Chronic airway infection leads to angiogenesis in the pulmonary circulation

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Hopkins, Natalie, Elaıne Cadogam, Shay Giles, and Paul McLoughlın. Chronic airway infection leads to angiogenesis in the pulmonary circulation. J Appl Physiol 91: 919–928, 2001.—In both pulmonary and systemic hypertension, the walls of the arteriolar vessels are thickened and the lumen size is reduced, leading to increased total vascular resistance. It has been reported previously that chronic airway infection and inflammation lead to increased wall thickness in the pulmonary vasculature, without the development of pulmonary hypertension. The aim of the present study was to examine quantitatively the remodeling of intra-acinar blood vessels in chronically infected rat lungs. Adult rats were anesthetized and inoculated intratracheally with Pseudomonas aeruginosa (n = 10) incorporated into agar beads to induce chronic airway infection. Control groups included rats inoculated with sterile agar beads (n = 8) and rats that were not inoculated (n = 6). Chronic infection caused vascular wall thickening without reduction in mean lumen radius. Furthermore, chronic infection led to increased total length of intra-acinar vessels and increased numbers of branch points, demonstrating that angiogenesis had occurred. Preservation of lumen size and formation of new parallel pathways in the vasculature of chronically infected lungs account for the maintenance of normal PVR despite vessel wall remodeling.

Pseudomonas aeruginosa; isolated-perfused lungs; stereology; pulmonary hypertension

CHRONIC AIRWAY INFECTION and inflammation lead to remodeling of the pulmonary vasculature characterized by an increased ratio of wall thickness to lumen diameter (2, 3, 7, 8, 26). This change in structure is similar to that seen in pulmonary and systemic hypertension, and it is commonly suggested that the thickened vessel walls encroach on the vessel lumen, thus increasing vascular resistance. However, we and others have reported that, in chronically infected lungs in rats, pulmonary hypertension did not occur and right ventricular hypertrophy was not observed, despite the development of thickened vessel walls (2, 8). In a similar observation in human subjects with chronic obstructive pulmonary disease, Wright et al. (32) have reported marked thickening of the walls of pulmonary arterial vessels in the absence of pulmonary hypertension.

One possible explanation of these findings is that wall thickening occurred in an outward direction so that the size of the vascular lumen was not compromised. This phenomenon, termed compensatory enlargement, has been reported previously in the systemic (7) but not in the pulmonary vasculature. A second possible explanation is that angiogenesis occurs in the pulmonary circulation in response to chronic airway infection leading to an increase in the number of parallel pathways through the lung, thus preventing an increase in PVR. Angiogenesis in response to chronic infection is well documented in the systemic circulation, including the bronchial circulation (4, 5, 18). However, it is standard teaching that angiogenesis does not occur in the adult pulmonary circulation (6, 10, 20).

The aim of the present study was to determine whether chronic airway infection in rat lungs caused angiogenesis or compensatory enlargement of the vessels in the pulmonary circulation. Such changes could account for the absence of pulmonary hypertension in these lungs, despite the development of vascular wall thickening. We used a previously described model to establish chronic airway infection with Pseudomonas aeruginosa in rats (3, 8) and compared the structure of lungs of chronically infected and control animals by using quantitative stereological techniques. In addition, in isolated, ventilated, blood-perfused lungs, we examined baseline pulmonary vascular resistance (PVR) and changes in PVR produced by hypoxia.

METHODS

Infection of Animals

A mucoid Ps. aeruginosa strain, isolated from a patient with cystic fibrosis, was used to prepare the inoculum for all experiments. Chronic infection was produced by incorporating the organism into agarose beads as previously described (2, 3, 8). In brief, a suspension of Ps. aeruginosa grown overnight in peptone water resulted in a concentration of ~3 × 10⁸ colony-forming units/ml. Nineteen milliliters of agarose (2.1% wt/vol) were prepared and maintained at 45°C.

