Active Transendothelial Transport of Albumin

Interstitium to Lumen

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SUMMARY. Cultured porcine pulmonary artery endothelial monolayers actively transport albumin from interstitium to lumen. The active process favors interstitial to luminal transport by a factor of 10 even in the face of a 2:1 luminal:interstitial albumin gradient. The active process is abolished by treatment with 1 mM sodium cyanide. Dextran is not actively transported. This process could be important in determining transvascular fluid balance and transfer of macromolecules across the endothelium. (Circ Res 57: 903-908, 1985)

WHEREAS the transendothelial movement of fluid is mostly explained by the balance of osmotic and hydrostatic forces across the endothelium, the transendothelial transfer of macromolecules is much less well understood and modeled. Because most endothelia are much more "leaky" than transporting epithelia, most analyses have assumed that transendothelial transfer of macromolecules is a passive process (Crone and Levitt, 1984). It has been suggested that pores of some kind conduct macromolecules across endothelium, and that the transendothelial movement is dominated by either convective or diffusive forces, depending on the author. However, in his recent review, Renkin (1985) concluded that calculated diffusive and convective transport through two different pore channels was not sufficient to model observed transport rates accurately. He suggested that nonhydraulically conductive pathways might contribute significantly to transendothelial transfer of macromolecules (Renkin, 1985).

One of the major problems in the study of transendothelial macromolecule transport has been that the measurements of transport have been indirect. In this report, we measured the transendothelial transfer of albumin across a cultured endothelium. We studied albumin because it is the most important macromolecule for determining the balance of oncotic forces across the endothelium. We studied transport across a cultured endothelium because it allowed us to make direct measurements of transendothelial transfer of macromolecules and thereby removed one of the major problems of in situ studies. Our results suggest that not all transendothelial transfer of macromolecules is a passive process, and hence it is not surprising that prior models have been difficult to fit to measured data.

Methods

Cell Culture

Porcine pulmonary arteries were obtained within a few minutes of slaughter at a local abattoir. The resected ends were clamped, the artery quickly immersed in 70% ethanol (EtOH) and then immediately rinsed in phosphate-buffered saline (PBS) with penicillin (300 U/ml) and streptomycin (300/ml). The arteries then were unclamped and placed in sterile PBS with 100 U/ml penicillin and 100 µg/ml streptomycin. Once in the laboratory, the arteries were opened longitudinally, the media were rinsed free of blood with PBS, and the lumen was gently stroked with a scalpel blade or a cotton-tipped applicator. The adherent cells were released into 35-mm diameter tissue culture plates with medium 199 supplemented with basal medium Eagle (BME) vitamins and amino acids, 100 U/ml penicillin, and 100 µg/ml streptomycin and 10% fetal bovine serum (FBS). The medium was changed after 1-2 hours depending upon the number of cells and again at 7 days. Cells were usually confluent after 10-14 days, at which time they were passed after brief (15 seconds) exposure to trypsin (0.25%) and EDTA (0.1%). Again the medium was removed after 30-60 minutes and replaced with M199 with 10% FBS.

We monitored cultures closely for homogeneous morphology, and discarded those in which other types of cells were present. Further identification of endothelial cells was done by quantitative measurement of angiotensin-converting enzyme activity (8-12 units/1.4-1.5 X 10⁶ cells, 3rd or 4th passage [3H]benzoyl-phe-ala-pro substrate from Ventrex Labs) and by positive fluorescence after uptake of fluorescein-labeled low-density lipoprotein (Dil-Ac-Ldl, Biomedical Technologies).

Micropore Filter Preparation

Polycarbonate micropore filters (0.8-µm pore diameter, 25-mm filter diameter) were purchased from Nucleopore and prepared as described (Shasby et al., 1982). Briefly, the filters were acid washed (0.5% acetic acid, 50°C, X20 minutes), gelatin impregnated (0.05% porcine gelatin, Sigma, 100°C X 60 minutes), air dried (100°C X 60 minutes), ethylene oxide sterilized, and then aerated for 5
days. The gelatin-impregnated filters were then used within 5 days.

