Lung albumin accumulation is spatially heterogeneous but not correlated with regional pulmonary perfusion

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Received 17 April 2001; accepted in final form 31 August 2001

Gerbino, Anthony J., and Robb W. Glenn. Lung albumin accumulation is spatially heterogeneous but not correlated with regional pulmonary perfusion. J Appl Physiol 92: 279–287, 2002; 10.1152/japplphysiol.00353.2001.—The contribution of pulmonary perfusion heterogeneity to the development of regional differences in lung injury and edema is unknown. To test whether regional differences in pulmonary perfusion are associated with regional differences in microvascular function during lung injury, pigs were mechanically ventilated in the prone position and infused with endotoxin (Escherichia coli 055:B5, 0.15 μg·kg⁻¹·h⁻¹; n = 8) or saline (n = 4) for 4 h. Extravascular albumin accumulation and perfusion were measured in multiple ~0.7-ml lung regions by injecting pigs with radiolabeled albumin and radioactive microspheres, respectively. Extravascular albumin accumulation was spatially heterogeneous but not correlated with regional perfusion. Extravascular albumin accumulation was greater in dorsal than ventral regions, and regions with similar albumin accumulation were spatially clustered. This spatial organization was less evident in endotoxemic than control pigs. We conclude that there are regional differences in lung albumin accumulation that are spatially organized but not mediated by regional differences in pulmonary perfusion. We speculate that regional differences in microvascular pressure or endothelial function may account for the observed distribution of extravascular albumin accumulation.

blood flow; interstitial; lipopolysaccharide

Methods

Animal preparation. Twelve pathogen-free pigs weighing 19–25 kg were chemically restrained with ketamine (20 mg/kg) and xylazine (2 mg/kg) and anesthetized with a thioental infusion (10 mg·kg⁻¹·h⁻¹) adjusted to achieve a surgical plane of anesthesia and suppress spontaneous ventilation (range: 10–17 mg·kg⁻¹·h⁻¹). Pigs breathed air and were mechanically ventilated with a piston-pump ventilator via tracheostomy without positive end-expiratory pressure. Respiratory rate was set so that arterial PO₂ was 30–35 Torr before endotoxin infusion and was not changed thereafter, and tidal volume was 12 ml/kg. Lungs were hyperinflated to twice tidal volume every 15 min to prevent atelectasis. Central venous, arterial, and pulmonary arterial catheters were placed. Pigs were given 20 ml/kg normal saline intravenously and placed in the prone posture to minimize the effects of the

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pleural pressure gradient. The University of Washington Animal Care Committee approved all experiments.

*Study protocol. Escherichia coli* O55:B5 endotoxin (Sigma Chemical, St. Louis, MO) was continuously infused at 0.15 μg·kg⁻¹·h⁻¹ through a femoral venous catheter for 4 h. The infusion rate was halved in one pig because severe pulmonary hypertension developed (mean pressure: ~70 mmHg) and doubled in another because neither hypoxemia nor pulmonary hypertension had developed by 90 min. Core body temperature, mean systemic and pulmonary arterial pressure, pulmonary arterial occlusion pressure, thermodilution cardiac output (in triplicate; Edwards COM 2, Baxter, Irvine, CA), and arterial blood gases were measured before endotoxemia and every 60 min during endotoxin infusion.

We used two radiolabeled tracers injected at different times to measure regional extravascular albumin accumulation between 3 and 4 h of endotoxemia. Two different albumin tracers were used to allow separation of total lung albumin into its intravascular and extravascular components. Human serum albumin labeled with 131I (131I-HSA) was injected over 1 min via a femoral venous catheter after 3 h of endotoxemia. Arterial blood was collected every minute for 5 min and then every 5 min thereafter. 125I-HSA was then injected over 1 min via a femoral venous catheter after 3 h and 50 min of endotoxiaemia, and arterial blood was collected every minute thereafter. As 125I-HSA circulated, ligatures were loosely placed around the aorta, pulmonary artery, and pulmonary veins that had been exposed just before injection of 125I-HSA by median sternotomy. When 125I-HSA had circulated for ~7 min, mixed venous and arterial blood were simultaneously collected, a 60-mg thiopental bolus was given to ensure deep anesthesia, and the vascular ligatures were tightened.

