Heterogeneous capillary recruitment among adjoining alveoli

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Baumgartner, William A., Jr., Eric M. Jaryszak, Amanda J. Peterson, Robert G. Presson, Jr., and Wiltz W. Wagner, Jr. Heterogeneous capillary recruitment among adjoining alveoli. J Appl Physiol 95: 469–476, 2003; 10.1152/japplphysiol.01115.2002.—Pulmonary capillaries recruit when microvascular pressure is raised. The details of the relationship between recruitment and pressure, however, are controversial. There are data supporting the idea that recruitment and pressure are correlated, but there is also evidence that recruitment can occur independently of pressure. The present study was designed to determine whether alveolar capillary networks recruit in a variety of ways or whether one model predominates. In isolated, perfused canine lung lobes, fields of six neighboring alveoli were recorded with video microscopy as pulmonary venous pressure was raised from 0 to 40 mmHg in 5-mmHg increments. The largest group of alveoli (42%) recruited gradually. Another group (33%) recruited suddenly (sheet flow). Half of the neighborhoods had at least one alveolus that paradoxically recruited when pressure was increased, even though neighboring alveoli continued to recruit gradually. At pulmonary venous pressures of 40 mmHg, 86% of the alveolar-capillary networks were not fully recruited. We conclude that the pattern of recruitment among neighboring alveoli is complex, is not homogeneous, and may not reach full recruitment, even under extreme pressures.

PULMONARY CAPILLARIES ARE recruited with increasing microvascular pressure. Different investigators, however, have found different patterns of recruitment. With the introduction of the zone model, West et al. (31) showed that pulmonary blood flow increased down a vertical gradient as pulmonary arterial and venous pressures increased relative to alveolar pressure. Glazier and colleagues (6) studied red blood cell distributions in rapidly frozen lungs and showed that there was gradual, homogeneous recruitment as capillary pressure increased, especially in zone 2. Fung and Sobin (5) developed the sheet flow theory, which suggested that the entire capillary bed opened suddenly and completely as capillary transmural pressure exceeded alveolar pressure. This hypothesis was supported experimentally by Sobin et al. (19), who studied latex casts of the capillary bed. Godbey et al. (9) showed in excised lungs that sheet flow tended to occur at low airway pressure when alveoli were not highly distended, whereas gradual recruitment tended to occur in more distended alveoli. Although the zone and sheet flow models predict homogeneous recruitment, Warrell et al. (28), studying rapidly frozen lungs under zone 2 conditions, found an uneven distribution of capillary red blood cells within areas likely to have been supplied by a common arteriole. The scatter in the data of Godbey et al. as capillaries recruited suggests confirmation of the Warrell data, i.e., that capillary recruitment could be heterogeneous. West et al. (32), using an elegant computer model, deduced that recruitment in a network of resistors could be heterogeneous. Nevertheless, the variety of models, each based on credible evidence, does not present a coherent picture of how alveolar capillaries recruit. The present study was designed to determine whether, at a given degree of alveolar inflation, alveolar capillaries recruit in a variety of ways, or whether one model predominates. To investigate these issues, we studied capillary recruitment directly, using video microscopy as capillary transmural pressure was gradually increased under zone 2 conditions into zone 3 conditions.

METHODS

Experimental preparation. In accordance with institutional guidelines, healthy adult male mongrel dogs (20–24 kg, n = 6) were anesthetized by intravenous injection of pentobarbital sodium dissolved in 0.9% saline (30–40 mg/kg). The animals were intubated and ventilated with room air (Harvard Apparatus 607D). After administration of heparin (1,000 U/kg), the animals were rapidly exsanguinated through the left common carotid artery. During exsanguination, the first 120 ml of blood removed were replaced with 120 ml of 10% Dextran 40 (40 kDa) in saline (4). After a left thoracotomy, the left lower lobar pulmonary artery was cannulated with a 6-mm ID Teflon fluorinated ethylene polypropylene cannula. The left lower lobe was then excised, along with a cuff of left atrium, and placed on a microscope stand. The left atrial cuff was secured around another Teflon cannula (10-mm ID), and the lobe was perfused with autologous heparinized whole blood. Care was taken to exclude all air bubbles from the circuit before perfusion was initiated. The time interval to reperfusion was <30 min.

