Susceptibility to pulmonary hypertension in inbred strains of mice exposed to cigarette smoke

Christine Nadziejko, Kaije Fang, Antonio Bravo, and Terry Gordon

Department of Environmental Medicine, New York University School of Medicine, Tuxedo, New York

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Nadziejko C, Fang K, Bravo A, Gordon T. Susceptibility to pulmonary hypertension in inbred strains of mice exposed to cigarette smoke. J Appl Physiol 102: 1780–1785, 2007. First published February 1, 2007; doi:10.1152/japplphysiol.01076.2005.—Cor pulmonale is a significant cause of morbidity and mortality in patients with emphysema, but it is not known whether alveolar destruction is directly involved in the disease pathogenesis. The purpose of this study was to examine the relationship between susceptibility to smoking-induced cor pulmonale and alveolar destruction in eight inbred strains of mice: 129X1/SvJ, A/J, A/HeJ, BALB/cJ, C3H/HeJ, C57BL/6J, DBA/2J, and SWR/J. The mice were exposed to filtered air or mainstream cigarette smoke at a concentration of 250 mg/m³ for 5.5 h/day, 5 days/wk for 5 mo, housed for 4 more months, and killed. The ratio of the weight of the right ventricle/left ventricle plus septum [RV/(LV + S)] was used to assess right ventricular hypertrophy. Alveolar mean linear intercept was used to quantify severity of alveolar destruction. Morphometric determination of blood vessel muscularization was done on sections from four mouse strains. Smoke exposure resulted in significant increases in RV/(LV + S) in the A/J and A/HeJ strains compared with air-exposed controls. The magnitude of the smoking-induced increase in RV/(LV + S) decreased as a function of the genetic distance of the other strains from the A/J and A/HeJ strains. Pulmonary vascular muscularization was significantly increased in smoke-exposed A/J and BALB/cJ mice but not in C3H/HeJ and C57BL/6 mice. Also, mouse strain susceptibility to smoking-induced pulmonary vascular muscularization did not correlate with changes in mean linear intercept. The data from this study suggest that alveolar destruction by itself is not sufficient to cause smoking-induced cor pulmonale in inbred mice.

cor pulmonale; cigarette smoking; inbred mice; emphysema; genetic susceptibility; animal model

Cor pulmonale is a form of pulmonary hypertension and right heart hypertrophy that results from chronic lung disease. This disease is a significant cause of morbidity and mortality in smokers who have chronic obstructive pulmonary disease (COPD). A study of patients with stable COPD showed that 25% developed pulmonary hypertension (measured by right heart catheterization) during a 6-yr follow-up (7). There is general agreement that patients who develop signs of cor pulmonale have a poor prognosis (5). A prospective study of patients with mild to moderate emphysema showed that mean pulmonary artery pressure was the only significant predictor of emphysema-related mortality in multivariate analysis of 14 parameters, including blood gases and pulmonary function measures (1).

The underlying mechanisms of cor pulmonale are not fully understood, but several pathophysiological processes are thought to be involved, namely: 1) structural alteration of the pulmonary vascular bed caused by lung destruction; 2) pulmonary arterial vasoconstriction secondary to alveolar hypoxia; and 3) vascular remodeling and proliferation of smooth muscle in pulmonary arterioles that are normally nonmuscular (11).

Destruction of pulmonary alveolar structures by emphysema is believed to be the initial step in the process that leads to cor pulmonale in smokers. It has been reported that 75% of subjects with severe COPD have signs of cor pulmonale detected by echocardiography (4). However, there is only a weak correlation between the severity of COPD and the prevalence of cor pulmonale (7). Understanding the role of alveolar destruction in cor pulmonale is an important issue for development of new treatment options. If loss of alveolar surface area is not directly linked to cor pulmonale, then treatments that target the pulmonary vascular system may be beneficial even in patients with advanced COPD. We have exposed inbred strains of mice to mainstream tobacco smoke to examine the relationship between susceptibility to cor pulmonale and alveolar destruction. A number of studies have shown that mice develop right heart hypertrophy and pulmonary hypertension in response to alveolar hypoxia (2), but cigarette smoke-induced cor pulmonale has not been previously described in mice. We screened mice from eight inbred strains for cor pulmonale 4 mo after a 5-mo exposure to tobacco smoke (or air) by measuring right ventricular weight. Although we did not determine pulmonary hypertension directly as a measure of cor pulmonale, pulmonary vascular muscularization was examined as a measure of pulmonary hypertension in four strains that showed differences in susceptibility to smoke exposure based on heart weights. We report here that some strains of mice do develop cor pulmonale after long-term cigarette smoke exposure and that genetic susceptibility to cor pulmonale in mice is not solely related to morphological measures of emphysema severity.