Plating Cells on Filters

The gelatin-impregnated filters were placed in the bottom of tissue culture plates (35 mm diameter). They were then exposed to human fibronectin (30 μg/ml, Collaborative Research) for 30 minutes. The fibronectin was washed from the filter with M199, and then a bead of a suspension of cells in M199 with 10% FBS (2 × 10⁶/cm²) was placed on the filter for 30 minutes. After 30 minutes, M199 with 10% FBS was added to the plate. Four hours later, this M199 was removed and was replaced with M199 + 10% FBS. Thereafter, the monolayers were fed daily and used 6–7 days after plating.

Measuring Albumin Transfer

Endothelial monolayers were mounted in Ussing chambers, and transendothelial albumin transfer was determined. In the initial experiments, albumin (145 μM, crystalline and essentially fatty acid free) was added to one side of the monolayer only, and the rate of transfer was determined by measuring the albumin concentration on the opposite side of the monolayer. In these experiments, albumin concentration was measured by following the change in absorbance at 530 nm following reaction of albumin in the sample with bromcresol green reagent (Shasby, 1982). The reaction was demonstrated to be linear with the volumes and concentrations of sample and reagent used.

In subsequent experiments, the rate of albumin transfer from lumen to interstitium was determined as above. Albumin was then added to both sides of the monolayer in the concentrations designated. After 1–2 minutes to allow adequate mixing, 125I-labeled albumin was added to one compartment (1 × 10⁶ counts/min per ml). The rate of albumin transfer was then determined by sampling the other compartment and counting the sample in a γ-counter (Beckman Instruments). All counts were corrected for that portion of the 125I activity that could not be precipitated by 10% trichloroacetic acid (≤5%). As demonstrated in the text, the spectrophotometric and 125I-albumin techniques gave equivalent measurements of albumin transfer. In data not reported, 14C-labeled albumin also gave identical transport rates to unlabeled albumin.

In the experiments in which 1 mM NaCN was added to the media to inhibit active transport, the lumens to interstitium albumin flux was measured first in the absence of 1 mM NaCN (0.60 ± 0.16 nmol/15 min) and then in the presence of 1 mM NaCN (1.1 ± 0.22 nmol/15 min). Equal concentrations of albumin were then added to both luminal and interstitial compartments, 125I-albumin was added to the interstitial compartment, and interstitial to luminal transfer was determined as above.

In experiments designed to determine transfer of dextran, the luminal to interstitial transfer of albumin was determined with 72.5 μM luminal and 0 μM interstitial albumin. The albumin concentrations on both sides were then equalized, and 1.0 mg/ml fluorescein isothiocyanate dextran (ave. mol wt 65,000) was added to the luminal or interstitial compartment. Transfer of dextran was then determined by measuring the concentration of fluorescein isothiocyanate (FITC) dextran in the initially dextran-free compartment using a Perkin-Elmer fluorescence spectrophotometer. Samples were excited at wavelengths 490 nm and the emitted light sampled at 530 nm with 10-nm slit widths.

All data are presented as the mean ± se. Differences in luminal to interstitial vs. interstitial to luminal albumin transfer were compared using paired t-test. Differences between experimental groups were compared using t-test. Differences were considered significant at P < 0.05.

Results

Polarized Transfer

In the initial studies, albumin (145 μM) was placed on only one side of the monolayer, and the net transfer to the other side of the monolayer was determined. Albumin was added alternatively to the luminal or interstitial side of 15 consecutive monolayers. As demonstrated in Figure 1, more albumin was transferred over 30 minutes from interstitium to lumen (25.1 ± 7.3 nmol, n = 8) than from lumen to interstitium (1.58 ± 0.37 nmol, n = 7). The time course of the transfer is listed in Table 1. There were no significant differences in the rates of transfer among the different time intervals for either direction. However, there was a trend for transfer to be greater during the initial 30 minutes. Consequently, all subsequent studies were carried out for 15 or 30 minutes.

The next series of experiments were designed to determine whether the presence of luminal albumin accounted for the difference in transfer from interstitium to lumen vs. lumen to interstitium. In these experiments, the transfer from lumen to interstitium was first determined with 145 μM albumin in the luminal compartment and 0 μM albumin in the interstitial compartment (1.96 ± 0.46 nmol/30 min).

