The Cellular Mechanisms Contributing to Lung Edema Clearance

Zaher S. Azzam MD[1,2] and Jacob I. Sznajder MD[1]

[1]Division of Pulmonary and Critical Care Medicine, Northwestern Memorial Hospital, University of Northwestern, Chicago, IL, USA; and [2]Department of Internal Medicine A, Rambam Medical Center and Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

Key words: Na,K-ATPase, lung edema clearance, active Na^+ transport, Na^+ channels, cytoskeleton, β-adrenergic agonists, dopamine

IMAJ 2000;2:235–239

Pulmonary edema is a life-threatening condition arising from diverse etiologies. During the last decade many physiologic, cellular and molecular biology studies have contributed to our understanding of the mechanisms responsible for lung edema clearance. In normal as well as injured lungs the alveolar epithelium has an important role in keeping the alveolar space clear of fluid by vectorial sodium transport. The sodium enters through the apical membranes of the alveolar epithelial cells via Na^+ channels and is extruded by the basolaterally located Na,K-ATPase with water following isosmotically. Specific pharmacological interventions can modulate the active Na^+ transport and thus effect lung edema clearance. β-adrenergic and dopaminergic agonists, dexamethasone, aldosterone, growth factors and gene transduction can enhance lung edema clearance by different mechanisms, whereas inhibitors of Na^+ channels and Na,K-ATPase such as amiloride and ouabain inhibit it.

Pulmonary edema develops as a result of changes in the hydrostatic and oncotic pressure gradients across the pulmonary circulation and the lung interstitium, or due to increased lung permeability [1]. The mechanisms affecting alveolar fluid resorption were investigated in recent years, and there is a significant body of knowledge suggesting that vectorial Na^+ transport across the alveolar-capillary barrier has an important role in keeping the air spaces free of edema [2].

In this review we will discuss some of the physiologic, cellular and molecular mechanisms contributing to lung edema clearance. We will focus on recent data suggesting that pharmacological means can accelerate lung edema clearance in models of normal and injured lungs.

Alveolar epithelium

The alveolar epithelium that lines the surface of the alveoli has a thin layer of fluid and surfactant interphase. The alveolar epithelium is composed of two main cell types, type I and II [3]. The alveolar type I cells are elongated, flat and thin. Their mean volume varies between 900 μm^3 in the rat and 1,800 μm^3 in humans. AT1 cell thickness does not exceed 0.2 μm and they cover approximately 90% of the alveolar surface. AT1 cells are relatively simple, containing a small nucleus, a few mitochondria, endoplasmic reticulum with ribosomes and an inconspicuous Golgi apparatus. The thin structure of AT1 cells facilitates gas exchange by minimizing the diffusion distance from alveolar gas to the blood [4].

The ATII cells are smaller and cuboidal with a size range of 370 μm^3 in rats to 900 μm^3 in humans. They account for approximately only 5% of the alveolar surface area and are functionally polarized. They contain organelles common to all cells, however their unique organelles are lamellar bodies that hold and secrete surfactant. ATII cells also have the ability to maintain the alveolar epithelium and recover from injury by proliferating and differentiating into ATI cells [5].

The alveolar epithelial cell plasma membrane consists of apical and basolateral domains. The apical part has short microvilli and comprises different pathways for ion transport, predominant among which are the apical Na^+ channels, whereas the basolateral domain is abundant with Na,K-ATPase proteins [2].

The alveolar epithelium functions as a barrier between the air spaces and the blood. The alveolo-capillary barrier allows the passive movement of small hydrophilic solutes. As depicted in Figure 1, the Na^+ ion uptake occurs via different pathways. The amiloride-inhibitable pathway consists of sodium channels and Na-H antiport; both are located on the apical side of the alveolar epithelial cells and have a major role in the sodium transport. Additional pathways for sodium entry include the Na-glucose co-transporters, Na-amino acid co-transporters, Na-K-Cl co-transporters and others [2,6]. Also, there are specialized water-transporting channels (aquaporins) located in the distal airways, the alveolar epithelium and the pulmonary microvessels. At least nine aquaporins, which are small proteins, have been identified in mammalian tissues. Aquaporin-1 is expressed in the pulmonary capillary endothelium. AQP-3 and AQP-4 are expressed in the distal airway epithelium, while AQP-5 is expressed in the alveolar epithelium. Although the full extent of their physiological role has not been elucidated, novel quantitative fluorescence methods have demonstrated very high water permeability of the alveolar and endothelial