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To this, 1 ml of the peptone broth was added, and the resultant agarose solution was injected into 20 ml of heated (50°C) mineral oil (Sigma Chemical) and mixed vigorously for a further 10 min. The mixture was then cooled rapidly by immersing the beaker in crushed ice while stirring continued, leading to the formation of agar beads. The beads were separated by centrifugation, and residual mineral oil was removed by washing in 5% (wt/vol) sodium deoxycholate solution in PBS (0.1 M) followed by a second wash in a 0.25% (wt/vol) solution and subsequently washed four times in PBS. Finally, the beads were resuspended in an equal volume of PBS for inoculation.

Adult male (300–400 g) specific pathogen-free Sprague-Dawley rats (Harlan, Bicester, UK) were anesthetized (Hypnorm: fentanyl citrate 0.25 mg/kg and fluanisone 0.08 mg/kg, midazolam 2.5 mg/kg sc), and a modified pediatric laryngoscope was used to introduce a polyethylene cannula (1.2-mm outside diameter) into the trachea via the larynx. In the group to be chronically infected, 10^4 colony-forming units of Ps. aeruginosa in agar beads suspended in PBS (total volume 200 µl) were inoculated intratracheally through this cannula in each rat, and the animals were then allowed to recover from anesthesia. A second group of animals (placebo-inoculated group) was anesthetized and inoculated with sterile agarose beads, that is, agar beads prepared in a similar manner except that Pseudomonas organisms were omitted. A third group consisted of animals that were not inoculated (noninoculated group). Isolation of lungs for hemodynamic, histological, and immunohistochemical analyses was carried out 10–15 days postinoculation.

Lung Isolation for Hemodynamic Studies

Rats were anesthetized (60 mg/kg sodium pentobarbitone) and mechanically ventilated (SAR-830P small animal ventilator, CWE, Ardmore, PA) at a tidal volume of 1.8 ml and a frequency of 80 breaths/min. The animals were then anticoagulated (300 IU heparin intravenously) and killed by exsanguination. The thoracic contents were then removed en bloc and suspended in fixative (4% wt/vol paraformaldehyde in PBS). The atria were removed, the right ventricle and septum (LV), and each ventricle was weighed separately.

Measurement of Right Ventricular Weights

The pulmonary circulation was then perfused with calcium-free normal saline at 37°C until the effluent was clear of blood. Lungs were fixed for morphometric examination by the technique of Meyrick and Reid (24). Paraformaldehyde (4% wt/vol) in PBS (300 mosM) at a pressure of 100 cmH2O and a temperature of 37°C was instilled through the pulmonary artery catheter to maximally distend the pulmonary vessels. During fixation, the lungs were simultaneously inflated through the tracheal catheter using the same fixative at a pressure of 25 cmH2O (24). After this, the pulmonary artery and trachea were ligated and the lungs were then stored in fixative until they were embedded in paraffin wax.

Morphometric Measurements

Estimation of lung volume and volumes of pulmonary tissue compartments. The vertical axis of each left lung was identified, and the lung was cut perpendicular to this axis into slices (4 mm thick) with a sharp blade beginning at a position chosen by random number in the first slice. The modal number of slices obtained was 7 (range 6–8). To determine the volume of the lung, an image of the surface area of each slice was obtained by using a JVC KY-F55B color video camera (Eurotek, Dublin, Ireland). The images were digitized and displayed on screen using Adobe Photoshop.
stored in eight-bit (256 level) format, and then imported into Stereology Toolbox (Morphometrix) for determination of surface area by use of a point-counting grid. Lung volume was then calculated by the Cavalieri method (1, 11, 12). The lung slices were cut into bars of tissue and every third bar was selected, beginning at a point identified by randomly choosing a number between one and three. The selected bars were cut into blocks using a vertical uniform random strategy, with cuts made at 2-mm intervals (1). Every fourth block was selected for embedding beginning at a start point identified by randomly choosing a number between one and four. The modal number of blocks selected from a single lung was 12 (range 11–17). The blocks were embedded in wax, and random sections (7 μm thick) were cut. To obtain isotropic uniform random sections, random blocks (one in four, as above) were initially cut in a randomly chosen direction about the vertical axis and then cut in a random direction chosen using a cosine-weighted orientator clock that was projected onto the block face exposed by the initial vertical uniform random cut (22). Sections (7 μm thick) were cut parallel to this direction and randomly selected sections were stained with an elastin stain (Miller’s) and neutral red.

