Frequency dependence of respiratory system mechanics during induced constriction in a murine model of asthma

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Evans, Kenda L. J., Richard A. Bond, David B. Corry, and Felix R. Shardonofsky. Frequency dependence of respiratory system mechanics during induced constriction in a murine model of asthma. J Appl Physiol 94: 245–252, 2003; 10.1152/japplphysiol.00593.2002.—Airway dysfunction in asthma is characterized by hyperresponsiveness, heterogeneously narrowed airways, and closure of airways. To test the hypothesis that airway constriction in ovalbumin (OVA)-sensitized OVA-intranasally challenged (OVA/OVA) mice produces mechanical responses that are similar to those reported in asthmatic subjects, respiratory system mechanics during intravenous methacholine (MCh) infusions. In control mice, MCh at 1,700 µg·kg−1·min−1 produced 1) a 495 and 928% increase of Rrs at 0.5 Hz and 19.75 Hz, respectively, 2) a 33% rise in Edyn,rs at 0.5 Hz, and 3) a mild frequency (f)-dependent increase of Edyn,rs. The same MCh dose in OVA/OVA mice produced 1) elevations of Rrs at 0.5 Hz and 19.75 Hz of 1,792 and 774%, respectively, 2) a 390% rise in Edyn,rs at 0.5 Hz, and 3) marked f-dependent increases of Edyn,rs. During constriction, the f dependence of mechanics in control mice was consistent with homogeneous airway narrowing; however, in OVA/OVA mice, f dependence was characteristic of heterogeneously narrowed airways, closure of airways, and airway shunting. These mechanisms amplify the pulmonary mechanical responses to constrictor stimuli at physiological breathing rates and have important roles in the pathophysiology of human asthma.

Airway closure; resistance; elastance; airway hyperresponsiveness

Airway narrowing is a major abnormality in the mechanism of diseases affecting pediatric and adult patients. Reductions in airway diameters can be predominately homogeneous or heterogeneous in nature; i.e., decreases in airway diameters can display a minimal or a large variability throughout the airway tree, respectively (5, 14). These categories of airway constriction bring about distinct patterns of frequency-dependent variations of lung mechanics. For example, homogeneously narrowed airways produce increases in lung resistance (Rl) at physiological breathing rates that are commensurate to those of Rl at high frequencies (5, 14). In contrast, heterogeneous airway narrowing is associated with increases of Rl at physiological breathing rates that exceed those of Rl at high frequencies (5, 14). Furthermore, a large variability in the reduction of airway caliber is more likely to produce closure of airways, which enhances the magnitude of dynamic lung elastance (Edyn,L) and may impair gas exchange.

Pulmonary resistance and Edyn,L spectra obtained in subjects with severe asthma during periods of clinical stability and in subjects with mild or moderately severe asthma during agonist-induced constriction are consistent with a pattern of severe and heterogeneous airway constriction and closure of airways (11, 12, 15). These manifestations of airway dysfunction are of paramount clinical importance because marked elevations of Rl and Edyn,L at physiological breathing rates may lead to respiratory failure and eventually death. Results from a model analysis of airway constriction in asthma suggest that airway smooth muscle shortening produces large variations in the magnitude of decrease in airway diameters throughout the airway tree as a result of inflammation and remodeling of asthmatic airways (6).

Murine models of asthma exhibit a Th2-lymphocyte-driven eosinophilic pulmonary inflammation and airway hyperresponsiveness, which are important features of human asthma. These animal models have been shown to be excellent tools to test hypotheses pertaining to mechanisms of this disease. Whether murine models of asthma exhibit heterogeneous or homogeneous airway narrowing in response to airway smooth muscle activation remains to be determined because pulmonary mechanical responses have generally been assessed at a single respiratory rate in previously reported studies (24). The purpose of the present study was to test the hypothesis that cholinergic-induced airway constriction in a murine model of eosinophilic pulmonary inflammation and airway hy-