AT1 = alveolar type 1

AQP = aquaporin

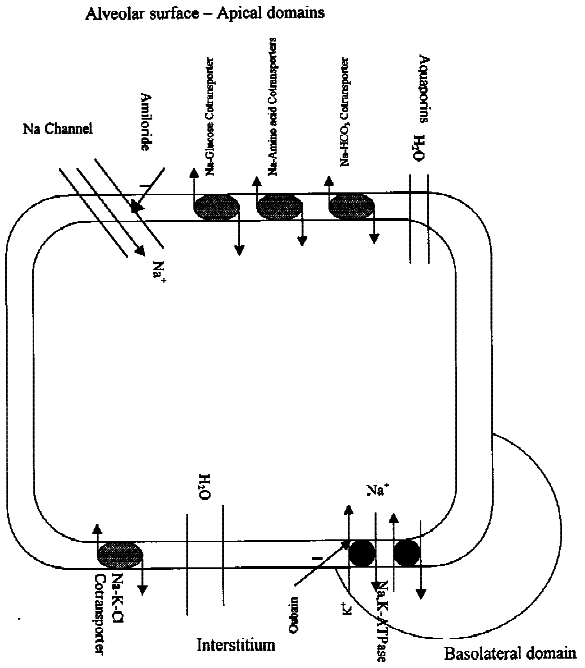

Figure 1. Schematic representation of the alveolar epithelial cell depicting the apical Na^+ channels, the basolaterally located Na,K-ATPase, the aquaporins and some of the co-transporters. Sodium enters through the apical membrane via Na^+ channels and is extruded by the Na,K-ATPase with water following iso-osmotically. The listed co-transporters are additional pathways of sodium movement in the alveolar epithelial cells coupled with various substances such as glucose, amino acids, bicarbonate and others.

cells and moderately high water permeability of the distal airways [7,8].

The physiological basis of fluid clearance

In lungs of anesthetized ventilated sheep instilled with 50 ml of an isosmotic solution, Matthay et al. [9] first demonstrated that active sodium transport is necessary for alveolar fluid clearance. The importance of Na^+ flux in lung edema clearance was confirmed later in mammalian lungs of rabbits [10], dogs [11], sheep [9], goats [12], pigs [13], rats [14,15] and humans [16,17].

The frequently used isolated perfused rat lung model was designed for the study of liquid clearance from the lungs [14,15,18]. Briefly, the heart and lungs of the anesthetized rat [14,19,20] are removed en bloc, the pulmonary artery and the left atrium are catheterized, and a tracheotomy is performed. The pulmonary artery is flushed continuously with a buffered salt albumin solution (perfusate, normal pH and osmolality) containing fluorescein isothiocyanate-tagged albumin to follow the movement of albumin from the pulmonary circulation into the air spaces through the alveolar capillary barrier. The lungs are instilled with a known amount of buffered salt albumin solution containing markers such as Evans blue dye or radioactive iodine (^{125}I or ^{131}I) tagged albumin, radioactive sodium (^{22}Na) and ^{3}H-mannitol. Since virtually all the EBD mass remains in the air space, any change in the EBD-albumin concentration will reflect a change in the air space volume.

Experimentally, we and others found that the sodium concentration is equal and relatively constant in all compartments, and since $^{22}Na^+$ is instilled in the air space the disappearance of the radioactive tracer from the air spaces reflects the total or unidirectional Na^+ outflux from the air space. The passive or bidirectional Na^+ flux between the air space and the other compartments is the difference between the unidirectional and active Na^+ outflux. The lungs' ability to clear edema, calculated from the active Na^+ transport in the different models, varies between ~10% per hour in the isolated rat lung model [14] and ~30% in the *in situ* models [9].

Cellular studies and the role of Na,K-ATPase pump in Na^+ flux

Culturing ATII cells in non-porous surfaces results in the formation of cell monolayers that adhere to the underlying surfaces and form a small fluid collection (domes). A change in the transepithelial potential was observed by using the Ussing chambers. This means that the net Na^+ flux from the apical to the basolateral membranes is electrogenic. The current that brings the potential to zero is called short-circuit current (I_{sc}) [6,21,22].

