Perspective

Respiratory Aquaporins in Lung Inflammation
The Night Is Young

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From the nasopharynx to the pleural space, alterations in fluid transport are central to a wide range of pathophysiologic processes in the respiratory tract. Despite intense investigation, details of the pathways for water transport in the respiratory tract remain unclear. Insight into molecular mechanisms of membrane water permeability was provided by the discovery of the aquaporins, a growing family of water-specific membrane channel proteins (1). In this issue, Towne and colleagues (2) provide a rigorous description of changes in aquaporin expression in the respiratory tract by using a murine model of pulmonary inflammation, the adenovirus infection.

The existence of water-specific membrane channel proteins was postulated by a small group of physiologists for several decades, but the molecular identity of such proteins remained enigmatic until recently. Aquaporin-1 (AQP1) was serendipitously discovered during studies of erythrocyte membrane proteins (3). Several features suggested that AQP1 could be the long-sought water channel, a suspicion that was confirmed by expression in *Xenopus laevis* oocytes. Transfer of AQP1-expressing oocytes from isotonic to hypotonic medium resulted in rapid swelling and rupture, whereas control oocytes changed little in volume (4). Subsequent investigations in oocytes, as well as proteoliposomes reconstituted with purified AQP1 protein, demonstrated that the channel is specifically permeable to water, but not to other small molecules, including protons, ions, urea, and glycerol (4, 5). More recently, it has been suggested that AQP1 is also permeated by CO_2 (6); however, the magnitude of this permeability may be much lower than that of water, and the physiologic relevance is debated.

Four aquaporins have been identified in the respiratory tract: AQP1, AQP3, AQP4, and AQP5, each with a unique distribution suggesting distinct physiologic roles (7, 8). AQP1 is expressed in the apical and basolateral membrane of the microvascular endothelium (Figure 1A), as well as in the visceral pleura. AQP3 is expressed in the basolateral membrane of basal cells found in the tracheal and nasopharyngeal epithelium. AQP4 is present in the basolateral membrane of ciliated columnar cells in bronchial, tracheal, and nasopharyngeal epithelium. AQP5 is expressed in the apical membrane of type I pneumocytes (Figure 1B), as well as in the apical membrane of acinar cells in submucosal glands of the airways and nasopharynx. The specificity of AQP5 for type I pneumocytes has also been demonstrated in studies of alveolar epithelial cell differentiation (9). The ontogeny of each of the aquaporins in the lung is likewise distinct. AQP1 is expressed from late gestation in rat lung and is induced by corticosteroids in both fetal and adult animals (7). AQP5 is expressed shortly after birth and is not steroid responsive (10). Both AQP1 and AQP5 are expressed at high levels in adult animals. AQP4 expression increases transiently after birth (10, 11) and is enhanced by corticosteroids and β-agonists (11). The complex distribution and ontogeny of aquaporins in the respiratory tract suggests potential involvement in a variety of pathophysiologic processes.

The first examples of rate-limiting aquaporin expression were derived from studies of the kidney. AQP2 is the vasopressin-responsive water channel that confers high water permeability on the collecting duct (12). Deen and colleagues (13) demonstrated that mutations in the AQP2 gene produce nephrogenic diabetes insipidus in humans. This observation has now been expanded by the demonstration that acquired forms of nephrogenic diabetes insipidus, including lithium therapy, chronic hypokalemia, and ureteral obstruction, all result from downregulation of AQP2 expression (12). More recently, upregulation of AQP2 has been shown in fluid retention states, including congestive heart failure, pregnancy, and cirrhosis (1). The physiologic relevance of AQP1 in the proximal tubule was recently confirmed in AQP1-null mice, in which a profound urinary concentrating defect became evident after water deprivation (14). Another example of rate-limiting aquaporin expression has recently been demonstrated in the salivary gland, where AQP5 is expressed in the apical membrane of acinar cells (8, 15). AQP5-null mice have a marked reduction in saliva formation (16), and transfection of an aquaporin gene into radiation-damaged salivary glands partially restores function (17).