Materials and Methods

Animals

Female mice, aged 6 to 8 wk, were purchased from Jackson Laboratory, Bar Harbor, ME, and allowed to acclimate for a minimum of 2 wk before the start of the study. Data from eight inbred strains are reported in this study: 129X1/SvJ (129), A/J, A/HeJ (A/HE), BALB/cJ (BALBc), C3H/HeJ (C3H), C57BL/6J (C57BL/6), DBA/2J (DBA), and SWR/J (SWR). Twenty mice per strain were exposed to cigarette smoke, and 10–20 mice per strain were exposed to filtered air. The mice were maintained in a climate-controlled room with a 12:12-h light-dark cycle in an animal facility accredited by the Association for...
Assessment and Accreditation of Laboratory Animal Care. The mice were housed in polycarbonate cages with corncob bedding with free access to food and water except during exposure to cigarette smoke or filtered air (filtered by a high efficiency particulate air filter). There was no excess mortality associated with the cigarette smoke exposure although some strains lost weight during exposure. Eleven of the 16 groups of mice that were exposed to air or cigarette smoke had one or more mice removed from the study during the 9-mo protocol because of unexpected deaths or euthanasia of moribund mice. This study was approved by the Institutional Animal Care and Use Committee of the New York University School of Medicine.

Cigarette Smoke Exposure

This study was added on to a protocol designed to examine strain susceptibility to cigarette smoke-induced lung tumors (results to be presented separately) using the exposure regimen developed by Witschi and coworkers (17). In brief, the mice were exposed to filtered air or mainstream cigarette smoke (target concentration 250 mg/m³ total smoke particulates, TSP) for 5.5 h/day, 5 days/wk for 5 mo. After the 5-mo exposure period, the animals were housed for an additional 4 mo for tumor development. Mainstream cigarette smoke was generated from unfiltered 1R3 reference cigarettes (Tobacco Research Institute, Lexington, KY) using a Baumgartner-Jaeger CSM 2070 cigarette smoke-generation system (CH rettes (Tobacco Research Institute, Lexington, KY) using the ISO puff profile. The Tobacco Research Institute provides a reference cigarette that is comparable to those used in many tobacco smoke research studies and stores large batches of reference cigarettes with complete characterization. The eight strains were exposed to cigarette smoke or filtered air in Laskin-type whole body exposure chambers while housed in polycarbonate cages with wire-mesh tops. The airborne mass concentration in the two cigarette smoke exposure chambers was measured every hour by collecting particles on Teflon filters that were weighed before and after sampling. Carbon monoxide (CO) was monitored using a Fluke CO monitor (Fluke, Everett WA). Mass concentrations averaged 258.1 ± 4.0 mg/m³ TSP (mean ± SE), and CO concentrations were 205 ± 4 parts per million. Temperature and relative humidity were 74 ± 0.3°F and 43 ± 1%, respectively. Measurements related to core pulmonale and emphysema were made on a randomly selected subset of the mice.

Heart Weight Measurements

Because hearts with intact aortas were required for atherosclerosis end points, hearts were available for measurement of ventricular weights from 5–6 mice per group for groups that had 10 mice and from 11–14 mice for groups with 20 mice (cardiac tissue from remaining mice in each group were archived for atherosclerosis end points), equaling a total of 169 mice (65 air exposed and 104 smoke exposed). The hearts were dissected and weighed immediately after each mouse was euthanized to avoid changes in weight from dehydration of the tissue. The atria were removed with a razor, and the ventricular cavities were gently aspirated to remove blood clots. The right ventricle (RV) was cut away from the septum (S) with fine scissors. The weights of the right ventricle and the left ventricle plus septum (LV + S) were measured using an analytical balance with a sensitivity of 0.0001 g, and the RV/(LV + S) ratios were calculated (2).

