Differential lung mechanics are genetically determined in inbred murine strains

CLARKE G. TANKERSLEY, RICHARD RABOLD, AND WAYNE MITZNER
Department of Environmental Health Sciences, The Johns Hopkins University
School of Hygiene and Public Health, Baltimore, Maryland 21205

Tankersley, Clarke G., Richard Rabold, and Wayne Mitzner. Differential lung mechanics are genetically determined in inbred murine strains. J. Appl. Physiol. 86(6): 1764–1769, 1999.—Genetic determinants of lung structure and function have been demonstrated by differential phenotypes among inbred mouse strains. For example, previous studies have reported phenotypic variation in baseline ventilatory measurements of standard inbred murine strains as well as segregant and nonsegregant offspring of C3H/HeJ (C3) and C57BL/6J (B6) progenitors. One purpose of the present study is to test the hypothesis that a genetic basis for differential baseline breathing pattern is due to variation in lung mechanical properties. Quasi-static pressure-volume curves were performed on standard and recombinant inbred strains to explore the interactive role of lung mechanics in determination of functional baseline ventilatory outcomes. At airway pressures between 0 and 30 cmH2O, lung volumes are significantly (P < 0.01) greater in C3 mice relative to the B6 and A/J strains. In addition, the B6C3F1/J offspring demonstrate lung mechanical properties significantly (P < 0.01) different from the C3 progenitor but not distinguishable from the B6 progenitor. With the use of recombinant inbred strains derived from C3 and B6 progenitors, cosegregation analysis between inspiratory timing and measurements of lung volume and compliance indicate that strain differences in baseline breathing pattern and pressure-volume relationships are not genetically associated. Although strain differences in lung volume and compliance between C3 and B6 mice are inheritable, this study supports a dissociation between differential inspiratory time at baseline, a trait linked to a putative genomic region on mouse chromosome 3, and differential lung mechanics among C3, B6 progenitors and their progeny.

control of breathing; C3H/HeJ; C57BL/6J; A/J; BXH recombinant inbred strains

LINKAGE ANALYSIS has revealed a candidate genomic region on mouse chromosome 3 that candidates a substantial proportion of the variance in inspiratory timing (TI) during eupneic ventilation between C3H/HeJ (C3) and C57BL/6J (B6) inbred mouse strains (24). With comparable baseline minute volumes between strains, differential ventilatory characteristics can be summarized as a slow, deep pattern in C3 mice compared with a rapid, shallow pattern in B6 mice. One possible mechanistic difference between these strains may be different compliant properties of the lung. Human and animal studies have consistently demonstrated an inverse association between the compliant properties of the lung and the inherent respiratory timing of eupneic ventilation (1–3, 6, 8, 12, 13, 21–23).

The presumed rationale for this association that inversely links TI to lung compliance (Cl) is based on optimization of breathing frequency to minimize the mechanical work of breathing. In all mouse species (6, 8, 15), the time constant is very small relative to other species. This allows rapid filling and emptying of the lung at an optimal breathing frequency of ~2 Hz (6). The breathing frequencies of the C3 and B6 progenitors are the extremes among phenotypes in a distribution of responses among eight standard inbred strains (25), and if the minimal work-of-breathing concept is correct, Cl of C3 mice ought to be considerably greater than that of B6 mice.

We have previously utilized several genetic approaches to assess the inheritance pattern of differential baseline phenotypes of C3 and B6 mice that involve analyses of segregant offspring classes (26). Parental baseline breathing frequency and TI phenotypes are conserved among recombinant inbred (RI) strains derived from C3 and B6 progenitors (i.e., BXH RI strains), and a two-gene model emerged from cosegregation analysis (26, 27). Subsequent studies described the phenotypic distribution among BXH RI strains to establish linkage between TI at baseline and mouse chromosome 3 (24). If the data support the hypothesis that differential TI at baseline is governed by covariation in Cl, we predict that Cl properties of the BXH RI strains 8 and 14 would resemble those of C3 and B6 parental strains, respectively. Such results would demonstrate cosegregation of these functional and structural phenotypes and would support a potential common genetic etiology. However, if a dissociation between function and structure were evident by the absence of phenotypic cosegregation among a major portion of the BXH RI strains, then the genetic basis for differential regulation of TI at baseline would not be solely derived from variation in structural or mechanical phenotypes.