Fields of view from each section were examined by light microscopy (Leica, Laboratory Instruments), captured using the JVC KY-F55B color video camera mounted on the camera port of the microscope. The images were digitized and displayed on screen and imported into StereoToolbox for analysis (see above).

Volume densities of specific tissues in the lung were estimated by point counting, and the absolute volume was calculated using the lung volumes measured by the Cavalieri method (1, 11, 12).

To determine the volumes of the vascular compartments of interest, a cascade approach was used (1, 11, 12). The volume densities of intra- and extra-acinar tissues (including all air-spaces) were initially estimated by point counting. Intra-acinar blood vessels were defined as those associated with respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli, i.e., vessels within the gas exchange region of the lung. The modal number of randomly chosen fields of view examined was 12 (range 11–17), one field from a randomly chosen section from each block. The volume densities of the intra-acinar pulmonary blood vessels excluding capillaries, alveolar walls, and intra-acinar air-spaces per unit volume of intra-acinar tissue were then measured by point counting on random fields of view obtained at higher magnification. The modal number of randomly chosen fields of view examined was 24 per lung (range 22–34), two from each of 12 randomly chosen sections from each block. To estimate the volumes of the vessel lumen, the tunica intima, tunica media, and tunica adventitia within each left lung, images of randomly selected intra-acinar vessels were acquired and placed randomly within point-counting grids. To randomly sample intra-acinar vessels, the following strategy was adopted: a search for vessels was begun at one of the four corners of the section that had been chosen randomly. Contiguous square fields of view were sequentially examined by sweeping across the section horizontally until the opposite edge was reached, then moving the section vertically by a distance equal to that of one field of view, and then returning across the section. This pattern was continued until the five vessels first encountered in each block were examined. The modal number of vessels examined in a lung was 60 (range 55–85). The tunica intima was defined as the internal elastic lamina and the cells of the endothelium internal to it. The tunica media was defined as the external elastic lamina and everything internal to it but external to the internal elastic lamina. The tunica adventitia was defined as the loose connective tissue investing the vessel external to the external elastic lamina.

Estimation of the length of intra-acinar blood vessels. To estimate the length density of the intra-acinar pulmonary blood vessels of each lung, the number of intra-acinar blood vessels, which transected counting frames randomly superimposed on isotropic uniform random sections, was counted (1, 11, 12). The modal number of fields of view examined in a lung was 24 (range 22–34).

Estimation of number of branch points of intra-acinar pulmonary blood vessels. The number of branch points of intra-acinar pulmonary blood vessels was determined using the physical double dissector (1, 11, 12). A branch point was defined as where two immediately adjacent vessels shared a common wall (Fig. 1). Vertically aligned images separated by 14 μm were examined by using a systematic random sampling strategy. The modal number of dissector pairs examined in a lung was 72 (range 66–102).

The number of fields of view from each lung that were examined differed for estimating volume, length, and numerical densities of the specific compartments of interest, but for all parameters equal numbers of fields of view from each block were examined. The counting strategies (number of tissue blocks and fields of view) were devised in preliminary studies so that the number of counts of the item of interest (point hits on a specific compartment, vessel intersections or counts on dissector pairs) was never <100–200 in any single lung. Increasing the number of counts above this range does not significantly improve the precision of the estimate obtained (11–13, 31). This aim was successfully achieved in all estimates of volume, numerical, and length densities except that of the media of intra-acinar vessels in control lungs. In the case of the volume density of the media of intra-acinar vessels, point counts in the chronically infected lungs exceeded 600 in all lungs (mean number 983 ± 67) but were <100 in all lungs of both control groups (mean number 24 ± 7). However, because the differences between the groups...
were large in this particular case, adequate precision of the estimates was obtained.