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Blood was pumped (Masterflex 7522-10 pump drive and 7024-20 pump head) through a windkessel to dampen pump vibrations and trap bubbles, a filter (20-μm pore size, Fenwal 4C2423) to remove microaggregates, and a heat exchanger (Bentley HE-100) to warm the blood to 37–38°C before it entered the lobe (Fig. 1). Venous blood drained passively from the lobe into a reservoir. Adjusting the height of the reservoir changed pulmonary venous pressure. The lobe was ventilated (Harvard Apparatus 607D) with a mixture of 6% CO₂-17% O₂-77% N₂. Blood gases were sampled from the pulmonary venous line and analyzed with an Instrumentation Laboratories model 1304 blood-gas analysis machine. Sodium bicarbonate solution (1 meq/ml) was added to the venous reservoir periodically to neutralize metabolic acid. Polyethylene catheters (40 cm long, 1.19 mm ID, 1.70 mm OD) were threaded via the arterial and venous cannulas so that their tips were just within the lobar artery and vein, respectively. These catheters were connected to transducers (Statham P23 XL) that were zeroed at the level of microcirculatory observation. Pulmonary arterial and venous pressures were monitored continuously by use of a personal computer and monitoring software. The lobe was suspended by two small spring-backed paperclips attached to opposite edges of the lobe and was raised until the uppermost pleural surface contacted a 1.3-cm² transparent window. The window was surrounded by a vacuum ring to prevent lateral tissue movement (22). The remainder of the lobar surface was covered with a thin plastic sheet to prevent drying and to slow the transpleural diffusion of gas.

Microcirculatory observations. The subpleural microcirculation under the window was observed with a Leitz Ultropak surface-illuminating microscope (×11 objective) coupled to a 200-W mercury arc lamp. The light was heavily filtered to prevent tissue damage by infrared and ultraviolet light. A narrow band-pass interference filter was used to illuminate the field with the mercury green line (546 nm). This wavelength is absorbed by hemoglobin, thereby increasing the contrast between the erythrocytes and surrounding tissue (23).

Video recordings of the subpleural microcirculation were made with a Sony SVO-5800 SVHS video recorder and a Videoscope (model 200E) charge-coupled-device camera attached to the microscope. The average final magnification of the video recording was ×400. In each preparation, a field was selected for observation that consisted of six neighboring subpleural alveoli in which the capillary segments were clearly visible and were fed by a single arteriole and drained by a single venule.

The pump flow rate was set to perfuse about one-half of the observed capillaries, placing the lobe in zone 2. To increase capillary transmural pressure, pulmonary venous pressure was raised in 5-mmHg increments from 0 to 40 mmHg. Airway pressure was held constant throughout the experiments at 5 mmHg. At a pulmonary venous pressure of 5.0 ± 0.1 mmHg (mean ± SE), the pulmonary arterial pressure was 13.0 ± 0.8 mmHg, producing an arteriovenous pressure difference of 8.0 ± 0.9 mmHg. At the highest pressure when pulmonary venous pressure was 39.9 ± 0.5 mmHg, pulmonary arterial pressure was 42.8 ± 0.4 mmHg, producing an arteriovenous pressure difference of 2.9 ± 0.5 mmHg. The field of six alveoli was recorded for 1 min at each of the nine venous pressures. Ventilation of the lobe was paused and pressures were allowed to stabilize before each recording.