Na^+ enters the alveolar epithelial cells through apical amiloride-sensitive Na^+ channels [18] and via a process that consumes energy is pumped out of the cell by the Na,K-ATPase located in the basolateral membrane in exchange for potassium entry ata ratio of 3:2 Na^+-K^+ against their chemical gradient [23]. The Na,K-ATPase is a transmembrane protein composed of two subunits β and α. The α subunit contains binding sites for ATP hydrolysis, Na, K and cardiac glycosides such as ouabain. There are at least three isoforms of α (α_1, α_2 and α_3), each differing by its affinity to sodium, ouabain and tissue distribution. The β subunit, of which there are three known isoforms, is believed to be responsible for incorporating the α-subunit into the plasma membrane [24].

The Na,K-ATPase is synthesized in polysomes that are related to the rough endoplasmic reticulum, stored in endosomal components, and upon demand are transported to the basolateral plasma membrane via the cytoskeleton network [25]. The intracellular microtubular transport of the α_1 subunit was demonstrated when colchicine and brefeldin A, which interfere with the microtubular cytoskeleton, inhibited the recruitment of the Na,K-ATPase from the late endosomal compartments into the plasma membrane. Colchicine, by inhibiting Na^+ pump recruitment from endosomal compartments to the basolateral membrane, resulted in decreased fluid clearance by ~50% [25,26].

EBD = Evans blue dye

The administration of ouabain, a specific Na,K-ATPase inhibitor, also decreased lung edema clearance by more than 50% in the isolated perfused normal rat lung model, suggesting that Na,K-ATPase has an important role in the fluid reabsorption across the alveolar epithelium. Similar effects were reported when the air spaces were instilled with amiloride, a sodium channel blocker [2,15,18,27]. Na^+ channels are glycoproteins that have a hydrophobic domain spanning the cell membrane and a hydrophilic site that extends beyond the plasma membrane and serves as receptor ligand or secondary messenger binding sites. Immunopurification studies have shown that the channel proteins contain an amiloride-binding site [6]. When experiments were conducted in hypothermic conditions that inhibit the active metabolic functions, or with amiloride and ouabain given together, the edema clearance was stopped completely [Figure 2] [14,22]. These studies confirm the transport of vectorial ions from the apical membrane by sodium entry via Na^+ channels and extrusion from the basolateral membrane by the Na, K-ATPase pumps.

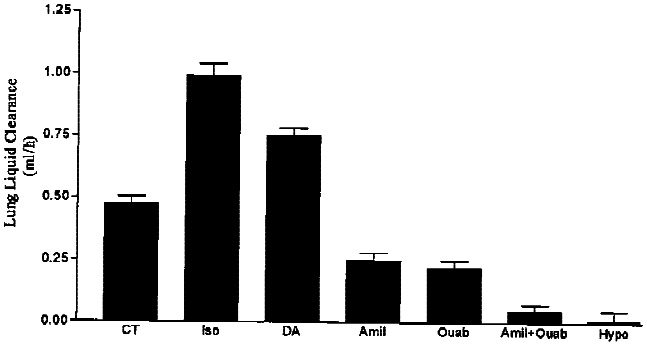

Figure 2. The effect of hypothermia and various pharmacological interventions on lung liquid clearance in isolated perfused rat lungs. Isoproterenol and dopamine increase the clearance, while amiloride (a Na channel blocker) and ouabain (a Na,K-ATPase antagonist) inhibit it. Hypothermia (which inhibits most active metabolic processes) also inhibits the lungs' ability to clear edema. Data are presented by means±SEM. (Adapted from previous publications, references 20,34 and 39). CT = control, Iso = isoproterenol 10^{-6} M, DA = dopamine 10^{-5} M, Amil = amiloride 10^{-4} M, Ouabain 5x10-4M, Hypo = hypothermia.

