Protein Transport Across Pulmonary Alveolar Epithelium

Kwang-Jin Kim, Ph.D.

Assistant Professor of Medicine and Physiology

Department of Medicine and Physiology, Cornell University Medical College,

1300 York Avenue, New York, NY 10021

The distal airways of the lung terminate in alveolar air sacs which must be kept relatively fluid-free for efficient gas exchange. Fluid balance in the alveoli can be altered due to injury to the alveolar-capillary barrier and/or by other cardiopulmonary complications (e.g., congestive heart failure). It has become appreciated recently that component of the alveolar-capillary barrier for regulation of alveolar epithelium is probable the single most important of the alveolar-capillary barrier for regulation of alveolar fluid and solute content, and for the prevention and resolution of alveolar pulmonary edema.

Until recently, the majority of investigations of alveolar fluid balance and solute (e.g., ions and nonelectrolytes) transport relied on intact lung models. The information obtained from these intact mammalian lung studies in vivo and in vitro, while useful, can be difficult to interpret mechanically, due largely to the anatomical complexity of the lung. In order to obtain more precise information about alveolar epithelial properties, studies of isolated preparations have provided useful additional approaches to the underlying transport mechanisms and pathways in the alveolar capillary wall. The barrier functions of the alveolar epithelium are therefore being studied in a number of ways, in addition to intact lung investigations, including monolayers of alveolar epithelial cell cultures as an in vitro model of alveolar epithelium and anatomically simpler amphibian lungs representing an intact preparation of alveolar epithelium. In this review, the bar-

rier properties of the alveolar epithelium relevant to alveolar protein transport and fluid balance, citing the pertinent supporting experimental data where appropriate, will be discussed.

Macromolecules in general are thought to traverse biological tissue barriers by pathways probable different from those used by small molecules (e. g., ions and sugars). Macromolecules are also not thought to be actively transported. It has recently become appreciated that macromolecule transport probably involves vesicular uptake and secretion pathways with or without modification of the molecules within the cells (e.g., via lysosomal processing of the proteins into amino acids.). There are experimental models for each of these two patterns of epithelial macromolecule transport^{1,2).}

Protein transport across the air-blood barrier in the lung may be of considerable importance, in that serum proteins (e.g., albumin) may be involved in the pathogenesis and resolution of alveolar pulmonary edema. Clearance of these proteins from the alveolar air spaces may become a formidable task for a damaged air-blood barrier. Even in normal lungs, it is now generally understood that alveolar fluid contains small concentrations of antioxidant proteins and immunoglobulins, which may be important for lung defenses. Unfortunately, little is known about the pathways and underlying mechanisms of macromolecule transport across alveolar epithelium.

Historically, it has even been controversial wheth-

ere macromolecules such as serum proteins are able to traverse the air-blood barrier at all. As early as 1936, Fox³⁾ utilized intact rabbit and dog lungs to show that intratracheally-instilled anti-sera largely remained in the air spaces for up to 48 hours. Thus, the air-blood barrier was thought to severely restrict the passage of the proteins studied. Drinker et al⁴⁾ instilled serum protein (e.g., hemoglobin, albumin) tracers tagged with a dye (T1824) intratracheally into canine lungs to demonstrate that virtually no tracers appear in the vascular space. Similar results were also obtained by Drinker and Hardenbergh⁵⁾, in that T1824-albumin instilled in canine lungs did not appear in the blood stream for up to 5 hours. Other more recent studies⁶⁻¹¹⁾ have been consistent with these earlier results, in that the air-blood barrier may be relatively impermeable to protein tracers within the physiological range of lung inflation volumes.

A number of morphological studies performed by electron microscopy using protein markers (e.g., cytochrome c, horseradish peroxidase) generally support the notions that the normal air-blood barrier may be virtually impermeable to proteins. For example¹²⁻¹⁴⁾, protein markers were found generally not to traverse pulmonary epithelial junctions, although they freely penetrate endothelial junctions (except for catalase and ferritin, which are stopped at endothelial junctions). The epithelial junctions, however, were seen to allow passage of protein markers when high vascular pressures are present¹²⁾.

