NHLBI Workshop Summary

Alveolar Epithelial Transport
Basic Science to Clinical Medicine

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In the last 10 years, since the National Heart, Lung, and Blood Institute (NHLBI) convened a workshop focused on mechanisms of alveolar microvascular injury (1), there has been an explosion of new knowledge in alveolar epithelial cell biology, in part because of advances in cell culture, electrophysiology, molecular biology, and success in applying these insights to the clinical problem of pulmonary edema. A recent NHLBI Workshop (Alveolar Epithelial Transport: Basic Science to Clinical Medicine) examined alveolar epithelial cell biology as it relates to transport and cell differentiation in the adult lung, with a particular emphasis on the relevance of this topic to the resolution of pulmonary edema. The program did not consider airway epithelial barrier function/biology, surfactant biology/metabolism, cystic fibrosis, or developmental biology since each of these areas merits a separate workshop. This report provides a synopsis of topics that were discussed at the workshop.

ALVEOLAR EPITHELIAL TRANSPORT: BASIC SCIENCE

Alveolar Type I and Type II Epithelial Cells

Approximately 99% of the large internal surface area of the lung (adult human lung $\sim$ 100 to 150 m^2) is lined by two morphologically distinct epithelial cells, Type I and Type II. Type I cells are large squamous cells (diameter of $\sim$ 50 to 100 μm and volume of $\sim$ 2,000 to 3,000 μm^3). Type II cells are smaller cuboidal cells (diameter $\sim$ 10 μm and volume of $\sim$ 450 to 900 μm^3). Both tight (2–4) and gap (5–7) junctions couple Type I and Type II cells, providing barrier functions and pathways for intercellular communication.

Although the precise functions of Type I cells remain largely speculative, this cell must play an important role in gas exchange. Initial immunocytochemical evidence *in situ* demonstrated localization of Na,K-ATPase in Type II cells, but not in Type I cells (8). From these observations, it was initially inferred that alveolar transport was regulated largely by Type II cells, with Type I cells playing only a passive role. Because of the presentation of more recent data, however, this hypothesis will need to be revisited. Freshly isolated Type I cells were reported to exhibit the highest known water permeability of any mammalian cell type, thereby likely to confer very high water permeability to the lung (9). On the basis of preliminary data, freshly isolated Type I cells express subunits of both Na,K-ATPase and the amiloride-sensitive epithelial Na channel (ENaC), suggesting that this cell type may play a role in vectorial ion and water transport as well (10). Detection of Na,K-ATPase in isolated Type I cells, but not *in situ* to date, is perplexing but could be due to the low density of Na,K-ATPase content per unit membrane surface area on the widely spread Type I cells *in situ.* Finally, the presence of both vesicles and caveolin in Type I cells suggests that this cell may also be involved in transport of macromolecules (11–13).

Type II cells synthesize, secrete, and recycle surfactant components and mediate repair to the injured alveolar epithelium. When Type I cells are damaged and sloughed from the alveolar surface, Type II cells divide, with cell progeny either maintaining morphologic characteristics of Type II cells or spreading over the denuded basement membrane and transdifferentiating into Type I cells (14). Ion transport has been studied in both freshly isolated and in cultured Type II cells by various methods, including radiolabeled probes (15, 16) and patch-clamp techniques (17–21).

When Type II cells are cultured on tissue culture plastic or on microporous supports, the cells flatten, lose differentiated Type II cell morphologic features, and cease synthesis and secretion of biochemical markers of surfactant (22–24). These metabolic changes begin within the first 24 h of culture and are progressive over several days in culture. By manipulating extracellular matrix components, soluble factors (such as growth factors), physical shape, mechanical forces, or the air-liquid interface cultured alveolar epithelial cells can be induced to retain (or regain) morphologic, biochemical, and molecular expression of markers of the Type II cell phenotype (25, 26).