Calculation of vessel radius and the ratio of blood vessel wall thickness to vessel radius. The vascular smooth muscle was fully relaxed by perfusion with calcium-free solution and then fixed in a fully distended state by infusion of fixative at high pressure, as already described. Thus the vessels were assumed to be cylindrical in shape, and the radius of the vessel was calculated from the standard formula for the volume of a cylinder and the known volume of the intracinar vessels (volume-derived radius). The ratio of the intracinar blood vessel wall thickness to the radius of the lumen was calculated as

\[ \text{WT/vessel radius} = \left( \frac{[V(\text{media}) + V(\text{intima}) + V(\text{lumen})]^{0.5}}{V(\text{lumen})} \right)^{0.5} \]

where WT is the blood vessel wall thickness measured from the innermost aspect of the tunica intima to the outside of the external elastic lamina.

Data Analysis

Values are expressed as means ± SE. Volumes of specific tissue compartments, vessel lengths, and numbers of branch points in the left lung are reported per 100 g body wt. Statistical comparisons of means were made using ANOVA, and, when this indicated significant differences between groups, the Student-Newman-Keuls post hoc test was used to assess the significance of the differences between specific means. Where appropriate (total and differential cell counts), data were log transformed before ANOVA. For clarity, the untransformed values are presented in the text. A value of \( P < 0.05 \) was accepted as statistically significant.

RESULTS

There were no significant differences in mean body weight and mean right ventricular weight between the three groups of animals under study (Table 1). Mucoid colonies of \( \text{Ps. aeruginosa} \) were grown on blood agar plates from the BAL fluid obtained from each chronically infected lung. Organisms were not isolated from either the noninoculated or the placebo-inoculated lungs. Differential cell counts showed that the mean percentage of neutrophils and lymphocytes in the \( \text{Pseudomonas}- \)infected lungs was significantly elevated above that in the other two groups (Table 2). The mean total cell count in the BAL fluid from \( \text{Pseudomonas}- \)infected lungs was significantly greater than that in the noninoculated and placebo-inoculated groups (Table 2). Mean baseline perfusion pressure and the mean hypoxic vasoconstrictor response were similar in the three groups of isolated lungs (Table 3).

Lung Histology

Lungs from noninoculated animals showed normal alveolar structure with no evidence of inflammatory cell infiltration (Fig. 2A). In contrast, the \( \text{Pseudomonas} \)-inoculated group showed evidence of extensive regions of chronic inflammation with extensive thickening of the alveolar walls due to the infiltration of inflammatory cells (Fig. 2B). Markedly increased numbers of inflammatory cells were observed in the alveolar walls of the \( \text{Pseudomonas} \)-inoculated group (Fig. 2, B and E). However, these inflammatory changes were not homogeneously distributed throughout the lung, so that some areas within an infected lung were relatively normal in structure (Fig. 2C), whereas others showed extensive inflammatory changes (Fig. 2, B and E). The placebo-inoculated group had normal alveolar structure without evidence of inflammation (Fig. 2D).

In the noninoculated group, the vessel wall of intracinar pulmonary blood vessels generally contained a single elastic lamina (Fig. 3A), although occasional vessels were seen with a double elastic lamina. In contrast, the \( \text{Pseudomonas} \)-inoculated group showed frequent thickening of the tunica media, with internal and external elastic laminae (Fig. 3B). The extent of the remodeling varied throughout the lungs with some vessels showing lesser medial thickening and still

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**Table 1.** Body weight, RV weight, RV/LV ratio, and hematocrit in noninoculated, \( \text{Pseudomonas} \)-inoculated, and placebo-inoculated animals