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per responsiveness induces a frequency-dependent pattern of mechanics consistent with that of heterogeneous airway narrowing and airway closures, thus resembling key features of human asthma. This hypothesis is based on the fact that the histological abnormalities found in murine models of human asthma, such as peribronchial accumulation of inflammatory cells, thickened airway walls infiltrated with eosinophils, lymphocytes, and monocytes, goblet cell hyperplasia, increased mucous production, and mucous and debris within the airway lumen, are distributed non-homogeneously within the lung (24). Thus, in the presence of nonhomogeneously distributed inflammatory changes, the activation of airway smooth muscle is expected to cause heterogeneous airway narrowing and closure in the animal model. The patterns of resistance and elastance spectra described by Gillis and Lutchen (5) in a model of human airways subjected to homogeneous and heterogeneous constriction were used to interpret the impedance spectra obtained in our mice, notwithstanding marked differences in airway morphology between humans and mice.

METHODS

Animals. Balb/cj mice aged 6 wk were purchased from Jackson Laboratories and housed under specific pathogen-free conditions. All the experiments were approved by the Animal Research Ethics Boards of Baylor College of Medicine and of the University of Houston.

Experimental protocol. The respiratory system resistance (Rrs) and elastance (Edyn,rs) spectra obtained under baseline conditions and during methacholine-induced constriction in a murine model of ovalbumin (OVA)-induced pulmonary eosinophilia inflammation and airway hyperresponsiveness were compared with those obtained in control mice. Seventeen mice, designated OVA/OVA, were systemically sensitized with a subcutaneous injection of 25 μg of chicken OVA adsorbed to aluminum hydroxide on protocol days 1, 7, and 14. Subsequently, they were given 50 μl of saline solution containing 25 μg of OVA intranasally on a daily basis, from protocol days 21 through 25 (31). Twenty-one mice, which served as controls, received either systemic sensitization with OVA or intranasal administrations of OVA following a timeline identical to that of the study mice. Before intranasal administrations, mice were sedated with halothane vapor. On protocol day 26, mice underwent pulmonary physiology studies. Subsequently, mice were euthanized and a bronchoalveolar lavage was performed with 2 ml of phosphate-buffered saline solution.

Animal preparation. Mice were anesthetized with an intraperitoneal injection of 0.5–0.7 ml/kg of a compound containing ketamine (42.8 mg/ml), xylazine (8.6 mg/ml), and acepromazine (1.4 mg/ml) (Center for Comparative Medicine, Baylor College of Medicine). An additional dose of anesthetic compound was given 30 min after the initial dose. As soon as the animal’s lack of both corneal reflex and motor responses to noxious stimuli was confirmed, a 27 × 3/8 butterfly needle (Abbott, Chicago, IL) was inserted in a tail vein for drug administration. Subsequently, the trachea was exposed through a midcervical incision, a tracheotomy was performed just below the cricoid cartilage, and a plastic cannula (Abbocath-T, 20G, VeniSystems) ~6 mm long was inserted into the trachea and secured with silk ties. The tracheal cannula was connected to a computer-controlled small-animal mechanical ventilator (Flexivent, Scientific Respiratory Equipment, Montreal, Canada). Mice were mechanically ventilated in the supine position with supplemental oxygen, the tidal volume and respiratory rate being 8–10 ml/kg and 180 breaths per min, respectively. To achieve a mean lung volume close to that during spontaneous breathing, the respiratory system was kept at a positive end-expiratory pressure of 2 cmH2O (18). This was achieved by connecting the expiratory port of the ventilator to a water column. Muscle paralysis was induced with an intraperitoneal injection of 0.1 mg of pancuronium bromide (Abbott).

Pulmonary physiology studies. Respiratory system mechanics was assessed by use of the forced oscillation technique. To this end, the regular mechanical ventilation was discontinued for 9 s, during which the respiratory system was allowed to expire passively down to the functional residual capacity determined by the applied positive end-expiratory pressure for 1 s (7), after which an 8-s volume perturbation was delivered to the airway opening by the small animal ventilator. Immediately thereafter, regular mechanical ventilation was resumed. Thirty seconds before data collection was begun, the respiratory system was standardized by inflating the lungs to total lung capacity twice. This was achieved by closing the ventilator’s expiratory line until a transrespiratory pressure of ~30 cmH2O was reached.