Contrary to the above conclusion that protein does not cross the air-blood barrier, there are numerous reports indicating that proteins are normally translocated in both directions across the air-blood barrier, although the exact transport mechanisms and pathways remain unclear. The earliest evidence in support of this alternate conclusion came from the studies of Courtice and Simmonds¹⁵⁾, where intratracheally-administered T1824-albumin was found, proportional to the rate of ventilation, in the

lymph and blood of intact rabbits. This result was essentially confirmed by Schultz et al¹⁶⁾ in dog lungs in situ, where instilled ¹²⁵I-albumin was found to slowly clear from the lung air spaces into plasma. These investigators repeated the same experiments later by utilizing isolated, perfused dog lungs with ¹³¹I-albumin instilled into the air spaces, and showed that about 6% of the instilled radioactivity appeared in the vascular space at the end of a 5 h period. Findings similar to these have been obtained by a number of investigators, utilizing intact lungs of rats¹⁷⁾, rabbits¹⁸⁻²¹⁾, guinea pigs²²⁾, dogs²²⁻³²⁾, and sheep³³⁻³⁶⁾.

The pathways and transport mechanisms underlying these apparent protein flows across the air-blood barrier are not yet clear. Early on, type II alveolar epithelial cells were suggested to be the potential site of phagocytosis and or endocytosis of macromolecules³⁷⁾. However, Lauweryns and Baert³⁹⁾ thought that type I pneumocytes, with the added advantage of occupying most of the alveolar surface, with the added advantage of occupying most of the alveolar surface, may also be the site of transcytosis of proteins and other macromolecules presented to the air spaces. Gil et al³⁹⁾ further suggested that numerous vesicles present in the alveolar epithelial and capillary barriers may be useful to transport macromolecules by pinocytosis. The possibility of receptormediated endocytosis by the alveolar epithelium was first raised by Simionescu and Simionescu⁴⁰⁾, who indicated that the alveolar surface of the Type II pneumocyte is decorated by cationic ferritin at the locale of coated and uncoated pits, as well as within the vesicles. Similar findings were presented by Williams^{41,42)}, utilizing in vivo rat lungs. She also noted cationized Maclura promifera agglutinin binds to these anionic sites located in pits and vesicles.

Bignon et al^{43,44)} clearly demonstrated that autologous albumin and immunoglobulin G (IgG) are present on the alveolar surfaces of the mammalian

lung. These investigators incubated horseradish peroxidase-labeled antibodies (against albumin or IgG) with prefixed lung blocks, and demonstrated the normal presence of IgG in alveolar epithelial cell coats, in tubular myelin, and in free granular deposits. Further noted was that autologous albumin is present on the surface of Type II pneumocytes and in vesicles in Type I pneumocytes, thereby supporting the possibility of albumin being normally translocated across the air-blood barrier. Interestingly, albumin was absent from lamellar bodies of Type II cells. These data suggest that proteins (e.g., albumin) may traverse the air-blood barrier under normal conditions, perhaps via vesicular pathways.

Apparent albumin permeabilities ranging from 5×10^{-10} cm/s in sheep lungs³⁶⁾ to $1.9 \sim 3.4 \times 10^{-9}$ cm/s in dog lungs²⁹⁻³²⁾ have been reported. The wide range of values may reflect in part the difficulties of obtaining precise values may reflect in part the difficulties of obtaining precise measurements in intact mammalian lungs. Characterization of protein permeability properties of the alveolar epithelium under more strictly controlled experimental conditions was first attempted by Kim et al⁴⁵⁾, who utilized the simpler amphibian lung model of the alveolar epithelium. These investigators found that apparent ^{14}C -albumin permeabilities across the bullfrog alveolar epithelium are unexpectedly large compared to those of a much smaller polysaccharide, ^{14}C -methylhydroxy-inulin. Furthermore, the permeability of albumin observed in the alveolar to the pleural direction is 2.3×10^{-7} cm/s, about four times greater than that in the opposite direction. Inulin permeability, on the other hand, is symmetric (3.1×10^{-8} cm/s) and much smaller than that for albumin in either direction. It was further noted that these asymmetric ^{14}C -albumin fluxes were not affected by different electrical gradients (-20 to 20 mV) imposed across the does not occur through passive, restricted diffusional routes across the alveolar epithelium. Very similar results were