<table>
<thead>
<tr>
<th></th>
<th>Non-inoculated (n = 8)</th>
<th>Pseudomonas-Inoculated (n = 10)</th>
<th>Placebo-Inoculated (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>362 ± 15.0</td>
<td>348 ± 8.0</td>
<td>369 ± 15.0</td>
</tr>
<tr>
<td>RV weight, mg/100 g</td>
<td>48.0 ± 4.0</td>
<td>51.0 ± 7.0</td>
<td>51.0 ± 6.9</td>
</tr>
<tr>
<td>body wt</td>
<td>0.16 ± 0.01</td>
<td>0.18 ± 0.03</td>
<td>0.18 ± 0.03</td>
</tr>
<tr>
<td>RV/LV ratio</td>
<td>44.0 ± 0.7</td>
<td>45.0 ± 0.6</td>
<td>44.0 ± 0.5</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td></td>
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</table>

Values are means ± SE. RV, right ventricular free wall RV/LV ratio, ratio of weight of the RV to left ventricular and septal weight. No significant differences between group mean values were observed.

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**Table 2.** Total and differential cell counts in BAL fluid from noninoculated, \( \text{Pseudomonas} \)-inoculated, and placebo-inoculated lungs

<table>
<thead>
<tr>
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<th>Placebo-Inoculated (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cell count, ( \times 10^7 ) cells/ml</td>
<td>4.2 ± 2.2</td>
<td>17.4 ± 12.0*</td>
<td>4.1 ± 1.1</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>2.7 ± 0.6</td>
<td>20.0 ± 8.0*</td>
<td>2.8 ± 0.7</td>
</tr>
<tr>
<td>Macrophages, %</td>
<td>97.0 ± 0.8</td>
<td>72.0 ± 13.0*</td>
<td>96.0 ± 1.4</td>
</tr>
<tr>
<td>Lymphocytes, %</td>
<td>0.52 ± 0.8</td>
<td>9.0 ± 4.0*</td>
<td>1.2 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significant difference from noninoculated and placebo-inoculated groups (\( P < 0.01 \), ANOVA).

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**Table 3.** Baseline and change in Ppa in response to hypoxia

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<tr>
<th></th>
<th>Non-inoculated (n = 8)</th>
<th>Pseudomonas-Inoculated (n = 10)</th>
<th>Placebo-Inoculated (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline Ppa, mmHg</td>
<td>16.0 ± 1.2</td>
<td>15.3 ± 1.1</td>
<td>17.3 ± 1.5</td>
</tr>
<tr>
<td>Hypoxia, ΔPpa, mmHg</td>
<td>23.4 ± 1.2</td>
<td>27.7 ± 4.2</td>
<td>27.5 ± 3.9</td>
</tr>
</tbody>
</table>

Values are means ± SE. Ppa, pulmonary arterial pressure; \( Δ\text{Ppa} = \text{change in Ppa} \). No significant differences between group mean values were observed.
other vessels having a normal structure. In general, vessel wall remodeling was most extensive in the most inflamed regions of lung, whereas in regions of lung that were not inflamed vessel structure was normal. The placebo-inoculated group showed vessels that predominately contained a single elastic lamina (Fig. 3C), similar to those seen in control lungs.

Morphometric Analysis

Volumes of pulmonary tissue compartments. Mean total lung volume of the Pseudomonas-inoculated group was significantly ($P < 0.05$) greater than those from both the noninoculated and placebo-inoculated groups (Fig. 4), whereas both control groups, the non-
inoculated and placebo-inoculated, did not differ significantly from one another. Extra- and intra-acinar tissue volumes were significantly greater in the *Pseudomonas*-inoculated group than in either of the two control groups (Fig. 4). Mean total alveolar wall volume was significantly greater in the chronically infected group than in both control groups. There was also a significant difference between the mean volume of intra-acinar pulmonary blood vessels in the infected and both control groups, whereas no significant differences were observed in air-space volumes between the three groups. These results indicate that the increase in intra-acinar tissue volume was due to the increase in the volume of alveolar walls and intra-acinar blood vessels.

Volumes of the tissue compartments of intra-acinar blood vessels. The mean total volume of the tunica intima, tunica media, and tunica adventitia of the intra-acinar pulmonary vessels showed significant increases in the *Pseudomonas*-inoculated group when compared with noninoculated and placebo-inoculated groups (Fig. 5).

