Lung Edema Clearance: 20 Years of Progress
Invited Review: Role of aquaporin water channels in fluid transport in lung and airways

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Borok, Zea, and A. S. Verkman. Invited Review: Role of aquaporin water channels in fluid transport in lung and airways. J Appl Physiol 93: 2199–2206, 2002; 10.1152/japplphysiol.01171.2001.—Water transport across epithelial and endothelial barriers in bronchopulmonary tissues occurs during airway hydration, alveolar fluid transport, and submucosal gland secretion. Many of the tissues involved in these processes are highly water permeable and express aquaporin (AQP) water channels. AQP1 is expressed in microvascular endothelia throughout the lung and airways, AQP3 in epithelia in large airways, AQP4 in epithelia throughout the airways, and AQP5 in type I alveolar epithelial cells and submucosal gland acinar cells. The expression of some of these AQPs increases near the time of birth and is regulated by growth factors, inflammation, and osmotic stress. Transgenic mouse models of AQP deletion have provided information about their physiological role. In lung, AQP1 and AQP5 provide the principal route for osmotically driven water transport; however, alveolar fluid clearance in the neonatal and adult lung is not affected by AQP deletion nor is lung CO2 transport or fluid accumulation in experimental models of lung injury. In the airways, AQP3 and AQP4 facilitate water transport; however, airway hydration, regulation of the airway surface liquid layer, and isosmolar fluid absorption are not impaired by AQP deletion. In contrast to these negative findings, AQP5 deletion in submucosal glands in upper airways reduced fluid secretion and increased protein content by greater than twofold. Thus, although AQPs play a major physiological role outside of the airways and lung, AQPs appear to be important mainly in airway submucosal gland function. The substantially slower rates of fluid transport in airways, pleura, and lung compared with renal and some secretory epithelia may account for the apparent lack of functional significance of AQPs at these sites. However, the possibility remains that AQPs may play a role in lung physiology under conditions of stress and/or injury not yet tested or in functions unrelated to transepithelial fluid transport.

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Fluid transport across cellular barriers in biological tissues results from water transport driven by osmotic gradients or hydrostatic pressure differences. In the lung and airways, fluid movement between the air space and cellular and/or interstitial and vascular compartments is important in the maintenance of air space hydration, the absorption of air space fluid near the time of birth and in clinical pulmonary edema, and the secretion of fluids onto the airway surface by submucosal glands. Although pressure-driven bulk fluid flow

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is an important mechanism in the development of lung edema and pleural effusions in heart failure associated with elevated left ventricular pressure, osmotically driven water transport across cell membranes is the principal mechanism of fluid transport under normal physiological conditions. Osmotic gradients produced by active and secondary active solute transport are dissipated by the transport of water across cell membranes. Although all cell membranes and lipid bilayers are modestly permeable to water, the water permeability of certain cell membranes, such as those in kidney tubules and alveoli, is 5- to 50-fold enhanced by aquaporin-type water channels. The aquaporins are a family of small (~30-kDa monomer), integral membrane proteins that function as water transporters. There are at least 11 homologous aquaporins in mammals, at least 4 of which are expressed in the airways and lung. This review is focused on the biology of these aquaporins with respect to airway and lung physiology.

**BARRIERS TO FLUID TRANSPORT IN BRONCHOPULMONARY TISSUES**

Figure 1 depicts the barriers to water transport between air space and interstitial and vascular compartments in the bronchopulmonary tree. The nasopharynx, trachea, and airways are lined by an epithelial cell layer that forms the principal barrier for water movement between the interstitial and vascular compartment and the airway surface liquid (ASL), the thin (<100 μm) layer of liquid lining the apical surface of the epithelium. Submucosal glands, which secrete fluid and glycoproteins onto the airway surface, are present in the larger airways. The alveolar epithelium provides the major surface area for gas exchange. The alveolar epithelium contains type I cells, which are flat cells comprising the majority of the alveolar epithelial surface, and type II cells, which transport salt actively and produce surfactant. Water movement between the air space and capillary compartments across the alveolar epithelium must also cross the interstitium and capillary endothelium. Permeability measurements have indicated exceptionally high water permeability across intact alveolar capillaries (8) and epithelium (5, 7) and moderately high water permeability across the airway epithelium (15, 16, 39). Water permeability in isolated alveolar type I cells was the highest reported of any mammalian cell type (12).