also found recently for excised canine bronchial epithelium by Johnson et al⁴⁶⁾. At least some of the radiolabel is transported across the alveolar (and bronchial) epithelium on subfragments of the originally intact ^{14}C -albumin. These findings on asymmetric albumin transport across the alveolar epithelium indicate that the normal air-blood barrier is capable of translocating proteins, and perhaps removing excess proteins from the alveolar fluid, thereby helping to maintain relatively dry alveolar air spaces for efficient gas exchange.

Preliminary results reported very recently indicate that mammalian alveolar epithelium *in vitro* also transports proteins rapidly and asymmetrically. Kim et al⁴⁷⁾ showed that tight mono layer cultures of isolated rat type II pneumocytes grown on tissue culture-treated Nuclepore membranes⁴⁸⁾ exhibit apparent ^{14}C -albumin permeabilities of 2.39×10^{-7} cm/s in the apical to basolateral direction and 1.66×10^{-7} cm/s in the opposite direction. These protein permeabilities are similar to the permeability observed for the much smaller ^{14}C -mannitol (about 2×10^{-7} cm/s). These data taken together clearly suggest that mammalian alveolar epithelium transports proteins rapidly, favoring net removal of proteins from alveolar fluid. The underlying specific mechanisms and pathways for macromolecule translocation across the mammalian alveolar epithelium, however, largely remain to be determined.

It has been shown that vascularly-injected ^{131}I -albumin appears in bronchoalveolar lavage (BAL) fluid much faster in adult respiratory distress syndrome (ARDS) patients than in congestive heart failure patients⁴⁹⁻⁵¹⁾. These findings have usually been interpreted to suggest damage to the air-blood barrier in non-cardiogenic pulmonary edema. Bell et al⁵²⁾ suggested that the air-blood barrier appears to restrict the passage of proteins in part by molecular size, based on their findings of extremely low concentrations of larger proteins (e.g., α_2 macroglobulin) compared to smaller molecules (such as

albumin) in the BAL fluid. Fowler et al⁵³⁾ and others^{54,55)} reported similar findings, in that large macromolecules (e.g., α_2 macro-globulin, immunoglobulin M, and fibrinogen) are present in BAL fluid at extremely low concentrations. The apparent selectivity of the air-blood barrier toward proteins of different sizes seems to be disrupted by lung injury⁵⁴⁾. Furthermore, the elevated concentrations of the larger proteins in BAL fluid following lung injury were found to persist even after the concentrations of other smaller proteins in the alveolar fluid returned to normal levels⁵⁴⁾. These results may suggest that the normal alveolar epithelium is virtually impermeable to larger protein species. While severe injury to the air-blood barrier leads to indiscriminate flow of proteins from the interstitial and vascular spaces into the air spaces. During the recovery period following ARDS, the alveolar epithelium may again become impermeable to macromolecules of the size of α_2 macro-globulins, thereby retaining them longer in the air spaces, whereas smaller protein species may be translocated across the barrier more rapidly, perhaps via increased pinocytosis.

Another important feature of the normal BAL protein profile may be the presence of functional macromolecules such as antioxidant proteins (e.g., ceruloplasmin and transferrin)^{56,57)} have used the observed shifts in protein concentrations in BAL fluid following lung injury as an index of the degree of lung injury. However, in view of the discussions above, the shift in BAL fluid protein concentrations may not result from lung injury, and may in fact be a desirable adaptive change in the rate of proteins translocation and/or selectivity by the alveolar epithelium. Accordingly, recognizing that protein transport across alveolar epithelium is probably very complex, caution should be exercised before interpreting a shift in alveolar fluid protein concentrations as an index of lung injury.