Length of intra-acinar pulmonary vessels. The mean total length of the intra-acinar pulmonary blood vessels per left lung was significantly greater in the *Pseudomonas*-inoculated lungs than in the noninoculated and placebo-inoculated groups (Fig. 6).

Number of branch points in intra-acinar pulmonary vessels. The mean total number of branch points in the intra-acinar pulmonary blood vessels per left lung was
significantly greater in the Pseudomonas-inoculated lungs than in the noninoculated and placebo-inoculated groups (Fig. 7).

**Blood vessel wall thickness and lumen radius.** The mean ratio of blood vessel wall thickness to vessel radius was significantly greater \( (P < 0.05) \) in the infected \( (0.31 \pm 0.01) \) than in the noninoculated \( (0.12 \pm 0.02) \) and placebo-inoculated \( (0.14 \pm 0.02) \) groups. However, the mean diameter of the vascular lumen in the infected lungs \( (36 \pm 1.3 \mu m) \) was not significantly different from that in the placebo \( (37 \pm 1.4 \mu m) \) and noninoculated \( (42 \pm 2.8 \mu m) \) groups. The mean thickness of the internal elastic lamina and the intima internal to it in the chronically infected lungs was \( 3.4 \pm 0.2 \mu m \), a value not significantly different from that of the placebo-inoculated \( (3.0 \pm 0.5 \mu m) \) and of the noninoculated \( (2.6 \pm 0.5 \mu m) \) groups.

The increased volumes of each of the three layers of the vessel wall that were observed may have arisen because of the increased total length alone. Thus we computed the volume of each compartment per unit vessel length; results are shown in Table 4. Only in the case of the tunica media was a significant increase in volume per unit length observed, demonstrating that this is the layer that became thickened as a result of chronic infection.

**DISCUSSION**

We demonstrated that chronic airway infection leads to vascular remodeling in the pulmonary circulation, i.e., a thickening of the vessel walls compared with the radius of the vessel. Despite these changes in vessel

![Fig. 5. Mean ± SE volumes of lumen and tunics of the intra-acinar pulmonary blood vessels in the left lungs of the noninoculated \( (n = 8) \), Pseudomonas-inoculated \( (n = 10) \), and placebo-inoculated \( (n = 6) \) animals. \( V(lumen) \), volume of intra-acinar blood vessel lumen; \( V(intima) \), volume of tunica intima; \( V(media) \), volume of tunica media; \( V(adventitia) \), volume of tunica adventitia. *Significant difference from noninoculated and placebo-inoculated groups \( (P < 0.01, \text{ANOVA}) \).]

![Fig. 6. Mean ± SE total length of intra-acinar pulmonary blood vessels in the left lungs of the noninoculated \( (n = 8) \), Pseudomonas-inoculated \( (n = 10) \), and placebo-inoculated \( (n = 6) \) animals. *Significant difference from noninoculated and placebo inoculated groups \( (P < 0.05, \text{ANOVA}) \).]

**Table 4. Volumes of tunica intima, tunica media, and tunica adventitia in intra-acinar pulmonary blood vessels per unit length of blood vessel**

<table>
<thead>
<tr>
<th></th>
<th>Noninoculated ( (n = 8) )</th>
<th>Pseudomonas-Inoculated ( (n = 10) )</th>
<th>Placebo-Inoculated ( (n = 6) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of intima per unit length, ( \times 10^{-7} ) cm(^2)/cm</td>
<td>3.5 ± 0.8</td>
<td>4.3 ± 0.4</td>
<td>3.8 ± 0.8</td>
</tr>
<tr>
<td>Volume of media per unit length, ( \times 10^{-7} ) cm(^2)/cm</td>
<td>0.2 ± 0.08</td>
<td>6.9 ± 0.8*</td>
<td>0.2 ± 0.9</td>
</tr>
<tr>
<td>Volume of adventitia per unit length, ( \times 10^{-7} ) cm(^2)/cm</td>
<td>8.6 ± 0.8</td>
<td>8.4 ± 0.6</td>
<td>7.3 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significant difference from noninoculated and placebo-inoculated groups \( (P < 0.01, \text{ANOVA}) \).
structure, pulmonary hypertension did not develop, as demonstrated by the absence of right ventricular hypertrophy (Table 1) and normal PVR in isolated lungs from chronically infected animals (Table 3). This absence of pulmonary hypertension is in agreement with previous reports of chronic airway infection in rats, which demonstrate, after a similar time interval, structural changes in the vascular wall, including medial thickening, without increases in PVR (2, 8).