Data analysis. The recordings made during the observation periods were replayed, and two independent observers traced the perfused capillary segments onto separate sheets of transparency film placed over a video monitor. A capillary was defined as a vessel crossing an alveolar wall and perfused only by single red blood cells moving in series. A capillary segment was defined as any capillary between junctions with another vessel or between a junction and the alveolar boundary. A capillary segment was considered to be perfused if one or more red blood cells passed through the segment during the 1-min video recording. A master tracing...
based on agreement between independent observers was made that included all of the capillaries that were perfused during any of the observation periods, and those capillary segments were assigned unique numbers; these tracings represented the maximum observable number of capillaries in each alveolar network.

To compare the levels of capillary recruitment between neighboring alveoli, the capillary perfusion index (1, 2, 11, 13, 17, 24, 25) was computed for each alveolus during every observation period. We measured the length of each perfused capillary from the master tracings with a digitizing pad (Houston Instruments Truegrid 1017), planimetry software (SigmaScan, Jandel Scientific), and a personal computer. The areas of the observed alveolar walls were also measured by using the same system. From these measurements, the capillary perfusion index during each observation period was calculated as

\[
\text{Capillary perfusion index (} \mu\text{m}) = \frac{\sum \text{perfused capillary lengths (} \mu\text{m})}{\text{alveolar wall area (} \mu\text{m}^2)/10^4 (} \mu\text{m}^2) \times \text{perfused capillary lengths (} \mu\text{m})}
\]

Because subpleural alveolar facets in the upper lung can be approximated by flat discs with an average diameter of 110 \(\mu\text{m}\) and an area of 10,000 \(\mu\text{m}^2\) (22), the alveolar wall area was divided by 10,000 \(\mu\text{m}^2\) to obtain the number of average walls in the observed alveolar facet. This normalization permitted us to compare results between individual alveoli and between animals. Dividing the total length of perfused capillaries by the normalized alveolar area indicated how many times perfused capillaries crossed an average alveolar wall at its diameter. The level of capillary recruitment, therefore, can be estimated from the capillary perfusion index. For example, a capillary perfusion index of 110 \(\mu\text{m}\) can be visualized as a capillary path length that would cross the 110-\(\mu\text{m}\) diameter of an average alveolar facet once. To compare alveoli, the capillary perfusion index for each observation period was converted into a percentage of maximum capillary perfusion index, which was calculated as the capillary perfusion index when all observed capillaries from the master tracing were perfused.

RESULTS

On average, at the alveolar pressure used in this study, alveolar capillary networks recruited gradually as pulmonary venous pressure was increased (Fig. 2). However, the recruitment pattern among neighboring alveoli was heterogeneous. Four different variations in the pattern of recruitment were observed with increasing venous pressure, i.e., capillary transmural pressure: 1) gradual recruitment, 2) recruitment in a steplike manner, 3) a stable level of recruitment without change, and 4) derecruitment.

Gradual recruitment. Capillaries in 15 of 36 alveoli (42%) recruited gradually (Fig. 3, alveolus B and Fig. 4). This pattern was similar to the average response of all subpleural alveoli to increasing capillary transmural pressure at this inflation pressure (Fig. 2). The gradual recruitment response plateaued at a pulmonary venous pressure of 25–30 mmHg.

Sudden recruitment (sheet flow). In 12 of 36 alveoli (33%), the capillary network recruited suddenly, like a sheet, with increasing capillary transmural pressure (Fig. 3, alveolus A and Fig. 5). Of these rapidly recruiting alveoli, 67% recruited at the 5–10 mmHg pressure step, the range at which alveolar pressure was exceeded. Of the remaining four alveoli that recruited in this manner, three did so at the 10–15 mmHg pressure step, somewhat above alveolar pressure.

Stable recruitment. The level of capillary recruitment of six alveoli (17%) remained relatively unchanged over the course of incremental increases in pulmonary venous pressure. In each case, as in the example shown in Fig. 6, alveolus A and Fig. 7, the alveolus was highly recruited at a venous pressure of 0 mmHg, indicating that the opening pressure for most segments in these particular alveoli was low.