Equal albumin concentrations (145 μM) then were added to both compartments, 125I-albumin was then added to the interstitial compartment and interstitial to luminal transfer determined (25.6 ± 5.7 nmol/30 min, n = 5) (Fig. 2). These experiments confirm the difference in transfer rates and demonstrate that the greater rate of interstitial to luminal albumin transfer was not due to an effect of luminal albumin on albumin permeability.

To be certain that some of the observed differences in transfer rates in the two directions were not due to differences in the spectrophotometric and 125I

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Lumen (145 μM) to Interstitium (0 μM)</th>
<th>Interstitium (145 μM) to Lumen (0 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–15</td>
<td>0.61 ± 0.20 nmol</td>
<td>14.8 ± 4.3 nmol</td>
</tr>
<tr>
<td>0–30</td>
<td>2.06 ± 0.32 nmol</td>
<td>27.1 ± 8.1 nmol</td>
</tr>
<tr>
<td>0–60</td>
<td>3.65 ± 0.67 nmol</td>
<td>43.5 ± 12.8 nmol</td>
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</tbody>
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* Data represent net transfer of albumin from lumen to interstitium or interstitium to lumen with initial concentrations of 145 μM albumin on one side and 0 μM albumin on the other.
techniques, we measured luminal to interstitial transfer spectrophotometrically and then repeated the measurement with $^{125}$I-albumin. The rate of luminal to interstitial albumin transfer measured spectrophotometrically (3.9 ± 0.52 nmol/30 min) was not different from the rate measured using $^{125}$I-albumin (2.9 ± 0.50 nmol/30 min).

To determine whether the polarized transfer had any specificity for albumin or whether it represented bulk transfer of interstitial fluid, we measured luminal to interstitial and interstitial to luminal transfer of FITC-labeled dextran. Luminal to interstitial transfer of dextran (42.2 ± 22.2 μg/15 min, n = 4) was not different from interstitial to luminal transfer of dextran (27.5 ± 9.6 μg/15 min, n = 4).

**Transfer against a Gradient**

Since plasma albumin concentrations are approximately twice albumin concentrations in lung lymph, we measured interstitial-to-luminal albumin transfer against a concentration gradient. The rate of luminal to interstitial albumin transfer with 72.5 μM albumin in the luminal compartment and 0 μM albumin in the interstitial compartment was determined spectrophotometrically (1.2 ± 0.23 nmol/30 min). The albumin concentration in the luminal compartment...
was then increased to 145 \mu M, 72.5 \mu M albumin and
\textsuperscript{125}I-albumin were added to the interstitial compart-
ment, and the rate of interstitial to luminal albumin transfer was determined (11.05 \pm 4.06 nmol/30 min, n = 5) (Fig. 3). Hence, even against a 2:1 concentra-
tion gradient, the rate of interstitial to luminal albumin transfer was approximately 10 times the rate from lumen to interstitium.

It was possible that the higher luminal albumin concentration caused some convective flow from interstitium to lumen and thereby enhanced albumin transfer. To determine this, we first measured luminal to interstitial albumin transfer with 72.5 \mu M luminal albumin and 0 \mu M interstitial albumin (2.15 \pm 0.77 nmol). We then added 145 \mu M albumin to the interstitium, \textsuperscript{125}I-albumin to the lumen (still 72.5 \mu M albumin), and measured luminal to interstitial albumin transfer (0.75 \pm 0.14 nmol/30 min, n = 4). Hence, enhanced transport via convective flow did not account for the interstitial to luminal albumin transfer against a concentration gradient.

**Inhibition of Transfer with NaCN**

To determine whether the greater rate of albumin transfer from interstitium to lumen than from lumen to interstitium required energy production by the cell, we observed the effect of 1 mM NaCN on the transport process. We first determined the luminal to interstitial albumin transfer with 145 \mu M albumin in the luminal compartment and 0 \mu M albumin in the interstitial compartment (0.60 \pm 0.16 nmol/15 min). We then added 1 mM NaCN to both sides and repeated the luminal to interstitial transfer rate determination (1.13 \pm 0.22 nmol/15 min). We then made both sides 145 \mu M albumin, added \textsuperscript{125}I-albumin to the interstitial compartment, and measured interstitial to luminal albumin transfer (1.43 \pm 0.17 nmol/15 min, n = 4, Fig. 4). NaCN caused the albumin transfer to be equal in both directions.