The structure of the pulmonary vasculature of the rat has been extensively studied previously, most notably by Reid and colleagues (15, 19, 24). They report that intra-acinar arterial vessels vary in external diameter from 15 to 150 μm in the rat (15). Given that the smaller vessels are more numerous than larger ones, our volume-averaged lumen diameter of intra-acinar vessels in noninoculated and placebo-inoculated control lungs is in good agreement with the range of vessel diameters reported previously (15). Our estimate of the mean luminal diameter of intra-acinar vessels also agrees well with that obtained from previously reported models of the pulmonary arterial tree (14, 16, 17, 33). The mean lumen diameter depends on the relative numbers of vessels of different diameters and their lengths. Haworth et al. (14) showed that the slope of the power law relationship between the numbers of intrapulmonary vessels and their diameters and between the segment lengths and their diameters is relatively constant across species and can be used to determine the numbers and dimensions of the pulmonary vessels. We used the model of Haworth et al. to determine the mean volume-derived diameter of the intra-acinar vessels. Assuming that the mean diameter of the immediate precapillary arterioles is 15 μm and that the intra-acinar vessels are not more than 150 μm in diameter in the rat (24), then the volume-derived mean diameter of the intra-acinar vessels predicted by the model is 35 μm; this value is in good agreement with that which we observed in control tissues. Using electron microscopy, Meyrick and Reid (24) found that the internal elastic lamina is up to 1.4 μm thick and that the mean thickness of intima internal to this ranges between 0.2 and 4.0 μm. This suggests that the thickness of the two compartments combined ranged from slightly greater than 1 μm to 5.4 μm along the intra-acinar vessels. On the basis of stereological analysis, we estimated that the thickness of the internal elastic lamina, the endothelium, and other cells internal to the internal elastic lamina in the control noninoculated lungs, was 2.5 ± 0.5 μm, a value in good agreement with the data of Meyrick and Reid. Taken together, these observations indicate that the stereological approach that we used provided information about intra-acinar vessel structure compatible with previous studies using other techniques.

In chronically infected lungs, we found increased medial volume per unit length of intra-acinar vessel wall (Table 4), a finding that agrees with those based on measurements of wall thickness and lumen diameter in histological sections (2, 3, 8). The increase in intimal and adventitial volumes observed was proportional to the increase in vessel length, implying that neither of these two tunics was thickened relative to the size of the blood vessels. However, the calculated mean lumen radius in the chronically infected group was not significantly reduced compared with the two control groups, suggesting that thickening of the vessel wall does not inevitably lead to encroachment on, and reduction of, the lumen. Rather, the enlargement of the wall occurred predominantly in an outward direction, causing an increase in the external diameter of the vessel. A similar behavior has been reported previously in atherosclerotic disease of systemic arteries, in which the vessel enlarged in an outward direction at the site of an atherosclerotic plaque so that the lumen size remained normal (7). This phenomenon has not previously been reported in the pulmonary circulation. Our findings also demonstrate that medial hypertrophy does not necessarily imply increased vascular reactivity, because hypoxic vasoconstriction was not augmented in the chronically infected lungs (Table 3).

There are a number of possible explanations for this observation. Vascular remodeling was not uniform throughout all vessels in the chronically infected lungs, and a large number of vessels were observed in which wall structure was relatively normal (see Lung Histology and Fig. 2). These normal vessels may have provided a low-resistance pathway through the lung that allowed normal pulmonary artery pressure to be preserved. Such preservation of normal pulmonary artery pressure would be in keeping with previous reports that pulmonary artery pressure remains normal after pneumonectomy, i.e., after loss of half the pulmonary vascular bed (21). A further potential explanation is that the newly developed medial smooth muscle was predominantly proliferative in phenotype and therefore had poor contractile function (29). We did not examine these possibilities in the present study, and further work is needed to elucidate this issue.