As a final note, we might suggest that the alveolar epithelium may be a particularly advantageous

route of administration of some newly-available bioengineered protein drugs (e.g., erythropoietin) which probably are not able to be taken orally. Hubbard et al⁵⁹⁾ found that an aerosolized, synthetic analog of protease inhibitor (α_1 -antitrypsin) is cleared from the lung air spaces and found in the circulating blood in sheep. These findings, and those cited above from our own laboratory, suggest that further studies of the characteristics and regulation of macromolecule transport across alveolar epithelium may help support the use of the lung as a new site for drug delivery.

ACKNOWLEDGMENTS

This work supported in part by the National Institutes of Health (Research Grant HL38658) and New York Lung Association Lung Research Fund. The author sincerely thanks the Editor for the heart-felt invitation to write this review, when the author recently sojourned his native country after a long absence.

REFERENCES

- 1) Wall DA, Maack T : *Am J Physiol* **248**:C12-C20, 1985
- 2) Breitfeld PP, Casanova JE, Simister NE, Ross SA, McKinnon WC, Mostov KE : *Am J Respir Cell Mol Biol* in press, 1989
- 3) Fox JP : *J Immunol* **31**:7-23, 1936
- 4) Drinker CK, Warren MF, MacLanhan M : *J Exp Med* **66**:449-458, 1937
- 5) Drinker CK, Hardenbergh E : *J Exp Med* **86**:7-17, 1947
- 6) Normand ICS, Olver RE, Reynolds EOR, Strang LB : *J Physiol* **219**:303-330, 1971
- 7) Egan EA, Olver RE, Strang LB : *J Physiol* **244**:161-179, 1975
- 8) Normand ICS, Reynolds EOR, Strang LB : *J Physiol* **210**:151-164, 1970
- 9) Egan EA, Nelson RM, Beale EF : *Pediatr Res* **14**:314-318, 1980
- 10) Egan EA : *J Appl Physiol* **53**:121-125, 1982
- 11) Egan EA, Nelson RM, Olver RE : *J Physiol* **260**:409