Regional perfusion was measured by injecting ~1.5 million 15-μm-diameter microspheres labeled with 141Ce (141Ce; NEN, Boston, MA) via a femoral venous catheter after 3 h of endotoxemia. We used radioactive rather than fluorescent microspheres because tissue radioactivity was already being measured to calculate extravascular albumin accumulation. Thus the use of radioactive microspheres allowed us to measure regional perfusion without introducing additional steps to measure tissue fluorescence. Microspheres were vortexed, sonicated, suspended in normal saline, and manually injected over 30 s.

We performed median sternotomy at the end of each experiment to ligate the pulmonary artery and veins, thereby trapping blood in the pulmonary circulation. We initially trapped blood in the pulmonary circulation for another purpose (which we subsequently abandoned) but continued to adhere to this protocol in all pigs because of concern that interstitial albumin would shift between lung regions more easily (either within interstitial lymphatics or via vascular drainage) if the pulmonary vascular compartment were open to the atmosphere at the end of the experiment. Because radiodine circulated only briefly (<15 min in all pigs) after the switch to the supine position for median sternotomy, this procedure should have had little effect on regional albumin flux. Although median sternotomy may have altered regional blood volume, thereby shifting intravascular radiiodine between lung regions, regional shifts in intravascular radioiodine do not affect calculated rates of regional extravascular albumin accumulation.

*Tissue processing. Lungs were removed from the chest with ligatures in place, inflated with 25-cmH₂O airway pressure, and air-dried for 48–72 h. Lungs were encased in foam and cut into ~1.2-cm-thick transverse slices, and cylindrical tissue samples with diameters of 0.86 cm (~0.7 ml) were obtained from each slice with a cylindrical coring device. Sampling locations were predetermined using a template containing multiple 0.86-cm-diameter holes arranged along rectilinear coordinates with centers separated by 1.2 cm. Tissue samples were rejected if they overlaid the lung edge or if >20% of the sample was composed of Airways. This algorithm yielded 258–373 and 220–407 tissue samples per pig in the control and endotoxin group, respectively, and sampled ~30% of the lung parenchyma (each core sampled 40% of the surrounding lung, and ~25% of cored tissue samples were inadequate based on the above criteria). To account for variability in slice thickness, we confirmed the height of each cylindrical tissue sample to the nearest 0.5 mm. The lobe and x-, y-, and z-coordinates of each tissue sample were recorded, as were the coordinates of the right and left hilum.*

**Measurement of radioactivity.** Radioactivity of plasma and tissue samples was determined in a gamma counter (Minaxi model 5550, Packard, Downers Grove, IL) and corrected for decay time and spillover by using the matrix inversion method. Tissue samples were counted for 5 min, resulting in mean counts per sample of 325,000, 85,000, and 42,000 for 131I, 125I, and 141Ce, respectively. Plasma samples were counted for 15 min, resulting in mean total counts per sample of 820,000 and 417,000 for 131I and 125I, respectively. Because the 131I-to-125I-HSA plasma ratio in blood drawn at the end of each experiment is critical to the calculation of extravascular albumin accumulation (see Eq. 3), we measured radioactivity in approximately thirty 1-ml arterial and mixed venous samples collected at the end of each experiment to minimize error in this measurement.

**Regional pulmonary perfusion.** Regional perfusion (ml/min) was estimated by multiplying thermodilution cardiac output by the fraction of lung sampled by our algorithm (i.e., ~0.3) and the 141Ce signal in a sample and dividing by the 141Ce signal in all tissue samples. Coefficients of variation (standard deviation/mean) were calculated by using volumenormalized measurements of regional perfusion.

