow–derived mesenchymal stem cells (MSCs). Several investigators have reported the beneficial effects of MSCs in reducing lung injury and enhancing repair, either with the cells themselves or with cell-free conditioned media (**Figure 3**). To translate these basic science studies to the clinical setting, translational work will be needed to establish safety and efficacy. This cell-based therapy approach has potential in part because there are multiple mechanisms by which MSCs may benefit the injured lung, including anti-inflammatory effects, antimicrobial effects, decreased apoptosis, and enhanced repair (157–163). One interesting article recently reported that MSCs can increase the number of bronchoalveolar stem cells both in vitro and in vivo (151). Another recent study showed that endotoxin-injured alveolar epithelium in mice could be rescued by MSC-delivered mitochondria that improved alveolar epithelial cell bioenergetics and increased survival (164). Thus, there is evidence for both cell contact–dependent mechanisms and cell contact–independent mechanisms to explain the therapeutic effect of MSCs in the setting of experimental ALI (**Figure 4**) (165, 166).

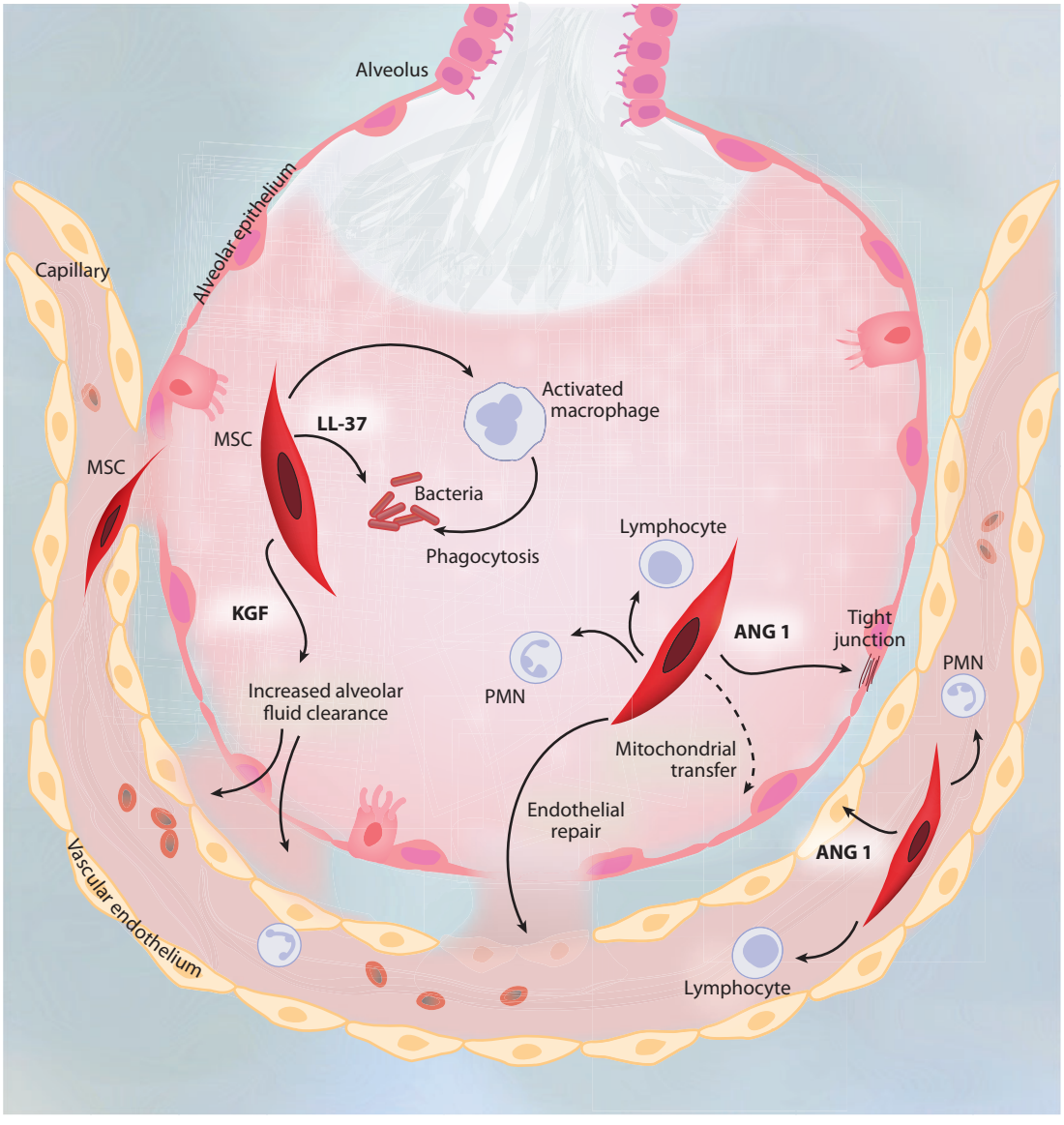

Figure 4

Beneficial effects of bone marrow–derived mesenchymal stem cells (MSC) in acute lung injury. Protein-rich edema fluid and inflammatory cells fill an injured alveolus after endothelial and epithelial injury. MSC exert immunomodulatory effects on neutrophils, lymphocytes, and macrophages; assist in the repair of the injured epithelial and endothelial barriers; improve alveolar fluid clearance; and secrete several molecules, including the antibacterial peptide LL-37, angiopoietin-1 (ANG 1), and keratinocyte growth factor (KGF). PMN denotes polymorphonuclear cells. Reprinted with permission from Reference 166.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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