Isolation and culture of alveolar Type I cells is much more difficult, in part because their cytoplasmic extensions are thin and the intercellular junctions may be very tight. Recent improvements for isolating Type I cells have facilitated the first studies of fresh Type I cells *in vitro* (9, 10). The properties of Type I cells have been inferred from studies done with freshly isolated Type II cells plated and grown on tissue culture plastic or porous substrata (27, 28). Alveolar epithelial cells cultured in this manner cease to express the Type II cell phenotype and, over time, increasingly express all the available markers associated with Type I cells *in situ* (29–33). These *in vitro* changes are reminiscent of the response of the adult alveolar epithelium to injury. Furthermore, under certain conditions, e.g., shape change and keratinocyte growth factor (KGF), these Type I-like cells revert to the Type II cell phenotype (25, 26, 34). Although use of alveolar epithelial Type I cell-like monolayers obtained by culturing freshly isolated

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Type II is valuable, studies of freshly isolated and cultured Type I cells are needed to advance our knowledge of Type I cell biology.

It is also important to apply the new understanding of the molecular structure of intercellular junctions and the maintenance of cell polarity to studies of alveolar Type I and II cells (35, 36). Both targeted delivery of vesicles to the appropriate membrane domain by sorting of proteins in the trans-Golgi apparatus and regulated turnover of specific proteins in membrane domains help maintain epithelial cell polarity. For example, localization of the Na pump (Na,K-ATPase) to the basolateral membrane is determined not only by targeting to this location but also by recycling of Na,K-ATPase delivered to the apical plasma membrane (37). Interestingly, there is colocalization of Na,K-ATPase and cytoskeletal elements (38), suggesting a functional interaction.

Regulation of Na Transport by the Normal and Injured Alveolar Epithelium

There is strong evidence that active Na transport across the alveolar epithelium creates an osmotic gradient that leads to fluid reabsorption from the alveolar space. Na ions enter the apical membranes of alveolar epithelial cells in part through amiloride-sensitive cation channels and are transported across the basolateral membrane by the ouabain-inhibitable Na,K-ATPase.

The Na,K-ATPase is a ubiquitous plasma membrane ion-transporting ATPase that maintains transmembrane gradients of Na and K by pumping Na out of the cell and K into the cell against their respective concentration gradients, fueled by hydrolysis of ATP. Removing edema fluid from the air spaces is accomplished by the polar distribution of Na pumps and channels on opposite poles of the epithelial cells (39). Na pump activity in the plasma membrane is regulated acutely by covalent or allosteric modification and/or acute trafficking of pumps between the plasma membrane and the intracellular endosomal pools, and chronically by regulation of the abundance of pumps in the membrane by changes in pump synthesis and/or degradation rates (40). Na pumps comprise one alpha and one beta subunit, although there are multiple isoforms of both subunits, usually expressed in a tissue-specific pattern. There is evidence for two alpha and three beta subunits expressed in lung cells (28), having potential for at least six heterodimer combinations. However, it is widely believed that a heterodimeric form made up of the alpha 1 and beta 1 subunits is the predominant Na pump isoform in alveolar epithelial cells, although expression of the alpha 2 subunit over time in culture has been reported by one group (41). Adenoviral gene transfer of the beta 1, but not alpha 1, subunit increases Na pump expression and function in the adult rat lung (42).

In situ hybridization studies, as well as Northern blot analyses, have identified the presence of mRNA for all three subunits of ENaC in alveolar epithelial Type II cells both *in vivo* and *in vitro* (24, 43–52). However, patch clamp studies in alveolar epithelial cells have failed to identify the classic amiloride-sensitive Na channel (with a single channel conductance of ~ 5 pS) in alveolar epithelial cells, suggesting that apical Na entry may also be mediated via processes other than ENaC such as nonspecific cation channels. There is usually parallel, independent regulation of apically localized Na transport processes and basolaterally located Na,K-ATPase in response to a variety of stimuli, including hormones such as dopamine, catecholamines, glucocorticoids, aldosterone, and thyroid hormone, as well as other pathologic conditions (49, 50, 51–73). Proposed mechanisms for stimulation of active Na absorption across the alveolar epithelium by beta agonists and catecholamines include increases in Na pump alpha subunit phosphorylation and quantity in the basolateral cell membrane (74, 75), augmented Na channel open probability (62, 76–78), delivery of more ENaC channels to the membrane by cAMP stimulation (79), and indirect stimulation of transcellular Na movement by stimulation of apical chloride conductance (80). Intracellular Na concentration has also been implicated in the coupling of Na pump and channel activities (81–83).