**EXPRESSION OF AQUAPORINS IN BRONCHOPULMONARY TISSUES**

At least four members of the aquaporin protein family are expressed in the respiratory tract, where they are distributed in a cell-specific manner throughout the nasopharynx, airways, and distal lung (Fig. 1). AQP1 was the first aquaporin to be identified in lung, where its transcript was initially demonstrated in alveoli of rat lung by in situ hybridization (20). AQP1 was subsequently immunolocalized predominantly to the microvascular endothelium adjacent to airways and alveoli (17, 25, 44, 50) and to microvessels and mesothelial cells of visceral and parietal pleura (44, 55). Expression of AQP1 has also been detected in occasional pneumocytes (13, 17, 25, 43). AQP3 is expressed in the basolateral membranes of basal cells of epithelium lining the rat trachea and nasopharynx but not distal lung (18, 26, 43) and in basolateral membranes of glandular and surface epithelial cells in nasal conchus (43). AQP4 is expressed in the basolateral membrane of ciliated columnar cells of bronchial, tracheal, and nasopharyngeal epithelium (18, 43). AQP5 is expressed in the apical membrane of type I cells in rat distal lung epithelium (19, 43). Consistent with localization of AQP5 to alveolar epithelial type I (but not type II) cells in vivo, AQP5 is upregulated in rat alveolar epithelial cells in primary culture during the in vitro transition from the type I toward the type II cell phenotype (5). Treatment with keratinocyte growth factor maintains the type II cell phenotype and prevents the increase in AQP5. In the rat, AQP5 has also been localized to apical membranes of acinar cells in nasopharyngeal subepithelial glands (43). Low levels of AQP5 were found in rat trachea by immunoblotting, although it could not be localized by immunohistochemistry (43). These four aquaporins are expressed in a similar distribution in the mouse (52). A recent immunohistochemical study in the mouse suggested more widespread expression of AQP5 in trachea, bronchi, as well as alveolar type II cells (28), although independent confirmation will be needed.

Some differences from the cellular distribution pattern described in the rodent lung were recently reported in humans (29). In a survey of AQP3–AQP5, the major differences in human samples compared with the rodent were AQP3 expression on the basolateral and apical membranes of bronchiolar cells in small airways and AQP5 expression at the apical membrane.
of bronchial acinar and ciliated duct columnar cells, as well as columnar cells of the superficial epithelium of the nasopharynx. In contrast to rodent lung, AQP4 was localized by in situ hybridization (but not immunofluorescence) to type I alveolar epithelial cells and AQP3 by both methods to type II cells. There was discordance between findings by in situ hybridization and immunofluorescence for AQP4 and AQP5 in bronchial superficial epithelium. Whether these apparent differences in cellular distribution and localization across species reflect functional differences remains to be determined. The largely nonoverlapping and complex pattern of aquaporin expression provides indirect evidence for a role of aquaporins in lung and airway physiology.