Interventions to increase the lungs' ability to clear edema in experimental models

Several pharmacological agents that are currently in clinical use for other purposes have been found to stimulate the active Na^+ transport and lung edema clearance [Table 1]. These agents may be of potential clinical importance for the treatment of patients with pulmonary edema. β-adrenergic receptor agonists such as dobutamine, isoproterenol and terbutaline have no proven beneficial effects on survival in patients with advanced chronic heart failure. Although not specifically studied to determine the outcome in patients with pulmonary edema, these agents were shown to increase the lungs' ability to clear edema by stimulating β-adrenergic receptors. The β_2 stimulation results in an up-regulation of the apical Na^+ channels and recruitment of Na,K-ATPase proteins from intracellular compartments into the basolateral membrane, thus improving Na^+ flux [25,28–30]. Subsequent studies have shown that the β_2 stimulation via c-AMP and protein kinase A pathway recruits Na,K-ATPase subunits within 15 minutes of β-adrenergic receptor stimulation [31]. For example, in physiological studies the instillation of terbutaline in sheep distal air spaces [32] or in isolated perfused lung model [28] significantly increased alveolar fluid clearance. Similar effects were achieved with salmetrol [33], isoproterenol and dobutamine [25,29]. The β-adrenergic agonist stimulation also increased edema clearance in a resected human lung model [16]. Recently several studies reported the beneficial effects of β-adrenergic stimulation in models of lung injury. It was shown that exposure of rats to 100% oxygen resulted in a decrease of the lungs' ability to clear edema, probably due to decreased Na,K-ATPase activity [19]. Saldias et al. [34,35] demonstrated that in rat lungs injured by hyperoxia, both isoproterenol and dopamine restored the lungs' ability to clear edema to normal levels, a finding that may contribute to alveolar epithelial repair.

Table 1. Pharmacological agents and interventional procedures that modulate the active sodium transport and lung edema clearance

Agents/interventions that increase lung liquid clearance	Agents/interventions that decrease lung liquid clearance
Aldosterone	Acute hyperoxia
α-adrenergic agonists:	Amiloride
Epinephrine	Atrial natriuretic peptide
Norepinephrine	Colchicine
β-adrenergic agonists:	Congestive heart failure
Isoproterenol	Endothelin
Dobutamine	Hypothermia
Terbutaline	Ouabain
Salmetrol	Ventilation-induced lung injury
Dexamethasone	
Dopamine	
Gene therapy	
Growth factors: EGF, KGF, α-TGF	
Interleukin-8 antibodies	

Dopamine has been used to induce natriuresis and diuresis in patients with pulmonary edema by inhibiting the Na, K-ATPase activity in the renal epithelium [36]. However, in the alveolar epithelium, dopamine appears to have an opposite effect — it enhances active Na^+ transport and lung edema clearance [30]. These effects are in part due to short-term stimulation of the dopaminergic receptors in the basolateral membrane and not by β-adrenergic stimulation [37].

Other factors such as aldosterone, glucocorticosteroids and growth factors have been shown to regulate Na,K-ATPase activity in several tissues [33]. Barquin et al. [38] have shown that the incubation of ATII cells with

dexamethasone up-regulated the Na,K-ATPase activity by increasing transcription translation of the Na,K-ATPase pump, which may have a role in edema clearance. Also, there is growing evidence that growth factors such as epidermal growth factor, keratinocyte growth factor and transforming growth factor-α [33,39] can up-regulate alveolar epithelial Na,K-ATPase function and increase lung edema clearance. In a recent study it was shown that delivering aerosolized EGF to the lungs for 48 hours increased active Na^+ transport and lung edema clearance in a rat lung model, possibly via transcriptional and/or translational regulation of the Na,K-ATPase in the alveolar epithelial cells [39]. Also, Factor et al. [40] demonstrated that gene therapy — by infecting alveolar epithelial cells *in vitro* as well as rat lungs *in vivo* with replication-incompetent, recombinant adenoviruses containing genes for Na,K-ATPase subunits — resulted in overexpression of Na,K-ATPase function and increased lung edema clearance.

In summary, understanding the vectorial Na^+ transport mechanisms and the important roles of apical Na^+ channels and Na,K-ATPase in the alveolar epithelial cells could contribute to the development of new strategies in the treatment of patients with pulmonary edema.

This research was supported in part by HL 48129 and HL 65161.