Derecruitment. Of the six dogs studied, three had one alveolus that paradoxically derecruited >20% in response to increased pulmonary venous pressure, while the neighborhood of alveoli continued to recruit (Fig. 3, alveolus C and Fig. 8).

Recruitment not reaching a limit. Capillary recruitment was not complete at pressures of 40 mmHg in 86% of the alveoli studied, a surprising finding. The maximum capillary perfusion index, as determined by the accumulation of all of the capillaries that were perfused at some point during the experiment, was 456 ± 19 \(\mu\text{m}\) (mean ± SE), whereas the capillary perfusion index at 40 mmHg was 378 ± 17 \(\mu\text{m}\), a difference of 15% (\(P < 0.05\) by two-tailed \(t\)-test).

Blood gases were in the normal range for this type of preparation. Measurements made at the beginning were not different from those made at the end of the study (Table 1).

DISCUSSION

We studied the patterns of recruitment of neighboring subpleural alveolar networks as capillary transmural pressure was raised, changing the perfusion conditions from zone 2 to zone 3. Although the average recruitment pattern was gradual, the recruitment patterns of individual networks were heterogeneous. With increasing capillary transmural pressure, some alveoli recruited gradually, some recruited suddenly as in sheet flow, some were fully recruited at low pressure
and did not recruit further, and some derecruited. A large majority of alveoli were not fully recruited, even at very high transmural pressures.

Several issues need to be considered in interpreting these data. First, we assumed that subpleural capillaries recruit and derecruit the same way as capillaries in the rest of the lung. The subpleural capillary network is less dense than interior networks (10, 14, 21) and therefore might not recruit in the same way as interior capillaries. However, Short et al. (18) demonstrated that recruitment in subpleural capillaries accurately reflected recruitment in interior capillaries, indicating that our observations of recruitment patterns represent the recruitment patterns of the lung as a whole.

Second, we assumed stable, equivalent perfusion conditions for every alveolus at each pressure step. If that were not the case, each alveolus would be responding to independent hemodynamic conditions. We assumed equivalent conditions because 1) the adjacent alveoli were in close proximity; 2) the neighborhoods of alveoli, on the basis of red blood cell flow patterns, appeared to have been fed by a single arteriole and drained by a single venule; 3) ventilation was suspended during the recording, which maintained con-

![Fig. 3. Neighborhood of 6 alveoli with perfused capillary segments drawn at each of 9 venous pressures ($P_v$) in 5-mmHg increments from 0 to 40 mmHg. Alveolus A, step recruitments; alveolus B, gradual recruitment; alveolus C, derecruitment.](image)

![Fig. 4. Capillary perfusion index as percentage of maximum at each 5-mmHg increment from 0 to 40 mmHg venous pressure for alveolus B, Fig. 3, the gradually recruiting alveolus.](image)

![Fig. 5. Capillary perfusion index as percentage of maximum at each 5-mmHg increment from 0 to 40 mmHg venous pressure for alveolus A, Fig. 3, the rapidly (step) recruiting alveolus.](image)
stant alveolar pressure; and 4) the arterial-venous pressure gradient narrowed to low levels as pulmonary venous pressure was raised (<3 mmHg at a flow rate of 250 ml/min at the highest pressure). This combination of conditions would be expected to produce uniform capillary transmural pressures across the observed field. It should be pointed out, however, that once red blood cells enter each capillary bed and begin their transit across the complex series of alveolar capillary networks toward the draining venule, holding inlet and outlet pressures constant does not assure that subtle pressure gradients will not be continually altering among individual capillary segments. In fact, there is substantial evidence that continual switching does occur among capillaries during steady perfusion conditions, which suggests that intranetwork gradients do alter rapidly (27).