**Preparation of radiiodine.** HSA (0.1 mg) was labeled with 125I or 131I (22) just before the experiment. Disposable PD-10 columns packed with Sephadex G-25M (Pharmacia, Piscataway, NJ) were used to remove unbound radioiodide, and the percent unbound iodide was quantified by electrophoresis with cellulose polyacetate paper. One and one-half millicuries of 131I-HSA and 100 μCi of 125I-HSA were used in each experiment. 131I-HSA and 125I-HSA contained 0.6 ± 0.3 (SD) and 0.4 ± 0.2% unbound radioiodide, respectively. The mean difference between unbound 131I and unbound 125I was 0.2 ± 0.3 (SD) % in the endotoxin group and 0.3 ± 0.5% in the control group. Modeling the effects of unbound radioiodide on albumin accumulation rates suggests that, when unbound percentages of 131I and 125I differ by 0.2%, albumin accumulation rates are overestimated by only 0.4%. This model assumes mean plasma and tissue activities for 131I and 125I and rapid passage of unbound radioiodide into all body tissues in proportion to weight.

**Regional albumin accumulation rates.** Regional extravascular albumin accumulation was measured by adapting a dual-tracer method validated by Graham and Evans (20) in whole rat lungs. In this model, the interstitial and vascular compartments are separated by a permeable membrane with interstitial albumin accumulation proportional to plasma albumin concentration. If 131I-HSA and 125I-HSA are given after T₁ and T₂ minutes of endotoxemia, respectively, and the pig is killed after T₃ minutes of endotoxemia (where T₃ is time at the end of the experiment), the activities of 131I (A₁) and 125I (A₂) in tissue sample i are related to their intravascular and extravascular components by Eqs. 1 and 2.
where $A_i$ is activity in piece $i$ ($\mu Ci$); $V_i$ is plasma volume in each extravascular space in sample $i$ ($\mu l$); $L_i$ is rate of albumin accumulation in the extravascular space in sample $i$ (expressed as a plasma clearance, $\mu l/min$); $C_i(T_{end})$ and $C_2(T_{end})$ are plasma activities ($\mu Ci/\mu l$) of $^{131}I$ and $^{125}I$, respectively, at the end of the experiment; and $C_i(t)$ and $C_2(t)$ describe plasma activities ($\mu Ci/\mu l$) of $^{131}I$ and $^{125}I$, respectively, over time and are integrated over the time the radioisotope circulated. $L_i$ is the mean rate of extravascular albumin accumulation in the lung and is assumed to be identical for $^{131}I$ and $^{125}I$. $C_i(T_{end})$ and $C_2(T_{end})$ were generated by curve fitting $^{131}I$ and $^{125}I$ plasma activity, respectively, over time to a double exponential using least squares regression. $A_i$ and $A_2$, $C_i(T_{end})$, and $C_2(T_{end})$ were measured directly in a gamma counter.

Equations 1 and 2 were solved for $L_i$, giving

$$L_i = \frac{[A_{2i} - [A_{1i} \cdot C_2(T_{end})/C_i(T_{end})]]}{\int_{r_2}^{r_1} C_2(t) \cdot dt - [C_2(T_{end})/C_i(T_{end})] \cdot \int_{r_2}^{r_1} C_i(t) \cdot dt}$$

$L_i$ was normalized for differences in the volume of each tissue sample, giving extravascular albumin accumulation rates in units of microliters of plasma per minute per milliliter lung tissue.

This simple model of protein movement has been compared (20) with a more complex description in the lung (5) that includes osmotic and hydrostatic water flux, convective and diffusive protein flux, back diffusion, and lymph flow. Results of this model are similar to those of the more complete model over a range of permeability surface area products and microvascular pressures. This method has two principal advantages over more commonly used methods. First, we used a molecular tracer to mark the intravascular space, eliminating error due to variability in lung hematocrit that significantly affects regional lung measurements (3, 14). Second, we do not assume that either $^{125}I$-HSA or $^{131}I$-HSA is confined to the vascular space. Typically, one tracer is assumed to remain in the vascular space, despite some degree of transvascular leak. Failure to account for transvascular leak introduces error (31), especially when endothelial permeability is increased or a small molecular tracer is used.

Variability in $L_i$ due to counting statistic error. To determine whether error due to counting statistics accounts for the observed variability in $L_i$, we introduced counting statistic error in each measurement of radioactivity used to calculate $L_i$ by assuming typical radioactive signals from experimental data. We recalculated $L_i$ using Eq. 3 and repeated this process 500 times, yielding a hypothetical distribution for $L_i$ consistent with propagation of counting statistic error. Variability due to counting statistic error was expressed as the coefficient of variation (standard deviation/mean) of this distribution.