References

1. Braunwald E, W.S. Colucci, W. Grossman. Clinical aspects of heart failure: high-output heart failure, pulmonary edema. In: Braunwald E, ed. Heart Disease: A Textbook of Cardiovascular Medicine. 5th ed, vol 1. Philadelphia: WB Saunders, 1997:445–70.
2. Saumon G, Basset G. Electrolyte and fluid transport across the mature alveolar epithelium. *J Appl Physiol* 1993;74(1):1–15.
3. Schneeberger EE. The Lung: Scientific Foundations. New York: Raven Press Ltd., 1991.
4. Mason RJ, Williams MC. The Lung. New York: Raven Press Ltd., 1991:10991.
5. Crapo JD, Young SL, Fram EK, Pinkerton KE, Barry BE, Crapo RO. Morphometric characteristics of cells in the alveolar region of mammalian lungs. *Am Rev Respir Dis* 1983;128(2 Pt 2):S42–6.
6. Matalon S, Benos DJ, Jackson RM. Biophysical and molecular properties of amiloride-inhibitable Na^+ channels in alveolar epithelial cells. *Am J Physiol* 1996;271(1 Pt 1):L1–22.
7. Verkman AS. Role of aquaporin water channels in kidney and lung. *Am J Med Sci* 1998;316(5):310–20.
8. Raina S, Preston GM, Guggino WB, Agre P. Molecular cloning and characterization of an aquaporin cDNA from salivary, lacrimal, and respiratory tissues. *J Biol Chem* 1995;270(4):1908–12.
9. Matthay MA, Landolt CC, Staub NC. Differential liquid and protein clearance from the alveoli of anesthetized sheep. *J Appl Physiol* 1982;53(1):96–104.
10. Smedira N, Gates L, Hastings R, Jayr C, Sakuma T, Pittet JF, Mathay MA. Alveolar and lung liquid clearance in anesthetized rabbits. *J Appl Physiol* 1991;70(4):1827–35.
11. Berthiaume Y, Broaddus VC, Gropper MA, Tanita T, Matthay MA. Alveolar liquid and protein clearance from normal dog lungs. *J Appl Physiol* 1988;65(2):585–93.
12. Serikov VB, Grady M, Matthay MA. Effect of temperature on alveolar liquid and protein clearance in an in situ perfused goat lung. *J Appl Physiol* 1993;75(2):940–7.
13. Ballard ST, Schepens SM, Falcone JC, Meininger GA, Taylor AE. Regional bioelectric properties of porcine airway epithelium. *J Appl Physiol* 1992;73(5):2021–7.
14. Rutschman DH, Olivera W, Sznajder JI. Active transport and passive liquid movement in isolated perfused rat lungs. *J Appl Physiol* 1993;75(4):574–80.
15. Effros RM, Mason GR, Sietsema K, Hukkanen J, Silverman P. Pulmonary epithelial sieving of small solutes in rat lungs. *J Appl Physiol* 1988;65(2):640–8.
16. Sakuma T, Okaniwa G, Nakada T, Nishimura T, Fujimura S, Matthay MA. Alveolar fluid clearance in the resected human lung. *Am J Respir Crit Care Med* 1994;150(2):305–10.
17. Matthay MA, Wiener-Kronish JP. Intact epithelial barrier function is critical for the resolution of alveolar edema in humans. *Am Rev Respir Dis* 1990;142(6 Pt 1):1250–7.
18. Basset G, Crone C, Saumon G. Significance of active ion transport in transalveolar water absorption: a study on isolated rat lung. *J Physiol (Lond)* 1987;384:311–24.
19. Olivera WG, Ridge KM, Sznajder JI. Lung liquid clearance and Na,K-ATPase during acute hyperoxia and recovery in rats. *Am J Respir Crit Care Med* 1995;152(4 Pt 1):1229–34.
20. Sznajder JI, Olivera WG, Ridge KM, Rutschman DH. Mechanisms of lung liquid clearance during hyperoxia in isolated rat lungs. *Am J Respir Crit Care Med* 1995;151(5):1519–25.
21. Filippatos GS, Hughes WF, Qiao R, Sznajder JI, Uhal BD. Mechanisms of liquid flux across pulmonary alveolar epithelial cell monolayers. *In Vitro Cell Dev Biol Anim* 1997;33(3):195–200.
22. Yue G, Shoemaker RL, Matalon S. Regulation of low-amiloride-affinity sodium channels in alveolar type II cells. *Am J Physiol* 1994;267(1 Pt 1):L94–100.
23. Olivera W, Ridge K, Wood LD, Sznajder JI. Active sodium transport and alveolar epithelial Na-K-ATPase increase during subacute hyperoxia in rats. *Am J Physiol* 1994;266(5 Pt 1):L577–84.
24. Ridge KM, Rutschman DH, Factor P, Katz AI, Bertorello AM, Sznajder JL. Differential expression of Na-K-ATPase isoforms in rat alveolar epithelial cells. *Am J Physiol* 1997;273(1 Pt 1):L246–55.
25. Bertorello AM, Ridge KM, Chibalin AV, Katz AI, Sznajder JI. Isoproterenol increases Na^+-K+-ATPase activity by membrane insertion of alpha-subunits in lung alveolar cells. *Am J Physiol* 1999;276(1 Pt 1):L20–7.
26. Saldias F, Lecuona E, Friedman E, Barnard ML, Ridge KM, Sznajder JI. Modulation of lung liquid clearance by isoproterenol in rat lungs. *Am J Physiol* 1998;274(5 Pt 1):L694–701.
27. Mircheff AK, Bowen JW, Yiu SC, McDonough AA. Synthesis and translocation of Na(+)-K(+)-ATPase alpha- and beta-subunits to plasma membrane in MDCK cells. *Am J Physiol* 1992:262(2 Pt 1):C470–83.
28. Crandall ED, Heming TA, Palombo RL, Goodman BE. Effects of terbutaline on sodium transport in isolated perfused rat lung. *J Appl Physiol* 1986;60(1):289–94.
29. Saumon G, Basset G, Bouchonnet F, Crone C. cAMP and beta-adrenergic stimulation of rat alveolar epithelium. Effects on fluid absorption and paracellular permeability. *Pflugers Arch* 1987;410(4–5):464–70.
30. Barnard ML, Olivera WG, Rutschman DM, Bertorello AM, Katz AI, Sznajder JI. Dopamine stimulates sodium transport and liquid clearance in rat lung epithelium. *Am J Respir Crit Care Med* 1997;156(3 Pt 1):709–14.
31. Matalon S. Mechanisms and regulation of ion transport in adult mammalian alveolar type II pneumocytes. *Am J Physiol* 1991;261(5 Pt 1):C727–38.
32. Berthiaume Y, Staub NC, Matthay MA. Beta-adrenergic agonists increase lung liquid clearance in anesthetized sheep. *J Clin Invest* 1987;79(2):335–43.
33. Matthay MA, Folkesson HG, Verkman AS. Salt and water transport across alveolar and distal airway epithelia in the adult lung. *Am J Physiol* 1996;270(4 Pt 1):L487–503.
34. Saldias FJ, Comellas A, Ridge KM, Lecuona E, Sznajder JI. Isoproterenol improves ability of lung to clear edema in rats exposed to hyperoxia. *J Appl Physiol* 1999;87(1):30–5.
35. Saldias FJ, Lecuona E, Comellas AP, Ridge KM, Sznajder JI. Dopamine restores lung ability to clear edema in rats exposed to hyperoxia. *Am J Respir Crit Care Med* 1999;159(2):626–33.
36. Meister B, Aperia A. Molecular mechanisms involved in catecholamine regulation of sodium transport. *Semin Nephrol* 1993;13(1):41–9.
37. Barnard ML, Ridge KM, Saldias F, Friedman E, Gare M, Guerrero C, Lecuona E, Bertorello AM, Katz AI, Sznajder IJ. Stimulation of the