Third, we assumed reproducibility of recruitment throughout the experiment. We verified this in three additional preparations in which pulmonary venous pressure was raised in 5-mmHg steps from 0 to 25 mmHg and then lowered back to 0 mmHg. Similar levels of recruitment were measured at each pressure step whether raising or lowering pulmonary venous pressure \( P = 0.66 \). These experiments showed that there was neither hysteresis in the pressure-recruitment relationship nor any effect caused by differing flow histories among the alveoli in our preparation. This is consistent with the study of Presson et al. (17), who found that capillary opening pressures were sta-

Fig. 6. Neighborhood of 6 alveoli with perfused capillary segments drawn at each of 9 venous pressures in 5-mmHg increments from 0 to 40 mmHg. Alveolus A, steady levels of recruitment; alveolus B, gradual recruitment. Arrow highlights a capillary segment in which perfusion turns on and off.

Fig. 7. Capillary perfusion index as percentage of maximum at each 5-mmHg increment from 0 to 40 mmHg venous pressure for alveolus A, Fig. 6, a stable alveolus.

Fig. 8. Capillary perfusion index as percentage of maximum at each 5-mmHg increment from 0 to 40 mmHg venous pressure for alveolus C, Fig. 3, a derecruiting alveolus.
Table 1. Arterial blood gas variables at beginning and end of experimental observations

<table>
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<th>Beginning</th>
<th>End</th>
<th>P</th>
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<tbody>
<tr>
<td>PaO2, Torr</td>
<td>121 ± 8</td>
<td>115 ± 10</td>
<td>0.44</td>
</tr>
<tr>
<td>PaCO2, Torr</td>
<td>33 ± 3</td>
<td>33 ± 2</td>
<td>0.54</td>
</tr>
<tr>
<td>pH</td>
<td>7.37 ± 0.02</td>
<td>7.38 ± 0.01</td>
<td>0.82</td>
</tr>
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Values are means ± SE; n = 6 dogs; PaO2, arterial PO2, PaCO2, arterial PCO2, pH, arterial pH. P determined by paired, two-tailed t-test.

There were, however, elements (capillary segments) that derecruited as pressure was raised. Flow in these elements depended on the gradient between the feeder and drainer capillaries. In a complex network, however, pressure gradients between feeders and drainers could fall to zero as elements in other parts of the network are recruited, which would cause that element to close (derecruit). In other circumstances, the pressure in the drainer could exceed the feeder pressure, thus reversing the direction of flow, as predicted by West et al. (32) and observed in these experiments.

By analogy, each alveolus in the midst of an array of other alveoli has to be fed and drained by other alveoli. This is the result of blood having to cross an average of a half-dozen alveolar walls to travel from the feeding arteriole to the draining venule (20). We never observed reversal of flow in an entire alveolar network, but we did find examples of flow stopping completely in some alveoli as pressure was raised (Fig. 3, alveolus C). There were a number of examples of single alveoli derecruiting by 20–40% during a 5-mmHg pressure rise. This observation can be accounted for by alterations of the pressure gradient between feeding networks and draining alveolar networks, analogous to the alterations in flow in single capillary segments.

The third pattern observed for some alveoli was gradual recruitment as pressure was raised until a plateau was reached. This finding supports the observations of Glazier et al. (6) studying rapidly frozen lungs and studies from our own laboratory using in vivo microscopy (3, 24–26). This pattern of gradual recruitment reflected the average pattern for all of the alveoli (Fig. 2). It is an intuitively attractive pattern; i.e., if there is a range of opening pressures in the capillary network, then capillaries will be recruited as their individual opening pressures are exceeded, and then a plateau will be approached as the maximum number of capillaries is approached.