Lung Morphometry

Lung fixation. After removal of the heart, the lungs were inflated with Telysinscizy’s fluid (640 ml ethanol, 55 ml PBS at pH 7.4, 174 ml water, 87 ml formaldehyde, and 44 ml glacial acetic acid) at a pressure of 25 cm H₂O for 1 h. The lungs remained in Telysinscizy’s fluid for a minimum of 24 h before being transferred into 100% ethanol for storage. The trachea was tied off, and the lungs were removed and stored in fixative. The total volume of the fixed lungs was measured by water displacement (15). The lung lobes were embedded in paraffin (one block per mouse) and sectioned in the midsagittal plane. A subset of mouse strains was selected for morphometric determination of blood vessel muscularization to examine the relationship between emphysema severity and pulmonary vascular muscularization. Mice from four inbred strains with diverse heart weight changes were examined (A/J, BALBc, C3H, C57BL/6) with 8–13 mice per exposure group per strain. Mice with heart weight data were selected first (heart weights were not measured on all mice because some hearts were preserved intact for examination of arterial atherosclerosis severity), and then additional mice were selected at random to fill in the groups.

Mean linear intercept. The lung lobes of mice from all eight strains were embedded in paraffin, and midsagittal sections of the left and right lower lobes were cut at a thickness of 5 μm and stained with hematoxylin and eosin. Quantitative assessment of emphysematous changes was performed using NIH Image software (developed at the National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/), a Macintosh computer, and a Scion analog-to-digital frame capture board (SG-5). Mean linear intercept was measured on images of a section from the left lower lobe projected onto a monitor at a final magnification of ×380. The image was overlaid with a transparency consisting of 21 equally spaced lines (13). The number of alveolar wall intersections with each test line was counted in 15–30 fields in the peripheral region of the lobe (approximately 30–40% of the cross-sectional area of the lobe). A pilot study was done on a subset of air and cigarette smoke-exposed mice from two strains (n = 6 per exposure per strain) to verify that mean linear intercept measured in peripheral lung fields did not differ from values obtained by examining the entire lobe. Mean linear intercept was calculated on the basis of the number of lines counted, the length of the test lines, and the sum of the alveolar intercepts (13). Line lengths were calibrated with a stage micrometer. Although tissue shrinkage occurs in paraffin-embedded tissue and affects absolute morphological measurements, it was assumed that tissue shrinkage occurred to a similar degree in air- and cigarette smoke-exposed mice.

Pulmonary vascular muscularization. Double-label immunohistochemistry was used to quantify pulmonary vascular muscularization using reagents and antibodies from DAKO/Cytomation (Carpinteria, CA). Smooth muscle actin was stained using a mouse monoclonal antibody against human α-smooth muscle actin (clone 1A4, diluted 1:50), and von Willebrand factor staining was used as a blood vessel marker to aid in the identification of nonmuscularized blood vessels (12). The paraffin sections were dewaxed and heated in antigen retrieval buffer (modified citrate buffer, pH 6.1) at 95–99°C for 20 min. α-Smooth muscle actin was visualized using the Animal Research kit (DAKO) according to the manufacturer’s protocol, except that 2% (wt/vol) dry fat milk powder was added to the streptavidin peroxidase solution to prevent nonspecific background staining. Then von Willebrand factor staining was used using the Envision alkaline phosphatase kit with permanent red as the substrate-chromagen. The slides were not counterstained.

Blood vessel staining for α-smooth muscle actin was scored using an Olympus BH-2 microscope with a ×40 objective. Fifteen randomly selected fields per lung lobe were examined in two lobes per slide (average of 50 blood vessels per animal). All blood vessels less than 125 μm in diameter were scored for smooth muscle actin staining. A pilot study was done on slides from 12 animals (3 air-exposed and 3 cigarette smoke-exposed mice from A/J and C57BL/6 strains) to determine the number of fields needed to obtain a reproducible estimate of vascular smooth muscle actin staining per animal. Fields that were adjacent to large conducting airways or that contained mostly nonalveolar tissue were skipped. A scale of 1–5 was used: 1 = no actin staining; 2 = faint stippled brown staining; 3 = dark staining ≤½ the circumference; 4 = dark staining ≤¾ the circumference; and 5 = intense dark staining of the entire circumference.
ence. Slides were scored in random order without knowledge of the mouse strain or treatment. There were no significant differences in the number of blood vessels scored per animal between strains or between air- and cigarette smoke-exposed groups. The average actin staining score per animal was computed from the percentage of blood vessels in each staining category.