Several lines of evidence suggest a physiologic role for aquaporins in the respiratory tract. Studies of *in situ* perfused sheep lungs (18) and perfused distal airway seg-

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ments (19) provided functional evidence of water channel–mediated transport across the alveolar membrane and airway epithelium, respectively. More recently, studies in AQP1-null mice demonstrated a 10-fold reduction in the osmotic water permeability of the pulmonary vascular bed compared with controls; a 2-fold reduction in permeability was noted after increases in hydrostatic pressure (20). Although each of these studies suggests a potential role for aquaporins in regulating lung water permeability, the precise nature of that role in normal and pathologic conditions remains unclear.

At first glance, aquaporins could provide the molecular pathway for transcellular water movement across the endothelial and epithelial barriers. Abundant expression of AQP1 in both the apical and basolateral membrane of endothelial cells in the microvasculature (Figure 1A) could certainly facilitate transcellular water movement. However, the long-held model for water transport across the pulmonary endothelium is one of predominantly paracellular transport (21). How does one reconcile abundant expression of AQP1 with predictions of paracellular transport? One possibility is that the paracellular transport model is not correct, or at the least, is not operative in all circumstances. Similar considerations led not only to recent reassessment of water reabsorption in the renal proximal tubule, but also the determination that transport is predominantly transcellular rather than paracellular (22). Other possibilities, however, certainly exist. For example, do endothelial transport mechanisms differ between normal conditions and either injury or repair? Could paracellular transport predominate in one set of circumstances, while transcellular water transport predominate in another? Alternatively, does AQP1 participate in cell volume regulation, and could AQP1-mediated changes in cell volume contribute to dynamic regulation of the paracellular pathway? Much work is needed before we will be able to define roles for AQP1 in the pulmonary vasculature.

Different but no less complex issues await evaluation of AQP5 function in type I pneumocytes. Isolated type I cells are highly water permeable (23). However, polarized expression of AQP5 in the apical membrane of type I pneumocytes raises many questions. Are there undiscovered aquaporins on the basolateral membrane, or could water-transport across the basolateral membrane occur by non-aquaporin-mediated mechanisms? In either case, does AQP5 participate in transcellular water movement across the type I epithelium? Perhaps the message of restricted expression of AQP5 to the apical membrane is that transcellular water movement does not occur across type I cells. Since aquaporins are not active transporters, solute trans-