FURTHER INDIRECT EVIDENCE FOR A PHYSIOLOGICAL ROLE OF AQUAPORINS IN THE TRACHEOBRONCHIAL TREE

Developmental regulation. Several studies have demonstrated developmental regulation of aquaporin expression in the respiratory tract. RNase protection assay and immunoblotting of rat lung membranes showed detectable AQP1 in distal fetal rat lung at day E19 (2 days before birth), increasing severalfold perinatally and into adulthood (25, 26, 59). AQP1 at all stages of development was upregulated by treatment with corticosteroids. AQP5 was barely detectable at day E21 or postnatal day 1 in distal lung but was present on postnatal day 2, gradually increasing through into adulthood (26). AQP4 mRNA and protein were strongly induced immediately postnatally (26, 59, 64). Increases in AQP4 were observed in rat fetal lung distal epithelial cells in vivo exposed to 21% oxygen (compared with 3%), suggesting a mechanism for postnatal upregulation of AQP4 (48). AQP4 mRNA was also upregulated in fetal lung at 24 h after treatment of maternal rats (day E20) with β-adrenergic agonists or glucocorticoids. Different kinetics of aquaporin induction have been noted in trachea, where transient upregulation of AQP5 occurred immediately after birth, was maximal at day 21, and then declined to very low levels (26). In contrast, AQP3 and AQP4 proteins were first detected in tracheal membranes at later times, on postnatal days 12 and 21, respectively. Peri- and postnatal induction of aquaporin in the lung and airways parallels the transition from fluid secretion to fluid reabsorption that occurs at birth. Together with increases in osmotic water permeability demonstrated in the first 24 h after birth in rabbit lung, these observations provide indirect evidence for a role of aquaporins in fluid clearance from the lung postnatally (9).

Regulation in the adult lung. Additional indirect evidence for a physiological role of aquaporins in respiratory physiology includes the regulation of aquaporin expression by growth factors, inflammatory mediators, and osmotic stress (5, 26, 57). AQP1 is induced by steroids in adult rat lung (25). Whether these steroid effects are mediated at the transcriptional level via glucocorticoid response elements in the proximal promoter, as shown in erythroid cells, is not known. Decreases in mRNA and protein for AQP5 and AQP1 were demonstrated in mouse lung following adenoviral infection, a model of pulmonary inflammation and edema (57). The reduction in aquaporin expression in the context of inflammation suggested to the authors a causal role for aquaporins in the development of lung edema associated with virus-induced inflammation. The observation that tumor necrosis factor-α down-regulates AQP5 expression in mouse lung epithelial cells (MLE-12) suggested a possible mechanism for AQP5 modulation by inflammatory cytokines following viral infection (58). Hypertonic induction of AQP5 mRNA and protein has been demonstrated in MLE-15 cells and in tissues from hyperosmolar rats through an extracellular-regulated kinase-dependent pathway (21). Similar induction by hypertonicity has been demonstrated in alveolar epithelial cells in primary culture, suggesting potential physiological relevance for AQP5 in the response of these cells to osmotic stress (32); however, it is unclear whether alveolar cells are exposed to hypertonicity in vivo. Upregulation of AQP5 in response to osmotic stress as well as its induction during transition from the type II to type I cell phenotype are not associated with changes in mRNA stability, suggesting transcriptional-level regulation. Promoters of the human AQP1 and AQP5 genes have been cloned and characterized (4, 60); however, definitive identification of cell- and/or stimulus-specific transcriptional regulation of these genes in lung requires further investigation.

TRANSGENIC MOUSE MODELS OF AQUAPORIN DELETION

Transgenic mice have considerable value in establishing the physiological role of specific genes. As reviewed recently (47), several general caveats should be noted regarding the use of transgenic mouse models to understand gene function and human physiology. Defects in organ function can result directly from protein deletion or indirectly from secondary factors such as altered organ development and/or structure, primary defects in other organs, or changes in the expression of other proteins. For example, changes in pulmonary fluid transport might result from altered cardiac function and hemodynamics, abnormal lung development, or changes in the expression of alveolar water and/or solute transporters. The extrapolation of results in mice to human physiology must take into account differences in patterns of protein expression, anatomy, and physiology. Notwithstanding these caveats, and until specific aquaporin inhibitors for in vivo use become available, transgenic mice probably represent the best model to define the role of aquaporins in mammalian physiology.