**Statistical Analysis**

All summary data are expressed as means ± SE. Statistical analysis was done using SYSTAT 10 (SPSS, Chicago IL) and S-PLUS 6.1 (Insightful, Seattle WA). A two-tailed t-test was used to compare air- and smoke-exposed groups. To examine whether there were strain differences in the pulmonary effects of cigarette exposure, data were normalized for baseline strain differences by dividing by the mean of the air group, and significant strain differences were identified by one-factor ANOVA with post hoc pairwise comparison using the protected least significant difference method. Linear regression was used to examine the relationship between mean linear intercept and pulmonary vascular muscularization scores within strains. P values < 0.05 were considered statistically significant.

**RESULTS**

**Right Ventricular Weight**

The ratio $RV/(LV + S)$ was determined to screen for pulmonary hypertension in eight inbred strains of mice that were killed 4 mo after a 5-mo exposure to mainstream cigarette smoke (or air). As shown in Table 1, there was a statistically significant increase in $RV/(LV + S)$ in smoke-exposed mice from the $A/J$ and $A/HE$ strains compared with air-exposed controls. $RV/(LV + S)$ was also increased in smoke-exposed mice from the $BALBc$ and 129 strains, but the difference was not statistically significant. There were minimal differences in $RV/(LV + S)$ between the air- and smoke-exposed groups for the other four strains in the study.

Figure 1 shows a dendrogram of the genetic relationships between the eight strains in this study (16). The average percent change in $RV/(LV + S)$ caused by smoke exposure for each strain is indicated in Fig. 1. The largest increases in $RV/(LV + S)$ were in the genetically similar $A/J$ and $A/He$ strains, and the magnitude of the smoking-induced increase in $RV/(LV + S)$ decreased as a function of the genetic distance of the other strains from the $A/J$ and $A/HE$ strains. This pattern of susceptibility suggests that there is a strong genetic component for smoking-induced cor pulmonale in inbred mice.

**Pulmonary Vascular Muscularization**

Lung sections from four strains were examined for pulmonary vascular muscularization, a morphological indicator of pulmonary hypertension. One strain ($A/J$) was positive for cigarette smoke-induced right heart hypertrophy, one ($BALBc$) was borderline, and two ($C3H$, $C57BL/6$) were negative, as shown by measurements of $RV/(LV + S)$ (Table 1). Pulmonary vascular muscularization was determined by scoring blood vessels for immunohistochemical staining for smooth muscle actin on a scale of 1 to 5 (nonmuscularized = 1, fully muscularized = 5). As shown in Fig. 2, pulmonary vascular muscularization was significantly increased in the smoke-exposed $A/J$ and $BALBc$ mice (compared with air controls) but not in the $C3H$ and $C57BL/6$ mice. There was a significant positive association between $RV/(LV + S)$ and vascular muscularization for individual smoke-exposed mice within the $A/J$ strain ($P < 0.05$, linear regression) but not in the other three strains (data not shown).