We also examined whether gamma-counter error (counting statistic error, machine error, error due to changes in tissue position within the scintillation vial, etc.) could account for observed variability in $L_i$ by calculating $L_i$ from radioactive signals measured five times on consecutive days in a subset of tissue samples in two pigs. Variability was expressed as the coefficient of variation for repeated measurements in each tissue sample.

Statistical analysis. Data are reported as means $\pm$ SD, except where noted. Physiological data from different time points were compared using ANOVA. Post hoc comparisons were evaluated for statistical difference using the Bonferroni correction for multiple comparisons. Differences between control and endotoxin groups were evaluated with unpaired t-tests. Distributions of albumin accumulation rates within each pig were characterized statistically by their mean, interquartile distance, and skewness (distributions could not be normalized by log transformation). We used interquartile distance rather than standard deviation as the measure of distribution breadth, because interquartile distance is a better measure of breadth when distributions are skewed.

Perfusion distributions were characterized by the coefficient of variation (standard deviation of perfusion/mean perfusion). Least squares linear regression was used to describe the correlation between regional albumin accumulation rates and regional pulmonary perfusion in each pig. In addition, least squares linear regression was used to determine the correlation between mean albumin accumulation rate and the following measurements: mean pulmonary arterial pressure at 3 h, arterial $P_{O_2}$ at 3 h, the percent difference in unbound $^{131}I$ and unbound $^{125}I$, and the number of tissue samples per pig.

Linear gradients. We analyzed rates of regional extravascular albumin accumulation as a function of cranial-caudal and ventral-dorsal position and as a function of distance from the ipsilateral hilum (i.e., hilar-peripheral position). On average, tissue samples were separated by a maximum of $12 \pm 1$ (SD) cm in the ventral-dorsal direction, $21 \pm 2$ cm in the cranial-caudal direction, and $14 \pm 2$ cm in the hilar-peripheral direction. Lungs were corrected for tilt before these relationships were calculated so that $x$, $y$, and $z$-axes reflected the true anatomic right-left, ventral-dorsal, and cranial-caudal directions, respectively. Slopes of least squares regression lines were used to describe rates of regional extravascular albumin accumulation as a function of ventral-dorsal, cranial-caudal, or hilar-peripheral position. Hilar-peripheral distance was defined as the Euclidean distance between the center of a piece and the ipsilateral hilum. A given slope was considered statistically different from zero if it lay outside 95% confidence intervals generated with a permutation test, as previously described (16).

Spatial correlation. The tendency for neighboring lung regions to have similar albumin accumulation rates was quantified by calculating the correlation between albumin accumulation rates in adjacent tissue samples (18). Briefly, pairs of tissue samples in the same lobe that were 1.2 cm apart (i.e., samples that were nearest neighbors) were identified, and the correlation between albumin accumulation rates from paired tissue samples was calculated. Spatial correlation was considered significantly different than zero if it fell outside 95% confidence intervals for a random spatial distribution that was calculated using a permutation test (16).

RESULTS

Physiological data. Endotoxemia significantly increased pulmonary arterial pressure and systemic arterial pressure and caused trends toward decreased arterial $P_{O_2}$ and cardiac output compared with the control group (Fig. 1). Pulmonary arterial, systemic arterial, and pulmonary arterial occlusion pressures and body temperature increased, and cardiac output,
arterial pH, and $P_{O_2}$ decreased, over time in endotoxemic pigs. In contrast, only cardiac output changed significantly over time in the control group and did not change significantly after the first hour.

**Pulmonary perfusion heterogeneity.** Pulmonary perfusion was spatially heterogeneous in endotoxemic and control pigs (Fig. 2, Table 1). Regional perfusion varied two- to threefold in the control lungs, consistent with previous reports in uninjured lungs (18). However, endotoxemia increased the fractions of lung that were poorly and well perfused, relative to the control group (Fig. 2).