The aims of the present investigation were threefold. First, we identified differential pressure-volume (P-V) characteristics among three standard inbred strains routinely used in lung biology. Second, we determined those phenotypes of the P-V relationship which are inheritable. Finally, we used cosegregation analyses to examine the association between TI at baseline (26) and lung volume and Cl in BXH RI strains.

METHODS

Animals. Standard inbred strains (A/J, C3, and B6) and the B6C3F1/J (F1) hybrid were purchased from Jackson Laborato-
ries (Bar Harbor, ME). On the basis of differential baseline Ti phenotypes, selected RI strains derived from C3 and B6 progenitors (BXH RI strains 4, 6, 8, 9, 11, and 14) were also procured. The BXH RI strains are propagated by inbreeding (i.e., >20 generations of brother-sister matings) filial offspring derived from C3 and B6 progenitors; therefore, these represent stable, segregant genotypes of the parental strains. All animals were male and were weaned within 4–5 wk. Water and chow (Agway Pro-Lab RMH 1000) were provided ad libitum.

Whole body plethysmography. Baseline ventilatory parameters were determined by whole body plethysmography, and a comprehensive review of the methods and results are described in previous communications (24–26). Briefly, Ti at baseline was determined by recording the time of inspiration as the ascending limb of a pressure signal by using a data-acquisition system and a dedicated computer. Data were acquired at an input frequency of 100 Hz. Each measurement was determined from ~15 consecutive tidal breaths, and the average of five replicate measurements represented a given individual response.

Quasi-static P–V curves. Animals were anesthetized with intraperitoneal injections of sodium pentobarbital at a dose of 70–80 mg/kg body weight. After the trachea was cannulated while the animal was in a supine position, each animal was ventilated with 100% O2 for 10 min before the cannula was sealed with a stopcock to degas the lung (11). Quasi-static P–V curves were immediately performed in situ, first with the respiratory system intact and then after widely opening the thorax. The rate of inflation and deflation was standardized by a dual infusion-withdrawal pump (model 900–610, Harvard Apparatus, Dover, MA), and the airway pressure was measured by using a differential-pressure transducer (model 8510B-2, Endevco, San Juan Capistrano, CA). The initial inflation rate was very slow (0.5 ml/min) to ensure that all lung regions were fully opened without excessive pressures. Once a pressure of 30 cmH2O was reached, the flow rate was increased to 2.1 ml/min for the remaining inflation and deflation maneuvers. The limits of the inflation and deflation airway pressures were 30 and −10 cmH2O, respectively. Sequential P–V loops, with and without chest wall effects, were generated for two to three complete cycles. Because repeated inflation and deflation curves were reproducible, the second or third cycle was used in the data analysis.

Residual volume and total lung capacity (TLC) were determined as the volumes on deflation at −5 and 30 cmH2O, respectively, with the respiratory system intact. Functional residual capacity was also defined as the deflation volume at 0 cmH2O. Both compliance of the intact respiratory system (Crs) and C1 were computed from the slopes of the P–V relationships between 0 and 5 cmH2O on deflation. Hysteresis in the P–V relationship was further quantified by integration of the area bordered by the inflation and deflation curves and normalization of volume to individual TLC (4).

Data analysis. Data were summarized in the figures and tables as means ± SE. Lung volume measurements at specific airway pressures were analyzed by two-way ANOVA corrected for repeated measures to test for strain and airway-pressure effects. Pressure-dependent mean comparisons among strains were detected by using Fisher's protected least significant difference test (SuperANOVA). For each statistical test, the confidence level was maintained at 95%.