Transgenic knockout mice lacking each of the four airway and lung aquaporins (AQP1, AQP3, AQP4, and AQP5) were generated by targeted gene deletion (34–37). Multiple extrapulmonary phenotypes were documented, as reviewed in Ref. 61. For example, in kidney, deletion of AQP1 or AQP3 produced marked polyuria.
molality, deduced from the perfused mouse lung was measured by a pleural sur-

Fig. 2. Water permeability and fluid transport in lungs of aquaporin knockout mice. A: strategy for measurement of osmotic water permeability between the air space-capillary barrier in isolated perfused lung as described in Ref. 7. A fluorescent volume marker (F) is present in the air space instillate, and pleural surface fluorescence is monitored in response to changes in pulmonary artery (PA) perfusate osmolality. B: osmotically driven water transport across the air space-capillary barrier in lungs from wild-type mice (+/+) and knockout mice (−/−) lacking the indicated aquaporin(s). Note the remarkable slowing of osmotic equilibration in mice lacking AQP1. As shown in Fig. 2B, osmotic water permeability of the air space-capillary barrier was ~10-fold reduced by deletion of AQP5 or AQP1 separately and >30-fold reduced by their deletion together (in double knockout mice).

FLUID TRANSPORT IN DISTAL LUNG

In the peripheral lung, the proposed aquaporin functions include alveolar fluid absorption at the time of birth and in the adult lung, gas (CO₂) exchange, and regulation of lung water content in response to acute and subacute lung injury (3, 40, 45). The main aquaporins in peripheral lung are AQP5 in type I alveolar epithelial cells and AQP1 in endothelial cells. Initial experiments indicated that these aquaporins provide the principal route for osmotically driven water transport across the alveolar epithelial and endothelial barriers, respectively (2, 33, 53). Osmotic water permeability between the air space-capillary barrier in isolated perfused mouse lung was measured by a pleural surface fluorescence method in which air space fluid osmolality, deduced from the fluorescence of a volume marker, was measured in response to changes in osmolality of the pulmonary artery perfusate (7) (Fig. 2A). As shown in Fig. 2B, osmotic water permeability of the air space-capillary barrier was ~10-fold reduced by deletion of AQP5 or AQP1 separately and >30-fold reduced by their deletion together (in double knockout mice).

It was found also that AQP1 deletion in mice resulted in decreased lung water accumulation in response to acute elevation in perfusate hydrostatic pressure (2, 53). Similarly, recent tomographic analysis of airway wall thickness after saline infusion showed a lesser increase in thickness in two AQP1-deficient humans compared with control subjects (27). It is unclear, however, whether the apparent AQP1-dependent changes in fluid accumulation result from altered capillary endothelial water transport rather than changes in capillary structure and function with AQP1 deletion. Pressure-driven fluid accumulation in intact airways and lung is a very inefficient process because the transported water is rapidly absorbed by osmotic forces. Fluid accumulation occurs primarily by bulk fluid movement through transient rifts in capillary and/or alveolar integrity.

The proposed physiological functions of aquaporins in peripheral lung were systematically investigated. Alveolar fluid absorption was measured from the increased concentration of an air space volume marker (radioiodinated albumin) at 37°C (Fig. 2C). Even with maximal stimulation of alveolar fluid absorption by β-agonists and pretreatment with keratinocyte growth factor (to increase the number of type II cells), there was no significant effect of aquaporin deletion (2, 33). Furthermore, the rapid absorption of fluid from the air space just after birth was not impaired by aquaporin deletion, nor was the accumulation of lung edema in response to acid-induced epithelial cell injury, thio-
urea-induced endothelial cell injury, or hyperoxic subacute lung injury (51). The remarkably slower rate of alveolar fluid absorption compared with proximal tubule fluid absorption in the kidney (49) and saliva secretion (34) was proposed to explain lack of effect of AQP1 and AQP5 deletion on alveolar fluid clearance. Because rates of fluid transport are relatively low in lung, the low intrinsic (aquaporin-independent) water permeability of the alveolar epithelial and capillary membranes appears to be adequate to allow fluid transport to occur without impairment under normal physiological conditions and in response to clinically relevant stresses.