EGF = epidermal growth factor

dopamine 1 receptor increases lung edema clearance. *Am J Respir Crit Care Med* 1999;160(3):982–6.

38. Barquin N, Ciccolella DE, Ridge KM, Sznajder JI. Dexamethasone upregulates the Na-K-ATPase in rat alveolar epithelial cells. *Am J Physiol* 1997;273(4 Pt 1):L825–30.

39. Sznajder JI, Ridge KM, Yeates DB, Ilekis J, Olivera W. Epidermal growth factor increases lung liquid clearance in rat lungs. *J Appl Physiol* 1998;85(3):1004–10.

40. Factor P, Saldias F, Ridge K, Dumasius V, Zabner J, Jaffe HA, Blanco G, Barnard M, Mercer R, Perrin R, Sznajder JI. Augmentation of lung liquid clearance via adenovirus-mediated transfer of a Na,K-ATPase beta1 subunit gene. *J Clin Invest* 1998;102(7):1421–30.

Correspondence: Dr. J.I. Sznajder, Division of Pulmonary and Critical Care Medicine, Tarry Building 14-707, Northwestern University, 300 E. Superior St., Chicago, IL 60616, USA. Tel: (1-312) 908 8163; Fax: (1-312) 908-4650; email: j-sznajder@nwu.edu