The stimulatory effect of beta agonists on transepithelial Na transport has been observed in lung injury concurrent with increases in the expression of Na,K-ATPase as well as Na channels (61, 84–87), raising the possibility that the endogenous stress response could be involved in modulating these changes. The impact of chronic stimulation with dexamethasone, as well as the impact of adrenalectomy, on changes in expression of Na pumps and channels in injury models and on lung liquid clearance are being investigated (45, 50, 52, 68, 72, 73, 88, 89). Identification of the cellular mechanisms by which cAMP/cGMP (77, 78, 90, 91), as well as other agents known to increase Na transport (91), are being studied. Also, mechanisms that can downregulate transport are being explored. For example, hypoxia decreases alveolar epithelial Na and fluid transport (92) and reactive oxygen species can reduce *in vivo* alveolar fluid transport (93).

Role of Water Channels in Alveolar Water Homeostasis

Water permeabilities have been measured across several of the major barriers in lung (94). Osmotically driven water movement across pulmonary epithelial barriers in the *in situ* sheep lung was found to be very fast (95, 96), and air-space-to-capillary osmotic water permeability in mouse lung is also high (osmotic water permeability coefficient Pf ~ 0.02 cm/s), weakly temperature-dependent, and mercurial-inhibitable (59). Microvascular Pf in mouse lung is equally high (0.03 cm/s) (97). Osmotic water permeability of isolated immunopurified Type I alveolar epithelial cells is exceptionally high (Pf ~ 0.07 cm/s) (9), although osmotic water permeability across the isolated alveolar epithelium per se is yet to be determined.

Recent studies in transgenic mice have begun to define the role of aquaporin (AQP)-type water channels in water transport in the intact lung. Four aquaporins have been localized in lung to date: AQP1 in microvascular endothelia and some pneumocytes (97-100), AQP3 in basal cells of nasopharynx, trachea, and large airways (100), AQP4 at the basolateral membrane of airway epithelium (101), and AQP5 at the apical membrane of Type I alveolar epithelial cells (100–102). Each of the four lung aquaporins has been deleted in mice by targeted gene disruption (103–106). Deletion of AQP1 or AQP5 each produced a 10-fold decrease in osmotically driven water transport between the air space and the capillary compartments (107–108). AQP1 and AQP5 thus appear to play major roles in osmotically driven water movement across the alveolar endothelial and epithelial barriers, respectively. AQP1 deletion also caused a moderate decrease in transcapillary water movement in response to hydrostatic pressure differences (107). AQP4 deletion alone had little effect on air-space-to-capillary water permeability, but studies comparing water permeability in AQP1 null mice versus AQP1/AQP4 double knockout mice showed a small contribution of the airways to lung water permeability (109). Interestingly, isosmolar alveolar fluid clearance was not affected by AQP1 or AQP5 deletion, even under conditions where clearance rate was maximized (107, 108).

Whether or not aquaporins play a role in the maintenance of alveolar fluid homeostasis is unknown. Although aquaporin expression is strongly upregulated near the time of birth (110–

112), AQP1, AQP4, and AQP5 knock out mice have the same ability to clear lung fluid in the perinatal period as wild type control mice (113). Although a major role of AQP5 in physiological transalveolar epithelial water movement appears unlikely, aquaporins may have effects on other cell functions, especially volume regulation, particularly in the highly specialized Type I cell (114). New studies also show no effect of AOP1, AQP4, or AQP5 deletion on the formation or clearance of lung edema after lung injury from hyperoxia, thiourea, or acid instillation (113). The role of aquaporin in airway hydration needs to be investigated and there may be some value in using aquaporin gene delivery for experimental studies of lung fluid balance.