Heterologous expression studies in Xenopus laevis oocytes have suggested that CO₂ exchange can be facilitated by AQP1 (42). However, abnormalities in CO₂ transport were not found in AQP1-null mice in arterial blood-gas measurements in anesthetized ventilated mice subjected to changes in inspired gas CO₂ content (62) or in rapid changes in air space fluid pH in isolated perfused lungs subjected to sudden changes in capillary CO₂ content (14).

FLUID TRANSPORT IN THE PLEURA

Fluid is continuously secreted into and cleared from the pleural space. The amount of fluid in the pleural space is normally very small (<0.2 ml/kg) despite its large surface area (4,000 cm² in humans, 10 cm² in mice) (42). Pleural fluid can accumulate in congestive heart failure, lung infection, lung tumor, and the acute respiratory distress syndrome. Fluid entry into the pleural space involves filtration across microvascular endothelia near the pleural surface and movement across a mesothelial barrier lining the pleural space, whereas fluid clearance is thought to occur primarily by lymphatic drainage (1, 24). RT-PCR screening and immunostaining revealed expression of AQP1 in microvascular endothelia near the visceral and parietal pleura and in mesothelial cells in visceral pleura (55). Osmotic water permeability across the pleural barrier was measured in anesthetized, mechanically ventilated mice from the kinetics of pleural fluid osmolality after instillation of hypertonic or hypotonic fluid into the pleural space. Osmotic equilibration of pleural fluid was rapid in wild-type mice (50% equilibration in <2 min) and slowed by approximately fourfold in AQP1-null mice. However, the clearance of isosmolar saline instilled in the pleural space (~4 ml·kg⁻¹·h⁻¹) was not affected by AQP1 deletion, nor was the accumulation of pleural fluid (~0.035 ml/h) in a fluid overload model produced by intraperitoneal saline administration and renal artery ligation. Also, pleural fluid accumulation was not affected by AQP1 deletion in a thiourea toxicity model of acute endothelial injury. Thus, although rapid osmotic equilibration across the pleural surface is facilitated by AQP1, as in peripheral lung, AQP1 did not appear to play a role in physiologically important mechanisms of pleural fluid accumulation or clearance.

FLUID TRANSPORT IN THE AIRWAYS

The proposed functions of aquaporins in the airways include humidification of inspired air, regulation of ASL volume and composition, and absorption of fluid from the airways. Evaporative water loss in the airways is thought to drive water influx from capillaries and interstitium into the ASL by the creation of an osmotic gradient. The depth and ionic composition of the ASL should depend theoretically on the ion-transporting properties of the airway epithelium and the rate of evaporative water loss, as well as the water permeability of the airway-capillary barrier (6, 11). Novel methods were developed to study airway humidification, ASL properties, and fluid clearance in the airways for measurements in mice lacking AQP3 and AQP4 (52). Lower airway humidification was measured from the moisture content of expired air during mechanical ventilation with dry air through a tracheostomy. Lower airway humidification was 54–56% efficient in wild-type mice and reduced only slightly (3–4%) in AQP1-AQP5 or AQP3-AQP4 double knockout mice. Upper airway humidification was measured from...
the moisture gained by dry air passed through the upper airways in mice breathing through a tracheotomy. Upper airway humidification decreased from 91% to 50% with increasing ventilation from 20–220 ml/min but was reduced only slightly (3–5%) in AQP3-AQP4 knockout mice. The depth and salt concentration of the ASL in trachea was measured in vivo with fluorescent probes and confocal and ratio imaging microscopy developed for measurements in cystic fibrosis mice (23). ASL depth was ~45 μm and Na⁺ concentration was ~115 mM in wild-type and AQP3-AQP4 knockout mice. Finally, active fluid absorption measured in nasopharyngeal airways (measured with a volume marker as done for alveolar fluid clearance) was not impaired by aquaporin deletion. The phenotype studies showed that aquaporins play at most a minor role in airway humidification, ASL hydration, and isosmolar fluid absorption. Recently, increased airway reactivity in response to bronchoconstricting agents was reported in AQP5-null mice (28). A mechanism was not established for the differences observed but may be related to indirect effects of AQP5 deletion on agonist-induced fluid secretion from submucosal glands (see below). The decreased rate of fluid secretion onto the airway surface in AQP5-null mice may alter apparent airway reactivity.