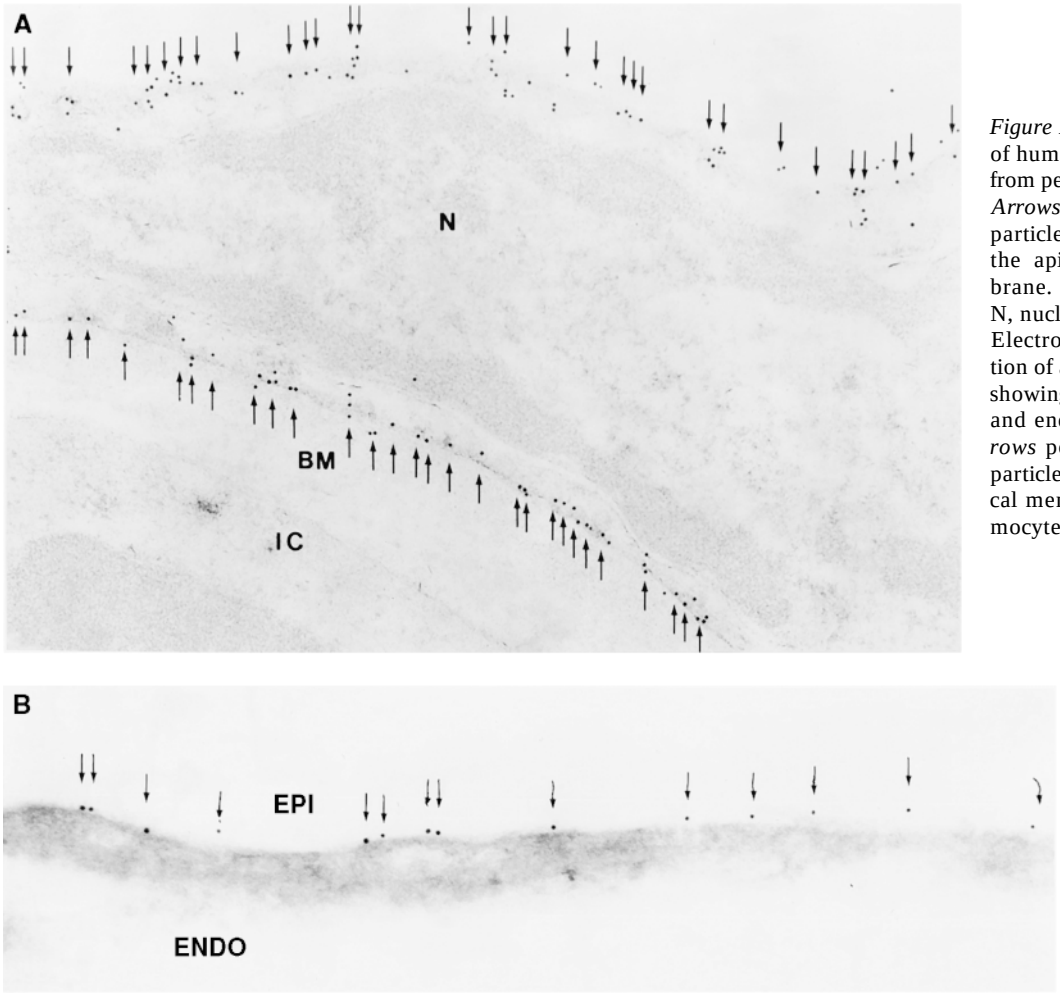

Figure 1. (*A*) Electron micrograph of human capillary endothelial cell from peribronchiolar vascular plexus. *Arrows* point to 10-nm immunogold particles labeling AQP1 on both the apical and basolateral membrane. BM, basement membrane; N, nucleus; IC, interstitial cell. (*B*) Electron micrograph of thin portion of alveolar membrane from rat showing type I pneumocyte (EPI) and endothelial cell (ENDO). *Arrows* point to 10-nm immunogold particles labeling AQP5 on the apical membrane of the type I pneumocyte.

porters must provide the driving force for water movement. The presence of both sodium transporters and sodium channels in type II pneumocytes is well established (24, 25). Although recent data suggest that type I pneumocytes express these transport proteins as well (26, 27), it remains to be determined whether their number or distribution is sufficient to provide the necessary driving force for transcellular water movement. Teleologically, there might be some advantage to having water transport pathways at other sites in order to preserve the thin part of the alveolar membrane. Rather than mediating transcellular water movement, AQP5 expression may contribute to regulation of the composition and volume of the surface liquid in the alveolus. Or, as suggested for AQP1, perhaps AQP5 participates in cell volume regulation, helping to maintain the attenuated state of the extended type I pneumocyte cytoplasm. The discussions relating to aquaporin-mediated gas permeability should also be considered. Could AQP5 be permeated by gas molecules and contribute to epithelial gas exchange? Although this seems unlikely at present, as with AQP1, many questions remain to be answered before we will be able to clearly assign or eliminate roles for AQP5 in the physiology of the alveolus.

Myriad transporters and mediators have been implicated in the process of edema formation during inflammation. It is likely that no single factor will sufficiently explain either formation or resolution of edema in all circumstances. In this context, however, the question of what path the water follows still remains. Towne and colleagues have provided an interesting and important addition to the ongoing evaluation of aquaporin biology in the respiratory tract (2). These investigators convincingly demonstrate that in mice infected with adenovirus, expression of both AQP1 and AQP5 in the lung is markedly reduced, concurrent with an increase in lung wet-to-dry weight ratios. The authors also observed that AQP1 and AQP5 expression were reduced at sites distant from foci of infection, consistent with the notion of humoral regulation of AQP expression. These studies do not allow discrimination of the specific role played by AQP1 or AQP5 in this process. They do, however, provide the first clear example that inflammation in the lung can alter aquaporin expression, an important observation that may prove relevant to future consideration of a role for aquaporins in the pathophysiology of the respiratory tract.

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