Macromolecule Transport Across the Alveolar Epithelial Barrier and Its Role in Pulmonary Drug Delivery

Systemic delivery of some macromolecule drugs (e.g., proteins) via the pulmonary route by intratracheal instillation and/or inhalation of aerosolized drugs has been well documented to provide higher bioavailability than via any other noninvasive port of entry (116, 117). In addition, peptides that have been chemically altered to resist peptidase activity exhibit high bioavailabilities by the pulmonary route, suggesting involvement of peptidases/proteases in peptide processing in the respiratory tract and/or elsewhere in the lung (118, 119). By contrast, proteins with molecular weights between 6,000 and 50,000 daltons are more resistant to most peptidases and have good bioavailabilities after inhalation (120). Although inhaled peptides and proteins are not yet commercially available for clinical use, trials of inhaled delivery of several drugs, including luteinizing hormone releasing hormone (LHRH) and insulin, are underway.

Caveolae are small plasma membrane invaginations with a cytoplasmically oriented protein coat (121). Three genes for caveolin have been cloned and are expressed in a regulable and tissue-specific manner (122). Multiple isoforms of caveolin are expressed in the same cell with distinct distributions (122). This implies the existence of functionally distinct subpopulations of caveolae. The functions of caveolin are still far from clear, although they may be involved in transcytosis or the vesicular movement of macromolecules across endothelial cells (123). Whether alveolar epithelial cells exhibit similar transport properties is unknown, but Type I and Type II cells have membrane apical and basolateral invaginations consistent with vesicular or caveolar transport capability.

Most exogenous macromolecules less than 40 kD are probably absorbed into the interstitial and vascular spaces from the air spaces through tight junctions by simple restricted paracellular diffusion (124–126). Recent data, however, suggest the presence of specialized transcellular transport processes for translocation of proteins greater than 40 kD. Albumin, transferrin and immunoglobulin G are absorbed intact, asymmetrically and at higher rates than expected from diffusional processes (127). By contrast, horseradish peroxidase, a widely used pinocytosis marker, exhibits low and symmetric permeability in either direction across the alveolar epithelium (128), suggesting that absorption of proteins/peptides via pinocytotic routes may be minimal.

ALVEOLAR EPITHELIAL TRANSPORT: CLINICAL MEDICINE

The workshop focused on opportunities to enhance alveolar epithelial fluid clearance in patients with pulmonary edema. There is considerable experimental evidence that beta adrenergic agonists could be effective in enhancing the resolution of pulmonary edema (129). In addition, there is experimental evidence that hormonal therapy with glucocorticoids, thyroid hormone, or aldosterone can augment alveolar fluid transport (72). Also, growth factors and cytokines increase the rate of fluid transport across the alveolar epithelium (129).

Alveolar fluid clearance may already be upregulated in the presence of clinical conditions that predispose to acute lung injury. For example, short-term studies of septic and hypovolemic shock in rats have indicated that endogenous release of epinephrine can markedly increase alveolar fluid transport (130, 131). Furthermore, endogenous release of epinephrine can markedly increase alveolar fluid clearance in experimental neurogenic pulmonary edema (132). Also, release of tumor necrosis factor-alpha in the presence of either pneumonia or peritonitis increases the transport capacity of the alveolar epithelium (133, 134). Therefore, in assessing the potential clinical application of therapeutic strategies designed to increase the rate of alveolar fluid clearance, more information is needed regarding the transport capacity of the injured lung. Also, the extent of interstitial edema may limit the transport capacity of the alveolar epithelium (135). Moreover, if alveolar epithelial injury is so severe that the barrier properties of the epithelium have been substantially altered (136, 137), then increasing the transport capacity of individual alveolar epithelial cells will not be effective until the epithelial barrier has been restored.

Beta-adrenergic Agonist Therapy

Beta adrenergic agonists are effective when delivered directly into the distal air spaces of the lung or when delivered intravenously. Although early studies indicated that the primary stimulating effect was mediated by beta-2 receptors, recent work indicates that beta-1 stimulation is effective in upregulating alveolar fluid clearance as well (138). Also, the receptors are present on the apical and basolateral surface of alveolar epithelium (139). Some species do not respond to beta adrenergic agonists with an increase in alveolar fluid transport (140, 141). However, beta-2 adrenergic agonists markedly enhance the rate of alveolar fluid clearance in *ex vivo* human lung preparations, by about 100% above baseline levels (142, 143).