RESULTS

In Figure 1, inflation and deflation P–V relationships are reported for the three inbred strains with the respiratory system intact. Relative to both A/J and B6 mice, the C3 strain demonstrates a significantly (P < 0.01) greater lung volume at every airway pressure between 0 and 30 cmH2O on both inflation and deflation. This is particularly evident at the peak airway pressure of 30 cmH2O, at which the lung volume in C3 mice (~1.5 ml) was 50% greater compared with the two other strains (~1.0 ml). The lung volumes of the B6 and A/J strains are not distinguishable at an airway pressure of 30 cmH2O.

Figure 2 shows average deflation curves of the three standard inbred strains while the respiratory system is intact and after the effects of the chest wall are removed by widely opening the thoracic cavity. This figure clearly demonstrates a negligible mechanical effect of the mouse chest wall in all strains. Thus any observed differences between strains in lung volume or C1 at airway pressures between 0 and 25 cmH2O are representative of genetic variation in lung mechanics. The lung volume of C3 mice is substantially greater than that of the other strains, and the A/J mice demonstrate a slightly but significantly (P < 0.05) greater lung volume compared with B6 mice at intermediate airway pressures of 5–10 cmH2O.

In Figure 3A, intermediate lung volumes on inflation and deflation are normalized for the volume achieved at 30 cmH2O (i.e., percent TLC). On lung inflation, C3
mice demonstrate significantly ($P < 0.05$) smaller lung volumes as a percentage of TLC at intermediate airway pressures of 10–15 cmH$_2$O. On lung deflation, B6 mice show a significantly ($P < 0.05$) reduced lung volume compared with A/J and C3 mice at airway pressures between 5 and 10 cmH$_2$O. The differential inflation and deflation characteristics among the strains contribute to significant strain variation in the P-V hysteresis; that is, the area enclosed by the inflation and deflation curves normalized for TLC is significantly ($P < 0.01$) smaller in the B6 strain relative to A/J and C3 mice (Fig. 3B).

Figure 4 illustrates the average deflation curves among C3 and B6 progenitors and the F1 offspring. The lung volume of the F1 progeny is significantly ($P < 0.01$) smaller compared with that of C3 mice and is indistinguishable from that of the B6 parental strain (Fig. 4A). To evaluate whether or not features that define the shape of the curve are inheritable, deflation lung volumes were normalized for TLC (i.e., the lung volume achieved at 30 cmH$_2$O). In C3 mice, lung volumes at intermediate pressures between 5 and 10 cmH$_2$O are significantly ($P < 0.05$) greater relative to both the B6 strain and the F1 offspring.

Table 1 summarizes the lung-volume parameters for the three inbred strains and the F1 progeny. There were no distinguishable differences among groups with respect to age, body weight, or residual volume. Both the Crs and the Cl, as well as an index of hysteresis, are significantly ($P < 0.01$) greater in C3 compared with B6 and F1 mice. The A/J strain demonstrated an intermediate Cl phenotype that is significantly ($P < 0.05$) different compared with both C3 and B6 mice.

Because TLC and the Crs are the predominant traits that define differences between strains and are inheritable, we examined the covariation of TLC and Crs as a function of baseline TI by cosegregation analysis (Fig. 5). In this analysis, informative BXH RI strains are included to examine the potential genetic linkage between functional and structural phenotypes. As predicted, several strains with a relatively rapid TI are characterized with an average TLC in the range of 0.9–1.0 ml, and BXH RI strain 14 represents a phenotypic profile comparable to the B6 progenitor. The other extreme of the strain-distribution pattern is occupied by the C3 strain and BXH RI strain 8 with respect to baseline TI; however, TLC and Crs are significantly ($P < 0.01$) smaller in BXH RI strain 8 relative to the C3 strain. It is important to note that four of the eight BXH RI strains did not demonstrate the predicted cosegregation between baseline TI and TLC phenotypes. Particularly, BXH RI strains 4, 9, and 11 demonstrate shortened TI phenotypes associated with a relatively large TLC and Crs phenotypes, whereas BXH RI strain 6 shows a prolonged TI with modest TLC and Crs phenotypes.