<table>
<thead>
<tr>
<th>Strain</th>
<th>RV/(LV + S) Air</th>
<th>RV/(LV + S) Cigarette smoke</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A/J$</td>
<td>0.162 ± 0.006 (12)</td>
<td>0.183 ± 0.007 (13)</td>
<td>0.02</td>
</tr>
<tr>
<td>$A/HE$</td>
<td>0.176 ± 0.004 (14)</td>
<td>0.196 ± 0.007 (13)</td>
<td>0.02</td>
</tr>
<tr>
<td>$BALBc$</td>
<td>0.180 ± 0.006 (5)</td>
<td>0.194 ± 0.005 (13)</td>
<td>0.14</td>
</tr>
<tr>
<td>129</td>
<td>0.175 ± 0.007 (6)</td>
<td>0.192 ± 0.008 (12)</td>
<td>0.16</td>
</tr>
<tr>
<td>$C3H$</td>
<td>0.224 ± 0.005 (6)</td>
<td>0.227 ± 0.007 (12)</td>
<td>0.78</td>
</tr>
<tr>
<td>$C57BL/6$</td>
<td>0.173 ± 0.013 (6)</td>
<td>0.168 ± 0.004 (14)</td>
<td>0.62</td>
</tr>
<tr>
<td>$DBA$</td>
<td>0.258 ± 0.007 (11)</td>
<td>0.266 ± 0.008 (14)</td>
<td>0.44</td>
</tr>
<tr>
<td>$SWR$</td>
<td>0.189 ± 0.013 (5)</td>
<td>0.192 ± 0.005 (13)</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Values are means ± SE; (n), no. of mice. $RV/(LV + S)$, ratio of right ventricular-to-left ventricular plus septal weights; $A/HE$, $A/He$; $BALBc$, $BALBc/J$; 129, 129XI/SvJ; $C3H$, $C3H/He$; $C57BL/6$, $C57BL/6J$; $DBA$, $DBA/2J$; $SWR$, $SWR/J$.  

![Fig. 1. A dendrogram of the relationship between mouse strain genetic similarity and the average percent change in the pulmonary hypertension index, the ratio of the weight of the right ventricle/left ventricle plus septum $[RV/(LV + S)]$ (in parentheses), after subchronic cigarette smoke exposure. The dendrogram of inbred mouse strain relatedness is based on a single-nucleotide polymorphism analysis (12). Mouse strains are 129XI/SvJ (129), $AJ$, $A/He$ (A/HE), $BALBc/J$ (BALBc), $C3H/He$ (C3H), $C57BL/6J$ (C57BL/6), DBA/2J (DBA), and SWR/J (SWR). Mouse strains with the greatest genetic similarity are physically closer in the dendrogram (e.g., $AJ$ and A/He mice have greater genetic similarity than $AJ$ and C3H mice).](#)
Relationship Between Emphysema Severity and Pulmonary Vascular Muscularization

Emphysematous changes in smoke-exposed mice were quantified by measuring the alveolar mean linear intercept (Table 2). Mean linear intercept measurements were also normalized for baseline strain differences by dividing by the mean of the air group for each strain (Fig. 3A). As shown in Table 2, mean linear intercept was significantly increased in smoke-exposed mice compared with air-exposed mice in all eight strains \((P < 0.05, 2\text{-}tailed \ t\text{-}test)\). Mean linear intercept was significantly greater in the smoke-exposed A/J mice compared with mice from the BALBc and C57BL/6 strains. Smoke-exposed C3H mice had a significantly greater mean linear intercept than C57BL/6 mice but not BALBc mice \((P = 0.06)\). More importantly, mean linear intercept was not significantly different in the smoke-exposed A/J group compared with the C3H group, indicating that the severity of alveolar destruction in response to cigarette smoke was similar in these two strains. Likewise, mean linear intercept was not significantly different in smoke-exposed BALBc and C57BL/6 groups. A similar pattern of strain susceptibility was seen for total lung volume; the smoke-exposed A/J and C3H groups were significantly increased compared with air controls, and there were no significant changes in lung volume in the smoke-exposed BALBc and C57BL/6 groups (data not shown).

A comparison of Fig. 3, A and B, indicates that mouse strain susceptibility to pulmonary vascular muscularization did not mirror susceptibility to alveolar destruction caused by cigarette smoke. Specifically, the smoke-exposed C3H mice had similar changes to mean linear intercept as the A/J mice, but the C3H strain did not develop increased pulmonary vascular muscularization, and the A/J mice did. The BALBc mice had a smaller increase in mean linear intercept in response to cigarette smoke than the A/J mice, but A/J and BALBc mice both had significant increases in pulmonary vascular remodeling.