**Spatial distribution of extravascular albumin accumulation.** Extravascular accumulation of radiolabeled albumin in the lung was spatially heterogeneous in the endotoxemic and control pigs (Fig. 3, Table 1). However, there was no correlation between regional extravascular albumin accumulation and regional pulmonary perfusion in either group (Fig. 4, Table 1). Albumin accumulation rates varied by an average of 5% because of counting statistic error and 7% (mean standard deviation: 0.008 μl·min$^{-1}$·ml lung$^{-1}$) because of gamma-counter error and, therefore, cannot explain regional differences in albumin accumulation observed in this study.

Variability in regional extravascular albumin accumulation was not randomly distributed in the lung. Adjacent lung regions had similar rates of albumin accumulation (i.e., showed positive spatial correlation; Fig. 5, Table 2) and rates were greater in dorsal (non-dependent) compared with ventral lung regions (Fig. 6, Table 2). There were no significant differences between cranial and caudal regions or between hilar and peripheral regions.

Spatial correlation and ventral-dorsal gradients were less impressive in endotoxemic pigs. Endotoxemia significantly decreased spatial correlation compared with the control group, and endotoxemic pigs with the highest albumin accumulation rates showed the weakest spatial correlation (Table 2). Endotoxemic pigs also exhibited a dorsal predominance of albumin accumulation less consistently (5 of 8 pigs) than did control pigs (4 of 4 pigs) (Table 2) and a trend toward less steep ventral-dorsal gradients ($P = 0.14$ for difference from zero).

The distribution of albumin accumulation rates was skewed to the right and constrained at the origin (Fig. 3). A fraction of tissue samples ($3 \pm 2\%$) had albumin accumulation rates less than zero, but two-thirds of these were close enough to the origin (i.e., within 0.016
muL.min^-1.ml^-1) to be explained by counting statistic error. The remaining tissue samples with albumin accumulation rates less than zero were not unique in terms of their location in the lung, physical appearance, or ¹²⁵I and ¹³¹I signals. Two pigs accounted for the majority of these samples, and most pigs had only zero or one such sample.

Variability in the response to endotoxin. The physiological response to endotoxin varied widely. Some endotoxemic pigs had little or no increase in extravascular albumin accumulation, whereas others had threefold increases relative to controls (Table 1). On average, the mean albumin accumulation rate was twofold greater in endotoxemic than control pigs, a difference that approached statistical significance (Table 1).

Variability in the response to endotoxin was mirrored by variability in the pulmonary vascular and gas-exchange response to endotoxin. Pulmonary arterial pressure (r = 0.69) and arterial PO2 (r = -0.73) at 3 h were associated with the mean albumin accumulation rate, suggesting that variability in albumin accumulation rates between pigs reflected biological variability in the response to endotoxin. In contrast, mean albumin accumulation rate was not correlated with the number of tissue samples per pig (r = 0.25) or difference between unbound ¹³¹I and ¹²⁵I (r = -0.01), suggesting that differences in lung expansion and unbound radioiodine do not explain variability in albumin accumulation rates between pigs.

**DISCUSSION**

We have demonstrated regional differences in the accumulation of extravascular albumin in endotoxemic and control pig lungs that are not explained by regional differences in perfusion. Variability in regional albumin accumulation is not randomly distributed in the lung but rather is spatially correlated and increased in dorsal (nondependent) lung regions. This spatial organization is present but decreased during endotoxemia.
than 131I or more rapid extravascular accumulation of 

that differences in unbound131I and 125I, which rapidly 

small and similar in magnitude, making it unlikely 

control pig. Each cube (n = 257) represents a single ~0.7-ml tissue 

sample. Samples are color coded according to their albumin accumu-

lation rate and are shown in their anatomic position in the lung. Note 

the slight dorsal predominance of albumin accumulation, variability 

that is independent of ventral-dorsal position, and tendency for 

nearest neighbors to have similar albumin accumulation rates (spa-

tial correlation = 0.73). Clustering of similar albumin accumulation 

rates in neighboring lung regions was less evident in endotoxemic 

lungs, and the dorsal predominance was less consistently seen (not 

shown).

Methodological error. Experimental error is unlikely 

to account for the observed variability in regional al-

bumin accumulation between tissue samples. The 

error estimated by simulating counting statistic error or 

by repeating experimental measurements was small 

compared with observed variability in albumin accumu-

lation rates. Fractions of unbound 131I and 125I were 

small and similar in magnitude, making it unlikely 

that differences in unbound 131I and 125I, which rapidly 

enter the extravascular space, impacted measure-

ments of extravascular albumin accumulation.