The therapeutic potential of beta-2 adrenergic agonists has been evaluated in some experimental models that are relevant for clinical pulmonary edema. In hydrostatic pulmonary edema, beta agonists can accelerate the resolution of alveolar edema experimentally (144) and perhaps in clinical hydrostatic edema (145). In the presence of acute lung injury, beta-2 adrenergic agonists have been reported to augment the rate of alveolar epithelial fluid transport in rats with moderate lung injury from hyperoxia by about 50% above basal levels (61, 69, 146).

In considering the potential use of beta adrenergic agonist therapy for treatment of alveolar edema in patients, the method of drug delivery is important. In one study in sheep, a lipid-soluble beta-2 agonist, salmeterol, was delivered by a simple nebulizer similar to the method used for aerosolization in ventilated, critically ill patients. Interestingly, 5 mg of salmeterol resulted in a high concentration (10^{-6} M) in alveolar edema fluid, even 3 h after administration of the aerosolized salmeterol (147). This concentration was at the plateau of the dose-response curve based on *ex vivo* human lung studies (143). Thus, these data suggest that aerosolization of a beta agonist might be sufficient to provide therapeutic concentrations of beta adrenergic agonists in the distal air spaces of the lung. However, there is a lack of information regarding concentrations of aerosolized agents in alveolar edema fluid from patients with pulmonary edema. Interestingly, preliminary data from a clinical study indicated that use of an inhaled lipid-soluble beta-2 agonist reduced the incidence of high alti-

tude pulmonary edema (HAPE) in patients with a prior history of HAPE (148).

Theoretically, beta-2 adrenergic agonists might be a safe therapy because aerosolized beta agonists are well tolerated, with minimal hemodynamic side effects. Furthermore, beta-2 agonists may also augment surfactant secretion, decrease lung endothelial permeability, and decrease airway resistance (149). More experimental studies are needed to test the potential efficacy of beta-2 agonists in patients with pulmonary edema from a hydrostatic mechanism or from acute lung injury. Exposure to beta-2 adrenergic agonists might result in downregulation of beta-2 receptors, with a diminishing therapeutic effect over time (150), although there was no evidence of downregulation when epinephrine was delivered to rats over a period of 4 h (151).

Hormonal Therapy

Because glucocorticoids upregulate alveolar fluid clearance experimentally (72, 73), there might be clinical value of this therapy in patients with pulmonary edema from acute lung injury. However, enthusiasm for this approach is diminished by clinical studies demonstrating that acute treatment with high doses of glucocorticoids has no beneficial effect on outcome in the early acute phase of clinical acute lung injury (152, 153). Furthermore, the potential deleterious effects of glucocorticoids on host susceptibility to infection makes this form of therapy less attractive, at least as a targeted approach to upregulating alveolar fluid clearance. Changing levels of endogenous levels of glucocorticoids, mineralocorticoids, or even thyroid hormone could alter endogenous expression and activity of ion transporters in the alveolar epithelium. However, it is difficult to assess the impact of hormonal factors in critically ill patients.

Growth Factors

Several growth factors can upregulate alveolar epithelial fluid transport. Epidermal growth factor increases Na transport across alveolar epithelial cells (39) and upregulates alveolar fluid clearance in rats (154). The effects seem to be mediated by transcriptional upregulation of vectorial sodium transport. Transforming growth factor-α (TGF-α) also upregulates alveolar fluid clearance in rats (155). Keratinocyte growth factor (KGF), an alveolar epithelial Type II cell mitogen, increases the transport capacity of the alveolar epithelium (87, 156), primarily by increasing the number of alveolar epithelial Type II cells, resulting in a greater transport capacity (87). Relatively high doses of KGF are required in mice and rats delivered by the intratracheal route and requires 48 to 72 h before the maximum effect is observed (157). However, KGF produces a sustained upregulation of alveolar fluid clearance. Furthermore, the combination of KGF and beta adrenergic agonists results in an additive effect, increasing alveolar fluid clearance in rats, for example, to 50% clearance in 1 h (87). Because KGF has other beneficial pulmonary effects, including cytoprotection (158, 159), increased surfactant secretion (160), and an antioxidant effect (161), KGF is appealing as a potential treatment for patients with acute lung injury.