Figure 4 shows the relationship between mean linear intercept and pulmonary vascular muscularization within each of the four strains. There was a significant positive association between mean linear intercept and blood vessel muscularization in the A/J and BALBc strains but not in the C3H or C57BL/6 strains. This is confirmed in Fig. 5, which shows no significant correlation when the mean linear intercept and right ventricular weight data for all strains are plotted \((R^2 = 0.05, P = 0.14)\). However, there was a close relationship between right ventricular weight and pulmonary vascular muscularization in the four strains studied (Fig. 6). These data suggest that the pathogenesis of cor pulmonale in smokers involves other factors in addition to the destruction of the pulmonary vascular bed.

**DISCUSSION**

This study demonstrates that subchronic exposure of mice to mainstream tobacco smoke results in cor pulmonale in a strain-dependent manner. A rodent model of smoking-induced cor pulmonale is an important research tool because this disease is difficult to study in humans because of its slow and variable course and risks involved in measuring pulmonary artery pressure. Numerous studies have shown that mice develop pulmonary hypertension and right heart hypertrophy after a few weeks of hypoxia (2, 12), but there have been no published reports describing cor pulmonale in mice after cigarette smoke exposure.

We found there are clear differences between the mouse strains in terms of susceptibility to cigarette smoke-induced cor pulmonale. This finding suggests there is a genetic basis for susceptibility that can be exploited to test disease mechanisms. While we did utilize right heart weights and pulmonary vas-

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mean Linear Intercept</th>
<th>Blood Vessel Muscularization</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/J</td>
<td>1.1 ± 0.1</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td>BALBc</td>
<td>1.2 ± 0.2</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>C3H</td>
<td>1.3 ± 0.3</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>1.4 ± 0.4</td>
<td>1.3 ± 0.3</td>
</tr>
</tbody>
</table>

Fig. 3. Mouse strain differences in mean linear intercept \(A\) and blood vessel muscularization \(B\). Data were normalized for baseline strain differences by dividing the values by the mean of the air exposed group for each strain. Bars indicate means ± SE for the smoke-exposed mice. Air group values are not shown because all air group means = 1.0. *Significant difference between air-exposed and cigarette smoke-exposed mice for that strain \((P < 0.05, 2\text{-}tailed \ t\text{-}test)\). Significant strain differences between smoke-exposed group means are indicated by the strains names listed on the bars. Differences between strains were determined by one-way ANOVA and pairwise post hoc comparison of data from the smoke-exposed mice.

**Table 2. Effects of subchronic cigarette smoke exposure on mean linear intercept in eight inbred mouse strains**

<table>
<thead>
<tr>
<th>Group</th>
<th>Strain</th>
<th>129</th>
<th>A/J</th>
<th>BALBc</th>
<th>C3H</th>
<th>C57BL/6</th>
<th>DBA</th>
<th>SWR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>86 ± 5 (10)</td>
<td>87 ± 4 (19)</td>
<td>86 ± 4 (18)</td>
<td>88 ± 3 (10)</td>
<td>86 ± 4 (10)</td>
<td>86 ± 9 (8)</td>
<td>86 ± 4 (19)</td>
<td>85 ± 3 (5)</td>
</tr>
<tr>
<td>CS</td>
<td>110 ± 6*(19)</td>
<td>106 ± 6*(18)</td>
<td>109 ± 5*(19)</td>
<td>106 ± 6*(18)</td>
<td>109 ± 5*(18)</td>
<td>101 ± 7*(20)</td>
<td>104 ± 4*(19)</td>
<td>105 ± 6*(18)</td>
</tr>
</tbody>
</table>

Values are means ± SD in μm; \(n\), no. of mice. *Statistically significant difference, cigarette smoke (CS)-exposed vs. corresponding air-exposed value.
cular muscularization as closely correlated indexes of pulmonary hypertension (Fig. 6), we did not directly assess pulmonary vascular pressure, and the absence of these measurements may hinder the interpretation of our findings. The present study examined whether there was a relationship between morphological measures of alveolar destruction and pulmonary vascular muscularization in smoke-exposed mice from four different strains. If the destruction of alveolar structures were directly involved in the pathogenesis of cor pulmonale, then new treatment options for cor pulmonale should target alveolar repair (6, 10). We found, however, that mouse strain susceptibility to smoking-induced pulmonary vascular remodeling is not closely linked to the severity of alveolar destruction (Fig. 5). This result is consistent with a recent cross-sectional study in patients with moderate emphysema indicating that the prevalence and severity of pulmonary hypertension is more closely correlated with airway obstruction (decreased percent forced expiratory volume in 1 s) than with measures of emphysema severity (1). Another study in patients with advanced emphysema showed there is a wide variation in resting pulmonary artery pressure that cannot be accounted for by hypoxemia or CAT scan scores of emphysema severity (14).