FLUID SECRETION BY AIRWAY SUBMUCOSAL GLANDS

Submucosal glands in mammalian airways secrete a mixture of water, ions, and macromolecules onto the airway surface. Glandular secretions are important in establishing ASL fluid composition and volume and are important in antimicrobial defense mechanisms. Abnormally viscous gland secretions in cystic fibrosis have been proposed to promote bacterial adhesion and inhibit bacterial clearance by impeding ciliary function (22). Submucosal glands contain serous tubules, where active salt secretion into the gland lumen creates a small osmotic gradient driving water transport across a water permeable epithelium, as well as mucous cells and tubules, where viscous glycoproteins are secreted. Immunocytochemistry revealed strong expression of AQP5 at the luminal membrane of serous epithelial cells (54). To determine whether AQP5 deletion affected submucosal gland fluid secretion, novel methods were developed to measure secretion rates and composition of gland fluid. In mice breathing through a tracheotomy, total gland fluid output was measured from the dilution of a volume marker present in the fluid-filled nasopharynx and upper trachea. Pilocarpine-stimulated fluid secretion was greater than twofold reduced in AQP5-null mice. Similar results were obtained by video imaging of fluid droplets secreted from individual submucosal glands near the larynx (Fig. 3). Analysis of secreted fluid showed a greater than twofold increase in total protein concentration in AQP5-null mice and a smaller increase in Cl⁻ concentration, suggesting intact protein and salt secretion across a relatively water-impermeable epithelial barrier. Submucosal gland morphology and density did not differ significantly in wild-type vs. AQP5-null mice. AQP5 thus facilitates fluid secretion in submucosal glands so that the luminal membrane of serous epithelial cells is the rate-limiting barrier to water movement. Modulation of gland AQP5 expression or function was proposed as a novel approach to treat hyperviscous gland secretions in cystic fibrosis and excessive fluid secretions in bronchitis and rhinitis.

PERSPECTIVE AND DIRECTIONS

The specific and regulated expression of aquaporins in airways and lung has provided indirect evidence supporting their physiological role. Water permeability measurements in mice lacking AQP1, AQP3, AQP4, and AQP5 have established that aquaporins provide a major pathway for osmotically driven water movement in the airways, alveoli, and pleura. However, except for impaired fluid secretion by airway submucosal glands in AQP5-null mice and increased airway reactivity, phenotype studies in aquaporin-null mice do not support a significant role for aquaporins in the airways, pleura, or distal lung. It remains unknown whether other (as yet unidentified) aquaporins may be important in lung physiology, whether the aquaporins may be important in stresses not tested, and whether there are situations in which aquaporins are important in human lung physiology. Selective, nontoxic aquaporin inhibitors, when available, will be useful to address these issues, as well as the general concern in transgenic mouse studies about possible compensatory changes in organ function and strain differences. Notwithstanding these caveats, the relatively slow rates of fluid transport in the airways, pleura, and lung compared with organs where aquaporins are important, such as kidney, support the conclusion that the lung and airway aquaporins are of little physiological significance for transepithelial water transport. The intriguing finding of AQP5-dependent fluid secretion in airway submucosal glands suggests that AQP5 inhibition may be useful in reducing fluid secretions in bronchitis and rhinitis and that AQP5 activation may be useful in treating the viscous glandular fluid secretions in cystic fibrosis.

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