- 424, 1976
- 12) Pietra GG, Szidon JP, Leventhal MM, Fishman AP : Science **166**:1643-1646, 1968
 - 13) Schneeberger-Keely EE, Karnovsky MJ : J Cell Biol **37**:781-793, 1968
 - 14) Schneeberger EE, Karnovsky MJ : J Cell Biol **49**:319-334, 1971
 - 15) Courtice FC, Simmonds WJ : J Physiol **109**:103-116, 1949
 - 16) Schultz AL, Grismer JT, Grande F : J Lab Clin Med **61**:494-500, 1963
 - 17) Giesecking R : Ergeb Allerg Pathol Anat **38**:92-98, 1958
 - 18) Gates L, Matthay MA : Am Rev Respir Dis **135**: A189, 1987 (Abstract)
 - 19) Braley JF, Dawson CA, Moore VL, Cozzini BO : J Clin Invest **61**:1240-1246, 1978
 - 20) Braley JF, Dawson CA, Moore VL : J Immunol **121**: 926-929, 1978
 - 21) Braley JF, Peterson LB, Dawson CA : J Clin Invest **63**:1103-1109, 1979
 - 22) Dominguez EAM, Liebow AA, Bensch KG : Lab Invest **16**:905-911, 1967
 - 23) Berthiaume Y, Broaddus VC, Gropper MA, Tanita T, Matthay MA : J Appl Physiol **65**:585-593, 1988
 - 24) Schultz AL, Grismer JT, Wada S, Grande F. Am J Physiol **207**:1300-1304, 1964
 - 25) Gillespie WJ, Lee G de J : Cardiovasc Res **1**:42-51, 1967
 - 26) Bensch KG, Dominguez EAM, Liebow AA : Science **157**:1204-1207, 1967
 - 27) Bensch KG, Dominguez EAM, Meyer EC, Liebow AA : Am Rev Respir Dis **101**:439-448, 1970
 - 28) Bensch KG, Dominguez EAM : Yale J Biol Med **43**: 236-241, 1971
 - 29) Goetzman BW, Visscher MB : J Physiol **204**:236-241, 1971
 - 30) Meyer EC, Dominguez EAM, Bensch KG : Lab Invest **20**:1-8, 1969
 - 31) Goodale RL, Goetzman BW, Visscher MB : Am J Physiol **219**:1226-1230, 1978
 - 32) Gee MH, Staub NC : J Appl Physiol **42**:144-149, 1977
 - 33) Matthay MA, Landolt CC, Staub NC : J Appl Physiol **53**:96-104, 1982
 - 34) Matthay MA, Berthiaume Y, Staub NC : J Appl Physiol **59**:928-934, 1985
 - 35) Berthiaume Y, Staub NC, Matthay MA : J Clin Invest **79**:335-343, 1987
 - 36) Gorin AB, Stewart PA : J Appl Physiol **47**:1315-1324, 1979
 - 37) Corrin B : Thorax **24**:110-114, 1969
 - 38) Lauweryns JM, Baert JH : Am Rev Respir Dis **115**: 625-683, 1977
 - 39) Lauweryns JM, Baert JH : Am Rev Respir Dis **115**: 625-683, 1977
 - 40) Simionescu D, Simionescu UM : Microvasc Res **25**:85-100, 1983
 - 41) Williams MC : Proc Natl Acad Sci USA **81**:6054-6058, 1984
 - 42) Williams MC : Proc Natl Acad Sci USA **81**:6383-6387, 1984
 - 43) Bignon J, Chahinian P, Feldmann G, Sapin C : J Cell Biol **65**:503-509, 1975
 - 44) Bignon J, Jaurand MC, Pinchon MC, Sapin C, Warnet JM : Am Rev Respir Dis **113**:109-120, 1976
 - 45) Kim KJ, LeBon TR, Shinbane JS, Crandall ED. J Appl Physiol **59**:1290-1297, 1985
 - 46) Johnson LG, Cheng PW, Boucher RC : J Appl Physiol **66**:2772-2777, 1986
 - 47) Kim KJ, Cheek JM, Crandall ED : Am Rev Respir Dis **139**:A477, 1989 (abstract)
 - 48) Cheek JM, Kim KJ, Crandall ED : Am J Physiol **256**: C688-C693, 1989
 - 49) Anderson RP, Holliday RL, Driedger AA, Lefcoe M. Reid B, Sibbald WJ : Am Rev Respir Dis **119**:869-877, 1979
 - 50) Sprung CL, Rackow EC, Fein IA, Jacob AI, Isikoff SK : Am Rev Respir Dis **124**:718-722
 - 51) Gelb AF, Klein E : Am Rev Respir Dis **114**:831-835, 1976
 - 52) Bell DY, Haseman JA, Spock A, McLennan G, Hook GER : Am Rev Respir Dis **124**:72-79, 1981
 - 53) Fowler AA, Walchak S, Ciclas PC, Henson PH, Hyers TM : Chest **81**:50S-51S, 1981
 - 54) Holter JF, Weiland JE, Pacht ER, Gadek JE, Davis WB : J Clin Invest **78**:1513-1522, 1986
 - 55) Merrill WW, Naegel GP, Olchowski JJ, Reynolds HY : Am Rev Respir Dis **131**:584-587, 1985
 - 56) Galdston M, Levytska V, Schwartz MS, Magnusson B : Am Rev Respir Dis **129**:258-263, 1984
 - 57) Pacht ER, Davis WB : J Appl Physiol **64**:2092-2099, 1988
 - 58) Lykens MG, Davis WB, Pacht ER : Am Rev Respir Dis **139**:A752, 1989 (Abstract)
 - 59) Hubbard RC, Casolaro MA, Mitchell M, Sellers SE, Arabia F, Matthay MA, Crystal RG : Proc Natl Acad Sci USA **86**:680-684, 1989