The fourth observed pattern in this study was capillary recruitment occurring suddenly as capillary transmural pressure exceeded alveolar pressure, consistent with the idea of sheet flow described by Fung, Sobin, and colleagues (5, 19). Godbey et al. (9) showed that, as capillary transmural pressure exceeded alveolar pressure, the capillary networks tended to recruit as a sheet when alveoli were relatively undistended. In more distended alveoli, the average recruitment was gradual as capillary pressure was raised. These observations resolved the lengthy conflict between sheet flow vs. gradual recruitment: the type of recruitment depended on the degree of distension of the alveoli. Of course, if the inflation pressure in the present study was set at a higher or lower value, the prevalence among the patterns would likely alter. We chose an inflation pressure of 5 mmHg and set the pump rate to perfuse about half of the capillaries when venous pressure was zero at the level of the observed capillaries. This placed the network at the boundary of zones 2 and 3 and provided us with the opportunity to study the pattern of recruitment as the network was exposed to increasing transmural pressure.
This concept of sheet flow in undistended alveoli and gradual recruitment in distended alveoli appears to be confounded by the present study, which shows that one alveolus may have recruited as a sheet while its next-door neighbor recruited gradually, even though the alveolar pressure was constant throughout the lobe. A potential way to resolve this conflict came from the study of Glazier and colleagues (7), who measured the vertical distribution of alveolar diameters in rapidly frozen lungs. In the lungs frozen at a positive pressure of 5 cmH₂O [Fig. 6 in their publication (7)], the curve for the vertical distribution of alveolar volumes closely approximated the distribution for normal lungs. The SD for alveolar volumes in the upper lung was five times as large as the SD for the lower lung, i.e., the range of alveolar sizes was much greater in the upper lung. Because our lungs were inflated to approximately the same degree (5 mmHg) and our observations were made of the upper lung, we thought our alveolar diameters might be heterogeneous as well. That was the case: our average alveolus was 111.3 μm in diameter with a large SD of 27.5 μm. From these data, we deduce that in the upper lung a given alveolus, embedded in an array of many other alveoli, is not likely to be distended to the same extent as its immediate neighbors, even though the alveolar pressure is the same between neighbors. If that is the case for our modestly inflated lung lobes, we reason that neighboring alveoli will recruit differently according to the individual degree of distension: some will recruit as a sheet, whereas more distended neighbors will recruit gradually as capillary pressure is raised.

Our observations also support the idea of the apparently “limitless” capillary reserve as described by Hsia et al. (12), or at least a limit not reachable even under extreme physiological conditions. They found that, after right pneumonectomy, the physiological reserve of a canine lung was not exceeded, even during peak exercise. In their study, diffusing capacity of carbon monoxide, pulmonary blood flow, and capillary blood volume continued to increase without evidence of reaching an upper limit, even at a cardiac output equivalent to 34 l/min. In our experiments, 86% of the alveolar capillary networks observed were not fully recruited at a pulmonary venous pressure of 40 mmHg.

We were surprised by the variability of the recruitment response among neighboring alveoli. This finding adds to the growing list of complex characteristics of the pulmonary circulation: the fractal character of the branching pattern of the vessels (30) and the resultant heterogeneous distribution of pulmonary blood flow (8); the fractal patterns of perfusion of individual capillary networks, which lead to independence of adjoining alveoli creating a robust design (27); and, in this study, the heterogeneity in recruitment shown by neighboring alveoli in response to increasing capillary transmural pressure. The complicated patterns we have observed in subpleural alveolar walls must be unimpressive compared with recruitment patterns in the interior of the lung, because our observations are limited to a two-dimensional, flat surface perfused through a relatively coarse network. In the interior, arterioles spray into a series of alveolar walls spread over three dimensions and comprised of doubly dense, partially fused networks (10), a considerably more complicated arrangement. Finally, we have not considered whether there are active components, such as locally released vasoactive factors, in response to increased shear or vessel wall stretch; if true, these add another layer of complexity, yet to be explored. One conclusion from this accumulation of data is that the ubiquitous one-airway, one-alveolus, one-capillary model of the lung, although so useful for teaching, is far removed from the complex reality of pulmonary perfusion on a microscopic level.

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DISCLOSURES

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