Gene Therapy

Another approach to increase Na transport and alveolar fluid reabsorption was recently tested using adenoviral-mediated overexpression of the Na,K-ATPase beta-1 subunit gene delivered to the lung epithelium to increase Na pump function *in vivo* in normal rats (42). Alveolar fluid clearance was increased in this model in normal lungs. Furthermore, preliminary data indicate that pretreatment was associated with increased survival in rats exposed to 64 h of hyperoxia. More work is needed to evaluate strategies to achieve gene expression in alveolar epithelium without inducing lung or systemic injury (162).

Vasoactive Agents

Dobutamine, a commonly used vasoactive agent in patients with acute heart failure, has been shown to markedly upregulate alveolar epithelial fluid clearance in rats by stimulating beta-2 receptors (163). In addition, dopamine can upregulate alveolar fluid clearance by stimulating the dopaminergic receptor D1, resulting in translocation of Na pumps from the intracellular endosomal compartments to the basolateral membrane of alveolar epithelial Type II cells with rapid upregulation of alveolar fluid transport (70). Clinically, patients are often given several vasoactive agents, and it can be difficult to determine the net effect of these agonists in hydrostatic or lung injury edema (145).

Upregulating Alveolar Fluid Transport in Clinical Acute Lung Injury: Potential Problems

Although several pharmacologic treatments might be successful in upregulating alveolar fluid clearance in the setting of clinical acute lung injury, there are potential problems. First, if the alveolar epithelium is extensively injured, then the lack of sufficient functional epithelial barrier would blunt the efficacy of any fluid transport-enhancing therapy. Second, under some conditions, the alveolar epithelium cannot respond to beta adrenergic agonists. Both *in vitro* and *in vivo* studies have demonstrated that oxidant-induced lung injury in rats results in submaximal alveolar fluid clearance that is associated with an inability to respond to beta adrenergic agonists (93). Third, the degree of epithelial injury may be so severe that relentless alveolar flooding may overwhelm any transport capacity of the epithelium. In one study, alveolar fluid transport capacity after acid-induced lung injury was reduced by approximately 50% (164). Another potential problem is that endogenous factors might maximally upregulate alveolar epithelial fluid transport. Short-term upregulation may occur because of elevated catecholamines, and sustained upregulation may occur from release of growth factors such as HGF, KGF, and TGF-α (165, 166) or endogenous release of glucocorticoids. Thus, exogenous delivery of an alveolar fluid transport-enhancing therapy might be ineffective because of the presence of endogenous factors that have already upregulated clearance.

Assessment of Strategies to Enhance Alveolar Edema Fluid Clearance

How should clinical studies evaluate the efficacy of treatments designed to enhance alveolar fluid transport? Changes in pulmonary infiltrates in the chest radiograph are qualitative (167, 168) and are likely to lag behind clinical improvement (169). During the resolution of clinical hydrostatic pulmonary edema, oxygenation improves as net alveolar fluid clearance occurs (145, 169), but the improvement may require 12 to 24 h to appreciate a beneficial effect on oxygenation. Similarly, the resolution of alveolar edema in patients with acute lung injury is usually followed by improvements in oxygenation, but there may be a lag time before the benefit is apparent (170). Also, a recent clinical study that reported an improved outcome from use of a lung protective ventilatory strategy indicated that oxygenation is a poor surrogate for a beneficial clinical effect (171). Improvements in the mechanical properties of the lung are likely to be insensitive indices of reduced alveolar fluid volume. Therefore, the best end points for a strategy designed to enhance alveolar fluid transport would be a reduced duration of mechanical ventilation and/or reduction in mortality (171).