The majority of tissue samples with albumin accu-

mulation rates less than zero were very close to zero 

and were, therefore, consistent with error due to count-

ing statistics. Negative albumin accumulation rates 

that were not explained by counting statistic error may 

have been due to a greater fraction of unbound 125I 

than 131I or more rapid extravascular accumulation of 

125I-HSA than 131I-HSA (violating the assumption that 

L2 in Eqs. 1 and 2 are identical).

Determinants of regional extravascular albumin ac-

cumulation. Regional extravascular albumin accumu-

lation is determined by the net effects of albumin 

movement from the vasculature into the interstitium 

and from the interstitium into adjacent interstitial 

and lymphatic spaces. Transvascular albumin movement is 

initially determined by diffusive and convective mech-

anisms, including endothelial permeability, microvas-

cular surface area, transendothelial water flux (itself 

depending on Starling forces), and the solvent-drag 

reflection coefficient for albumin. Once vascular-inter-

stitial equilibrium has been reached, however, ex-

travascular albumin content is determined by the 

plasma albumin concentration and the interstitial vol-

ume accessible to albumin.

To directly assess the contribution of transvascular 

albumin flux, interstitial volume, and lymph albumin 

clearance to extravascular albumin accumulation, in-

terstitial albumin concentration and lymph drainage 

would have to be measured in each tissue sample. 

Although measurement of total lung lymph flow has 

been made in some species (8, 9, 26, 27), measurements 

of regional lymph flow are not possible. Although re-

gional interstitial volume could have been measured 

with a molecular tracer such as diethylenetriamine 

pentaacetic acid, the interstitial volume accessible to 

albumin would still be unknown.

Inference from published data suggests that trans-

vascular albumin flux is more important than lymph 

drainage or interstitial volume in determining ex-

travascular albumin accumulation during the 1-h cir-

culation time for 131I-HSA in this study. Interstitial 

volume determines extravascular albumin accumula-

tion only as plasma-interstitial equilibrium is ap-

proached. However, studies in dogs (26, 28) and sheep 

(33) suggest that it takes >1 h for the vascular and 

interstitial spaces to reach equilibrium [half-time >2 h 

for normal and 0.7 h for abnormal lungs (26, 28, 33)]. 

Because the interstitial concentration [and, therefore, 

lymph concentration (34)] of 131I-HSA is small shortly 

after injection of 131I-HSA, lymph clearance of 131I-

HSA is also unlikely to have had a major influence on 

extravascular albumin accumulation. In addition, 

lymph drainage of radiolabeled albumin toward the 

hila should have resulted in a hilar-peripheral gradi-

ent in albumin accumulation, but such a gradient was 

not observed. For these reasons, we believe that re-

gional extravascular albumin accumulation primarily 

reflects local transvascular albumin flux, with smaller 

contributions from lymph albumin clearance and re-

gional interstitial volume.

Relationship between pulmonary perfusion and re-

gional albumin accumulation. Endotoxemia increased 

the number of lung regions that received either high or 

low levels of perfusion compared with perfusion in 

uninjured lungs (Fig. 2). The increase in poorly per-

fused regions is consistent with endotoxin’s effects on 

microvascular dysregulation observed in other studies 

(13, 21, 24) and may, in part, explain organ dysfunction

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Spatial distribution of albumin accumulation rates