**Discussion**

Albumin is the most important osmotically active macromolecule in the blood, and its osmotic forces are an important determinant of the forces determining the flux of water and electrolytes across the vessel wall. We have observed that a cultured porcine pulmonary artery endothelium transfers albumin at a greater rate from the interstitial space to the luminal space than from the luminal space to the interstitial space. The enhanced interstitial to luminal transfer persisted in the presence of a 2:1 concentration gradient from lumen to interstitium and the enhanced rate of interstitial to luminal transfer wasabolished by 1 mM NaCN. In contrast to the polarized albumin transfer, the transfer of dextran was equal in both directions. We believe these data demonstrate that this cultured endothelium actively transports albumin from interstitium to lumen and that this process has some solute selectivity.

Most of the current descriptions of transendothelial transfer of macromolecules assume that the endothelium functions as a passive, symmetric filter (Taylor and Granger, 1984). There is much dispute as to whether transfer of macromolecules is primarily via convective or diffusive pathways and the anatomic correlates of the pathways are not known (Renkin, 1985). Attempts to model transfer of macromolecules using pathways that model water and electrolyte transfer have not been successful (Renkin, 1985). One of the major problems in past studies has been the indirect nature of the measurements (Taylor and Granger, 1984). Because the cultured endothelium used in this report allows ready access to both sides of the endothelium, we were able to observe the asymmetric transfer which would not be detectable in situ.

Because it is so difficult to measure interstitial to luminal macromolecule flux in situ, there is very little literature to support our observations. Perry
and Garlick reported efflux rates of labeled γ-globulin from tissues. The tissues were initially perfused with the γ-globulins for 24 hours. The tissues then were perfused with γ-globulin free perfusate and the efflux rates were determined. Permeabilities of the tissues to γ-globulin calculated from the measured efflux rates were 4- to 20-fold those that had been determined measuring γ-globulin flux from the vascular space to the lymph. Hence, these data are consistent with a greater rate of transfer from interstitium to lumen (Parker et al., 1984).

King and Johnson (1985) just reported that bovine aortic endothelial cells, in a model similar to our own, transported insulin from lumen to interstitium via a receptor-mediated process. The insulin transfer is in the opposite direction of the albumin transfer, and hence it is unlikely that the model artificially causes polarized transfer. In our own experiments, the rates of transfer were the same in the presence of 1 mM NaCN, also making it unlikely that the polarized transfer is an artifact of the model.

We have not addressed the issue about what anatomic entity transports the albumin or what series of steps generates the energy for the transfer. As discussed by Taylor and Granger (1984) and Bundegaard (1983), macromolecule transport may occur via vesicles, transendothelial channels, or some may occur via the paracellular pathway. Since the interstitial to luminal albumin transfer is an active process, it is difficult to conceive of it occurring via the paracellular pathway. Our studies with dextran suggest that the process is not simply bulk endocytosis of interstitial media followed by non-discriminate vesicular transfer. However, since the dextran is heterogeneous regarding molecular size, and since the dextran molecule may be linear rather than globular, the dextran data do not necessarily indicate discrimination for a molecule of a size similar to albumin. Further studies will be necessary to determine if albumin accumulates in the cells and the capacity of the process.

In summary, a cultured pulmonary artery endothelium transports albumin preferentially from interstitium to lumen, it transports albumin against a concentration gradient, and energy is necessary for the endothelium to transfer albumin asymmetrically. Since our studies were done with large vessel endothelial cells, we cannot be certain this same active transport occurs in the microvasculature. Since we used cultured cells, we cannot be certain that this process is always expressed by endothelial cells, or whether it might be expressed only under certain conditions. However, it is not likely that culturing caused the cells to develop a process they did not have the genetic potential for before culture. If this process occurs in the microvasculature in situ it could help determine the balance of forces determining fluid balance in the lung.

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