The present results also show a substantial increase in total intra-acinar vessel length (Fig. 6). If this occurred because of increased length and tortuosity of existing vessels, then PVR would have risen, because mean lumen radius remained unchanged. However, if the increase in vessel length resulted, at least in part, from the formation of new vessels that ran in parallel to the preexisting pathways, then PVR could be maintained at normal values. Our finding that there were a significantly increased number of branch points in the pulmonary circulation supports the hypothesis that angiogenesis occurred as a result of chronic airway infection (Fig. 7). The relative increase in the number of branch points in infected lungs appears somewhat greater than that of vessel length (Figs. 6 and 7).

This finding suggests that the mean length of new vessels may be shorter than that of the preexisting vessels. This evidence of angiogenesis in the pulmonary circulation is in contrast to previous reports that neither chronic hypoxia (10, 19, 30) nor chronic inflammation (27, 28) causes new vessel formation in this circulation. Both of these stimuli are powerful initiators of angiogenesis in the systemic circulation (9, 20). It is inter-

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esting to note that, in the setting of metastatic lung disease, Milne and Zerhouni (25) have reported that most such tumors in the lung receive all, or a major part, of their blood supply from the pulmonary circulation.

It is likely that the explanation for these differing reports lies in the difficulty of identifying new vessels in an organ that is as vascular as the lung. When many blood vessels are already present in a highly vascular tissue, the addition of a small number of new vessels is very difficult to detect by direct inspection of two-dimensional sections, the method used in previous studies (27, 28, 30). Furthermore, counting the number of vessels viewed per high-power field will also fail to detect change in total vessel length, because such an approach does not allow for the effects of changes in the volume of the reference space, e.g., the size of the lung or the size of the alveolus. However, the techniques of stereological morphometry circumvent these difficulties and allowed us to obtain quantitative information on the three-dimensional structure of the lung vasculature demonstrating increased total length and new branch-point formation. Neither of these changes could have been detected by simple inspection of random histological sections.

The role played by vascular remodeling in the development of pulmonary hypertension in chronic lung infection is complex. Our data suggest that, under certain conditions, wall remodeling may not result in reduction of the mean lumen diameter. Furthermore, the addition of parallel vascular pathways through the lungs may serve to oppose any increase in total PVR that might result from reductions in vascular lumen diameter in some vessels. When these two processes occur together, vascular resistance would not increase despite thickening of the vessel wall. However, in other circumstances, such as more persistent or more severe infections, remodeling of the vascular wall, including the wall of newly formed vessels, might lead to significant reductions in mean lumen diameter. Under such conditions, the increase in resistance caused by narrowed vessels might outweigh the extent to which new vessel formation could reduce resistance and the net effect might then become an increase in total PVR. Graham et al. (8) found that, in chronic Pseudomonas infection of rat lungs, pulmonary hypertension was not observed 2 wk after infection but was present after 6–9 wk, suggesting that more prolonged inflammation caused increased vascular resistance. However, the duration of infection is clearly not the only factor that influences the development of hypertension. McCormack and Paterson (23) found significantly increased PVR 7–10 days after intratracheal inoculation of Pseudomonas incorporated into agar beads. This suggests that other influences, such as severity of infection, virulence of the infecting organism, and host factors, also play important roles.

In conclusion, we have demonstrated, for the first time, that chronic airway infection may cause pulmonary vascular remodeling together with angiogenesis in the intra-acinar vasculature. These changes can maintain lumen cross-sectional area and provide new vascular pathways through the lung, which prevent the development of pulmonary hypertension despite the development of significant thickening of the vessel wall. Our findings also suggest that pulmonary angiogenesis may play an important role in lung disease, including neoplastic and chronic inflammatory disorders.

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