<table>
<thead>
<tr>
<th>Group</th>
<th>Pig No.</th>
<th>Spatial Correlation</th>
<th>Cranial-caudal</th>
<th>Ventrail-dorsal</th>
<th>Hilar-peripheral</th>
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<tr>
<td>Endotoxin</td>
<td>1</td>
<td>0.67±</td>
<td>-0.0045±(2.4)</td>
<td>0.013±(8.5)</td>
<td>0.002±(1.3)</td>
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<td>2</td>
<td>0.43±</td>
<td>-0.0002±(1.1)</td>
<td>0.007±(3.4)</td>
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<td>3</td>
<td>0.33±</td>
<td>0.003±(1.4)</td>
<td>-0.010±(1.6)</td>
<td>-0.003(1.2)</td>
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<td>4</td>
<td>0.81±</td>
<td>0.001±(1.8)</td>
<td>-0.004±(1.7)</td>
<td>-0.001(1.2)</td>
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<td>5</td>
<td>0.49±</td>
<td>0.002±(2.4)</td>
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<td>-0.002±(1.7)</td>
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<td>6</td>
<td>0.21±</td>
<td>-0.0005±(2.6)</td>
<td>0.015±(3.0)</td>
<td>0.011±(2.1)</td>
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<td>7</td>
<td>0.41±</td>
<td>-0.00039±(2.6)</td>
<td>0.013±(5.3)</td>
<td>0.007±(2.8)</td>
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<td></td>
<td>8</td>
<td>0.23±</td>
<td>-0.00062±(1.7)</td>
<td>0.009±(1.5)</td>
<td>0.001(1.1)</td>
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<tr>
<td>Mean ± SD</td>
<td></td>
<td>0.45±0.21</td>
<td>-0.002±0.0044</td>
<td>0.006±0.009</td>
<td>0.002±0.005</td>
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<td></td>
<td>P</td>
<td>0.001</td>
<td>0.25</td>
<td>0.13</td>
<td>0.35</td>
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<td>Control</td>
<td>1</td>
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<td>0.000±(1.0)</td>
<td>0.006±(3.3)</td>
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<td>Mean ± SD</td>
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<td>0.70±0.08±</td>
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<td>0.006±0.003</td>
<td>0.001±0.005</td>
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<tr>
<td></td>
<td>P</td>
<td>0.001</td>
<td>0.15</td>
<td>0.02</td>
<td>0.83</td>
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</table>

Values in parentheses adjacent to slopes indicate the fold-change in albumin accumulation rates between extremes of the lung. P, P value for comparison of mean with zero.  *Significantly greater than zero using permutation test. †Significantly less than zero using permutation test. §Significant difference in mean correlation between control and endotoxin groups, P = 0.046.

Lung albumin accumulation is spatially heterogeneous even when total perfusion to an organ is preserved. These changes in perfusion heterogeneity during endotoxemia potentially magnify the effect of regional perfusion on local transvascular albumin flux.

Although regional differences in pulmonary perfusion may be associated with local differences in endothelial shear stress (11), vascular pressures (36), neutrophil sequestration (23, 37), and perfused vascular surface area, our data argue that regional differences in pulmonary perfusion are not associated with local accumulation of extravascular albumin. These data represent the first comparison of regional pulmonary perfusion and extravascular protein accumulation but are consistent with previous data showing no association between regional extravascular lung water and regional pulmonary perfusion in normal and edematous dog lungs (32). Because the spatial resolution of our methods is limited, we cannot exclude an association between regional perfusion and protein accumulation at the capillary level.

Possible causes of regional differences in lung albumin accumulation. The spatial distribution of extravascular albumin accumulation observed in this study is in agreement with previous data (2) demonstrating spatial heterogeneity in lymph and, therefore, interstitial albumin concentration (34). Albertine et al. (2) measured regional lymph albumin concentration in sheep lung and found increased concentrations in nondependent regions. Because extravascular lung water does not vary with vertical position in the lung (3, 14), increased albumin concentration measured by Albertine et al. (2) is consistent with increased albumin content observed in this study. Albertine et al. also reported considerable variability in albumin concentration in lung regions with the same vertical position, consistent with the variability that we observed in isogravitational lung (Figs. 5 and 6).

Any explanation for regional differences in albumin accumulation must account for the vertical gradient, isogravitational heterogeneity, and positive spatial correlation in regional albumin accumulation. The nondependent predominance of lymph and interstitial albumin has been explained by the presence of a vertical, gravitationally mediated vascular pressure gradient (35) in the absence of a vertical interstitial pressure gradient (6). The resulting vertical gradient in microvascular driving pressures increases transvascular flux of water out of proportion to protein flux in dependent regions, thus increasing regional lymph flow and “washing out” interstitial albumin (7). This mechanism decreases interstitial albumin in dependent regions if equilibrium has already been established (7) and slows albumin accumulation in dependent regions if equilibrium has not yet been reached.