The present study demonstrates the value of using multiple inbred mouse strains when developing mouse models of complex human diseases. In our study, only two of eight strains developed all of the features of cor pulmonale even though the mice were exposed to high concentrations of mainstream tobacco smoke for almost one-quarter of their life span. Exposing sufficient numbers of mice from so many inbred strains did impose some limitations. The study was restricted to female mice, and the pattern of susceptibility seen in this study may not hold for male mice. Also, all the mice were killed at a single time point in the disease process, 4 mo after the end of the 5-mo-long smoke exposure. It is not known whether there was alveolar repair and/or recovery from pulmonary hypertension in some strains during the 4-mo period after the end of

Fig. 4. Trellis graph of relationship between air-group normalized mean linear intercept values and pulmonary vascular muscularization scores for air and smoke-exposed mice from each strain. P values shown in each panel were determined by linear regression.

Fig. 5. Relationship between right heart weight [RV/(LV + S)] and mean linear intercept for individual mice in eight strains. Data were normalized for baseline strain differences by dividing the individual cigarette smoke (CS) values by the mean of the air-exposed group for that strain. Using linear regression, there was no correlation between RV/(LV + S) and mean linear intercept (P = 0.14).

Fig. 6. Relationship between right heart weight [RV/(LV + S)] and pulmonary vascular muscularization for individual mice in 4 strains. Data were normalized for baseline strain differences by dividing the individual cigarette smoke values by the mean of the air-exposed group for that strain. Using linear regression, there was a significant correlation between RV/(LV + S) and pulmonary vascular muscularization (P = 0.005).
smoke exposure. However, the delay between smoke exposure and morphological measurements reduced the possibility of confounding effects from decreased food intake during smoke exposure. There is recent evidence that alveolar destruction and repair can occur very rapidly in mice in response to changes in food intake (9). In the present study, the mouse strains showed varying degrees of retarded weight gain during cigarette smoke exposure (data not shown), which was probably due to decreased food intake. However, when the mice were euthanized 4 mo after the end of cigarette smoke exposure, there were no differences in weight due to smoke exposure in any strain.

We used changes in right ventricular weight to examine the strains for smoking-induced cor pulmonale in part because this measurement is more suitable for screening large numbers of mice than morphological measures of pulmonary vascular remodeling. However, increased right ventricular weight was not sufficient by itself to examine mouse strain differences in cor pulmonale because it is a relatively insensitive and nonspecific indicator. Pulmonary vascular muscularization is considered to be the central feature of cor pulmonale in experimental animal models and human disease. We measured pulmonary vascular remodeling in four strains that were expected to show differences in susceptibility on the basis of smoking-induced-changes (or lack of change) in right heart weight. Strain differences in smoking-induced right heart hypertrophy did correlate with differences in pulmonary vascular remodeling, as expected. Our use of double-immunohistochemistry labeling for von Willebrand factor and smooth muscle actin in the measurement of pulmonary vascular remodeling was not specific, however, for arterial changes. Although this nonspecificity hinders the interpretation of the vascular remodeling end point if taken into context alone, this technique is widely used (12), and the results are consistent with our findings of right heart hypertrophy.

The data from this study suggest that alveolar destruction by itself is not sufficient to cause smoking-induced cor pulmonale in inbred mice. For example, smoke-exposed mice from the A/J and C3H/HeJ strains had similar increases in mean linear alveolar diameters (10). In the present study, the mouse strains showed varying degrees of retarded weight gain during cigarette smoke exposure (data not shown), which was probably due to decreased food intake. However, when the mice were euthanized 4 mo after the end of cigarette smoke exposure, there were no differences in weight due to smoke exposure in any strain.

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