**DISCUSSION**

The overall objective of the present study was to examine lung mechanical phenotypes of inbred mice strains that are routinely studied. Our focus on C3, B6, and A/J inbred strains is based on many recent studies (e.g., Refs. 9, 10, 16, 17, 24) that have used these strains.
to investigate genetic determinants central to various aspects of lung biology. After we identified both volume-dependent and -independent characteristics of the lung, we tested the hypothesis that the differential baseline breathing patterns between C3 and B6 parental strains and their segregant and nonsegregant offspring are genetically linked to or associated with differential lung mechanics. In short, the results are not consistent with the hypothesis that differential TI at baseline segregates with phenotypic variation in lung volume or CL. This conclusion is based on the BXH RI strain-distribution pattern, which shows a spectrum of discordant mechanical and ventilatory phenotypes. For example, the slow baseline TI characteristic of both BXH RI strain 8 and the C3 progenitor occurs coincident with significant strain differences in TLC and Crs (Fig. 5). In contrast, BXH RI strains 4, 9, and 11 demonstrate significantly larger lung volumes and higher CL compared with the B6 progenitor, whereas Ti at baseline is similar among these strains. This strongly suggests that genetic factors that determine differences in Ti at baseline between C3 and B6 mice are not genetically linked to loci responsible for differential lung mechanics. Therefore, the putative genomic region on mouse chromosome 3, which substantially influences the variation in baseline Ti between C3 and B6 mice (24), is not likely to be acting to determine differential lung volumes or compliant properties observed among these strains.

The differences in lung mechanics that we observed among mouse strains may deserve closer examination by using future genetic approaches. The differential lung volume and CL of C3 mice are the most prominent phenotypes, being significantly greater relative to age- and weight-matched B6 and A strains. More precisely, maximal lung volume (at 30 cmH2O) was 50% greater in C3 mice relative to the two other strains and the F1 progeny (Fig. 4 and Table 1). Similarly, the CL of C3 mice is two to three times greater than in the B6 parental strain and the F1 hybrid. Relative to other rodent species (2), the weight-corrected CL of C3 mice is two to three times greater than that in the guinea pig and rat (4.24 vs. 1.50 and 1.94 ml·cmH2O⁻¹·kg⁻¹, respectively). The normalized CL of B6 and F1 mice (1.74 ml·cmH2O⁻¹·kg⁻¹) more closely matches that in these other rodents. Therefore, the C3 phenotype appears to be the outlier among these other rodent species and mouse strains. Because there are no distinguishable differences in TLC and Crs between B6 and F1 mice, these phenotypes are dominant relative to the C3 parental strain. The CL of the A/J strain (2.93 ml·cmH2O⁻¹·kg⁻¹) represents an intermediate phenotype relative to the C3 and B6 strains.

More subtle strain differences are detectable in the shape of the P-V relationship. After normalization for strain difference in TLC, a significantly greater pulmonary hysteresis is evident in the C3 strain relative to both B6 and A/J strains. In particular, at intermediate airway pressures between 10 and 15 cmH2O, C3 mice demonstrated a larger hysteresis compared with B6 and A/J strains (Fig. 3A). At lower intermediate airway pressures, between 5 and 10 cmH2O, B6 mice show significantly less hysteresis compared with the other strains. The hysteretic characteristics of the B6 progenitor are not different in the F1 progeny (Fig. 3A and Table 1).