We speculate that regional differences in microvascular driving pressure explain variability within iso-
gravitational lung regions and positive spatial correlation, as well as the vertical gradient in albumin accumulation. Recent observations suggest that vascular geometry plays an important role in determining the spatial heterogeneity of pulmonary perfusion (4, 19), including heterogeneity within isogravitational regions (19) and positive spatial correlation (18). We hypothesize that regional differences in vascular geometry (e.g., differences in regional vascular diameters, branching angles, vessel lengths, etc.) also cause heterogeneity in regional microvascular pressures, including heterogeneity within isogravitational regions. Because neighboring lung regions share a similar heritage in the vascular tree, regional microvascular pressures resulting from local differences in vascular geometry are more alike in adjacent than distant lung regions and, therefore, show positive spatial correlation. Thus the theoretical relationship between microvascular pressure and interstitial albumin proposed by Blake and Staub (7) and the hypothesis that vascular geometry, as well as gravity, determines local microvascular pressure together explain the isogravitational heterogeneity, positive spatial correlation, and vertical gradient that we have observed.

Regional differences in microvascular permeability are another plausible explanation for variability in extravascular albumin accumulation. Yoneda (38) postulated the existence of regional differences in endothelial permeability because extravascular lung water was uniformly distributed, despite the gravitational gradient in vascular driving pressures. Seeking structural correlates of endothelial permeability in uninjured lungs, he found the complexity of interendothelial tight junctions, which is associated with endothelial barrier function (12), to be greater in dependent than nondependent lung. If microvascular pressures are determined by both vascular geometry and gravity, and microvascular endothelium develop structural differences in response to local microvascular pressures, then spatial heterogeneity in endothelial structure and function may also explain the isogravitational heterogeneity, positive spatial correlation, and the vertical gradient that we have observed.

If pulmonary perfusion and microvascular pressure are linked because they both depend on vascular resistance, and microvascular pressure determines extravascular albumin accumulation, it seems intuitive that regional perfusion and albumin accumulation should be closely associated. How then do we explain the observed lack of correlation between regional perfusion and extravascular albumin accumulation? Although regional microvascular pressure and perfusion both depend on vascular resistance, they do so in different ways. Local microvascular pressure depends on the relative resistance in upstream (arterial) and downstream (venous) vessels, whereas local perfusion depends on the sum of arterial, venous, and capillary resistances supplying one region relative to this sum in other regions of the lung. For example, two lung regions in which the sums of arterial, venous, and capillary resistances are equal will have identical perfusion. However, if the ratio of arterial-to-venous resistance in the first region exceeds that in the second region, vascular pressure would be lower in the first than in the second capillary bed. Thus the design of the pulmonary circulation does not require a tight association between regional microvascular pressure (and, therefore, extravascular albumin accumulation) and regional perfusion.

Implications. Regional differences in extravascular albumin accumulation may reflect regional differences in the propensity of lung to develop edema and, in turn, develop ventilation-perfusion mismatch, hypoxemia, and ventilator-induced lung injury. Consequently, determining the etiology of these differences may improve the basic understanding of acute lung injury. Although perfusion heterogeneity does not explain regional differences in lung albumin accumulation, other possible mediators should be identified and pursued.

Variability in regional albumin accumulation in the lung provides additional evidence that spatial heterogeneity is fundamental to biological systems and should be considered when overall organ function is assessed. In particular, researchers should exercise caution when attempting to draw conclusions about whole organ function from measurements in a single region.

In conclusion, this study demonstrates that there are spatially organized regional differences in extravascular albumin accumulation in the lung. Although pulmonary perfusion is heterogeneous, regional differences in perfusion do not explain regional variability in extravascular albumin accumulation.

The authors acknowledge the excellent technical assistance of Dowan An, Shen-sheng Wang, and Dr. Susan Bernard. We are indebted to Dr. Kenneth A. Krohn for providing expertise regarding preparation of radiolabeled albumin and for use of his radiochemistry laboratory and to Dr. Michael M. Graham for expert advice in methods development.

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LUNG ALBUMIN ACCUMULATION IS SPATIALLY HETEROGENEOUS