RECOMMENDATIONS FOR FUTURE DIRECTIONS FOR BASIC SCIENCE AND CLINICAL RESEARCH

Intercellular Junctions/Cell Polarity

Better knowledge of the structure and function of the intercellular junctions between constituent cells of the alveolar epithelium is critical for understanding how the alveolar epithelial barrier is reestablished after injury. Studies are needed to characterize the proteins that make up the tight junctions, their relationships with the cytoskeleton, and the effect of injury on tight junction structure-function relationships. The role of gap junctions and possible differences in junctions between Type II cells or Type I cells versus those between Type I and Type II cells need to be studied. In addition, a better understanding of how alveolar epithelial cell polarity develops and is maintained, as well as how cell polarity is reestablished after injury, is likely to be important for understanding lung injury and repair. Approaches to study the intact lung may be facilitated by newer imaging systems (172).

Type I and Type II Cells in Alveolar Epithelial Transport

The change in expression of Na or other ion transporters during the transition from the Type II to Type I cell phenotype should be better characterized. Changes in the biophysical properties of the Na channels or the channel types themselves during the transition of Type II to Type I cells also remain to be determined. Differential regulation of ion transporters in Type II and Type I cells in response to injury (e.g., hyperoxia, hypoxia, cytokines, oxidants) and exogenous agents (e.g., beta agonists, growth factors) must be explored in detail. Identification of additional lung-specific transporters that can account for amiloride-insensitive current observed in freshly isolated Type II cells and in Type I cell-like monolayers, as well as in intact lung studies, is an important area (173). The relative contributions of Type I or Type II cells to overall alveolar ion (sodium and chloride) transport and net fluid clearance in normal and injured lungs need to be explored. It is likely that significant differences exist and that these differences play a role in the response of alveolar epithelium to injury.

Improved models for study of alveolar epithelium and its constituent cells, especially Type I cells, require further attention. The development of innovative techniques to isolate enriched populations of Type I cells of sufficient viability for use in cell culture, and of sufficient purity to allow for isolation of mRNA to facilitate characterization of their molecular phenotype, is necessary.

Coupling of Na Entry/Exit Pathways and Chloride Transport

Several independent regulatory pathways have been identified that are able to upregulate Na channels and pumps in parallel. It is crucial to explore the coordinate regulation of transalveolar epithelial Na transport properties. Coupling between apical entry pathways and basolateral ion extrusion, and the signal transduction pathways involved in their linkage, if any, is worthy of further study. Gene transfer investigations may be helpful in this regard. The mechanisms that upregulate and downregulate ion transport in alveolar epithelium will have substantial clinical relevance.

Although progress has been made in understanding the molecular and cellular basis for sodium transport, very little is known about how chloride is transported across the alveolar epithelium. New studies are needed to define the role of cystic fibrosis transmembrane conductance regulator (CFTR) and other potential chloride channels under both basal and stimulated conditions, and their potential contribution to transport across alveolar and distal airway epithelia in regulating clearance of edema fluid from the air spaces of the lung.

Other areas worthy of study include: (*1*) the ion and macromolecular contents of the alveolar subphase (the liquid between the film of surfactant and the surface of alveolar epithelial cells); (*2*) the dynamics and directionalities (from the air space to the vasculature or vice versa) of ion, macromolecule, and fluid transport in the normal lung and in lung injury; (*3*) the mechanical factors such as lung distention, contraction, or phasic volume and pressure changes that may influence transport, including the role of the important potential role of the air-liquid interface versus the fluid-filled alveolus; (*4*) the determination of channel biophysical properties by expression in different experimental systems (e.g., frog oocytes); (*5*) the development of additional antibodies to alveolar epithelial cell Na channel proteins for biochemical and immunohisto/cytochemical characterization of these channels in the alveolar epithelium.

Role of Water Channels (Aquaporins) in Alveolar Transport and Biology

Considerable progress has been made in a relatively short time in studying the potential contribution of aquaporins to lung fluid balance. The studies so far demonstrate that active isoosmolar alveolar fluid clearance in the newborn or adult lung does not require lung water channels, and the role of aquaporins in lung fluid balance in acute lung injury is negligible. The contribution of aquaporins to transepithelial water movement must be further clarified relative to airway hydration, and the role of aquaporins in cell volume regulation and in response to osmotic stress remain important areas to be investigated. Identification of other aquaporins in Type I and Type II cells would also be useful.