The strain-specific phenotypes in lung mechanics are not appreciably modified by removing influences of the chest wall (Fig. 2). These results are consistent with previously described P-V relationships in mice in which chest wall compliance was shown to be much greater than Cl (8, 15). In a comparison of species, Crosfill and Widdicombe (8) reported that chest wall compliance of mice, although quite variable, was on average six to seven times greater than Cl. In our experiments, the chest wall compliance was at least this much greater than that of the lung. Indeed, in the normal transpulmonary pressure range, we could not consistently detect
any differences between the P-V curves done with and without the chest wall.

In the present study, strain differences are largely attributable to genetic variation in the elastic recoil properties of the lung; in turn, these properties are characterized by tissue and surfactant protein properties as well as surface tension components (12). Tissue elastic fibers of the lung are primarily composed of the protein tropoelastin, which is encoded by the Eln gene on mouse chromosome 5. Allelic forms of the Eln gene have been shown to be similar between the B6 and A/J strains (28), but it is unknown whether or not the C3 strain is characterized with an alternative allelic form at this locus.

Pulmonary surfactant, a complex mix of phospholipids and proteins, contributes to the P-V hysteresis of an air-filled lung by modifying surface-tension characteristics (5, 7). More specifically, the normalized lung volume on deflation has been used as an indicator for the presence of functional surfactant (18, 19). The expression of polymorphic genes that encode a number of surfactant proteins may also contribute to strain variation in P-V hysteresis. At intermediate airway pressures, between 5 and 10 cmH₂O, the lung volume normalized for TLC was significantly greater in C3 mice than B6 mice (Fig. 4B). These results suggest that, on deflation, elastic recoil in the lungs of C3 mice is inhibited by different surface properties relative to B6 and F₁ mice (Fig. 4).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Age, days</th>
<th>Weight, g</th>
<th>Residual volume, ml</th>
<th>Functional residual capacity, ml</th>
<th>Total lung capacity, ml</th>
<th>Crs, ml/cmH₂O</th>
<th>Cl, ml/cmH₂O</th>
<th>Index of hysteresis, cmH₂O²</th>
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<tr>
<td>C3H/HeJ</td>
<td>54.2 ± 3.4</td>
<td>25.0 ± 0.8</td>
<td>0.14 ± 0.02</td>
<td>0.37 ± 0.03</td>
<td>1.14 ± 0.05</td>
<td>0.106 ± 0.005</td>
<td>0.126 ± 0.005</td>
<td>7.39 ± 0.30</td>
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<td>A/J</td>
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<td>C57BL/6J</td>
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<td>B6C3F₁/J</td>
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Values are means ± SE. Crs, compliance of the respiratory system; Cl, compliance of lung. *C3H/HeJ vs. other 3 strains, P < 0.01; †A/J vs. C57BL/6J, P < 0.05.
difference in ventilatory hypercapnic responsiveness does not appear to be explained by variation in lung volume or mechanics.

In summary, differential lung mechanics in inbred mice are characterized by volume-dependent and volume-independent components. The phenotype of lung mechanics in C3 mice represents greater lung volume and Cl than in other inbred strains. Influential genetic factors have been proposed to enhance lung volumes in human subpopulations that are isolated by altitude (14). Enhanced lung volumes in both mice and humans are likely the result of a greater number of alveoli recruited for a given airway pressure. Whether or not lung growth contributes to a greater alveolar number in C3 mice is one hypothesis requiring future study. In contrast, B6 mice are characterized by a TLC similar to other inbred strains; however, the P-V hysteresis of this strain is markedly reduced and results in attenuated intermediate lung volumes on deflation. Because the index of hysteresis used in the present study is a volume-independent component, the phenotype of lung mechanics in C3 mice represents greater lung volume and Cl than in other inbred strains. Influential genetic factors have been proposed to enhance lung volumes in human subpopulations that are isolated by altitude (14).

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Address for reprint requests and other correspondence: C. G. Tankersley, Div. of Physiology, The Johns Hopkins University School of Hygiene and Public Health, 615 N. Wolfe St., Baltimore, MD 21205.

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