Development of Novel Transgenic Approaches for Studying Alveolar Epithelial Transport

Transgenic murine models have been very helpful in assessing the functional role of aquaporins in lung fluid balance. Transgenic approaches in which SP-C promoters have been used to ablate or overexpress functional genes in a cell-specific fashion in Type II cells have provided new insights into mechanisms that determine alveolar epithelial structure and function. Similar studies using promoters specific for Type I cells (e.g., AQP5 promoter) may provide insight into alveolar epithelial cell biology. Transgenic systems that allow temporally and spatially regulated gene expression using such cell-specific promoters in combination with a regulable on-off system (e.g., tetracycline) provide an exciting additional opportunity to evaluate the importance of selected genes within specific cells in the adult alveolar epithelium. Type I and Type II cell-specific promoters to generate transgenic mice, in which the gene of interest can be regulated in a cell-specific fashion in the adult animal, would be useful to delineate the contribution of individual transporters to alveolar fluid clearance in the adult lung and to explore the potential for modulation of alveolar epithelial barrier properties through overexpression of specific transporter genes.

Mechanisms for Macromolecule Transport Across Alveolar Epithelial Barrier

The mechanisms for transalveolar epithelial macromolecule transport via transcytotic absorption and/or secretion in health and disease must be clarified. Studies of the role of caveolae in transcytosis of macromolecules will yield important information for targeting protein drugs via vesicle-mediated processes for systemic absorption through the lungs. These studies will provide the tools for improved design and delivery of protein

drugs intended for systemic absorption via the pulmonary route. Also, more information is needed regarding the pathways for clearance of soluble and insoluble protein from the lung after acute lung injury.

Recommendations for Clinical Studies

Experimental studies are needed to provide further guidelines for studies in patients. For example, longer-term studies of the effect of beta-adrenergic agonists on alveolar fluid clearance are needed to determine whether downregulation occurs in response to beta adrenergic agonist therapy. Further work is needed to determine if aerosolized beta-adrenergic agonists given to patients will achieve therapeutic levels in alveolar edema fluid. Studies are needed to assess the feasibility of gene therapy targeted approach to enhance alveolar fluid transport with delivery of Na,K-ATPase to the alveolar epithelium. Adenoviral vectors may not be sufficiently efficient and/or safe; new approaches and careful assessments are needed. Experimental studies are needed to determine whether KGF, or a comparable epithelial-specific mitogen, would be effective as a treatment strategy in the presence of acute lung injury. Experimental models need to be used in which animals can be maintained in an environment that simulates intensive care unit for patients with clinical lung injury.

Patient studies are needed to assess several issues. (*1*) The concentration of beta-2 adrenergic agonists in pulmonary edema fluid after aerosolization in ventilated patients is needed to determine whether therapeutic concentrations can be achieved. (*2*) More data are needed regarding the endogenous levels of plasma epinephrine and norepinephrine in patients with hydrostatic pulmonary edema and acute lung injury. This is especially important because experimental studies indicate that alveolar fluid clearance reverts to baseline levels soon after plasma epinephrine normalizes. (*3*) Biologic markers that can be measured in plasma or pulmonary edema fluid may identify patients with substantial alveolar epithelial injury and may therefore prove useful in selecting patients for therapy (174). (*4*) Gene therapy may provide novel approaches for treatment of pulmonary edema. Short term expression may be sufficient for clinical benefit. However, the required levels of expression and the thresholds for beneficial effect and duration are not yet defined. An additional concern with gene therapy is the potential to increase inflammation in response to viral vectors in an already damaged lung. Studies to date have administered the viral expression vector prior to the injury. The efficacy of transfection and expression and the benefit need to be established when administered after injury. Further, it is important to determine whether transfection of Type I or Type II alveolar epithelial cells, or both, is important for upregulation of alveolar fluid clearance. Also, the optimal delivery method in pulmonary gene therapy remains to be determined. (*5*) Potential clinical strategies to provide a sustained upregulation of alveolar fluid clearance need to be based on longer-term experimental models of acute lung injury that replicate the conditions of the intensive care unit. Ultimately, the value of clinical therapies to enhance alveolar fluid clearance in patients with acute lung injury will need to be demonstrated with improved clinical outcomes such as decreased duration of mechanical ventilation and/or decreased mortality.

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