The 8-s signal used to drive the piston of the ventilator for measurements of respiratory input impedance (Zin,rs) was composed of 18 sinusoids having mutually prime frequencies from 0.5 to 19.75 Hz (1, 7). The amplitude of the sinusoids decreased hyperbolically with frequency so that the value of the power spectrum of flow was similar in the frequency range used. The phases of the sinusoids were chosen to minimize the peak-peak amplitude of the volume perturbation signal, which was ~30% of the tidal volume used during mechanical ventilation.

Before each mouse was connected to the mechanical ventilator, calibration signals were collected by applying the volume perturbation signals through the tracheal cannula, first with the cannula fully closed and then with it open to atmosphere, to estimate the elastance of the measuring equipment and the airflow resistance of the tracheal cannula (23).

Airway smooth muscle activation. To induce airway constriction, a solution containing acetyl-β-methylcholine chloride (methacholine) (Sigma Chemical, St. Louis, MO) at a concentration of 150 μg/ml was infused intravenously by use of a syringe infusion pump (Raze Scientific Instruments, Stanford, CT), starting at a rate of 0.008 ml/min. The rate of infusion was doubled in a stepwise fashion up to a maximum of 0.272 ml/min. Each dose of methacholine was administered at a constant rate for 3–5 min, during which data were sampled at 1-min intervals. Thus each reported index of respiratory system mechanics response was the mean value of those obtained during steady-state methacholine-induced airway smooth muscle activation. The coefficient of variation of the measurements obtained during a given methacholine dose was <0.08. After the administration of methacholine was discontinued, mice were monitored until the respiratory system mechanics returned to values within 30% of those at baseline, which occurred within 6–8 min. On average, experiments lasted 45 min.

Data analysis. Each data set consisted of 8-s signals of piston volume displacement (Vcyl) and cylinder pressure (Pcyl). These were low-pass filtered at 30 Hz and sampled at 128 Hz before being stored on a personal computer (Dell 5133, Austin, TX). The complex Zin,rs was computed as previously described (23). Briefly, the Pcyl and Vcyl signals

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were subdivided into four blocks of 4 s, with adjacent blocks overlapping each other by 50%. The linear trend due to continuous oxygen consumption was removed from each block by fitting a straight line to the data in each block and subtracting it from the block. The cross-correlation between pressure and flow (Cvp) was calculated by multiplying the fast Fourier transform (FFT) of each Cvp block by the complex conjugate of the FFT of the corresponding Cpp block. The autocorrelation of volume (Cvv) was estimated as the product of the FFT of each Cpp block and its conjugate. The results of Cvp and Cpp for all blocks were then averaged. Finally, the transfer function (H) between volume and pressure was calculated as 
\[ H = \frac{Cvp}{Cvv} \]  
Zin,rs was finally computed by dividing H by \( -2\pi f_i \), where \( f_i \) is the positive square root of \(-1\) and \( 2\pi f \) is angular frequency. The Rrs was determined as the real part (Re) of Zin,rs \( Rrs(f_k) \). Coherence was calculated between the Flexivent’s cylinder pressure and piston flow; data that had a coherence of \(<0.95\) were discarded (\(\sim2\%\) of all data). A linear model containing frequency-independent resistance (Raw) and inertance (Iaw) in series with a constant-phase tissue compartment containing a tissue damping (Gti) and stiffness (Hti) was fitted to the Zin,rs spectra by minimizing the absolute difference between the measured and the modelled impedance data.

\[ Z_{in,rs} = \text{Raw} + j\omega \text{Iaw} + \left(\frac{\text{Gti}}{f} - \frac{\text{Hti}}{f^2}\right) \]  

A custom-made software written by J. H. T. Bates was used to compute the Zin,rs spectra and to perform the model-fitting analysis. The respiratory system hysteresivity \( (\eta) \), a dimensionless number that reflects the relationship between energy dissipation and energy storage within the respiratory tissues, was estimated as \( \eta = \frac{Gti}{Hti} \). The model parameters were used to characterize the airway and lung tissue properties under control conditions. The constant-phase model did not fit the Zin,rs data obtained in OVA/OVA mice during induced constriction. Thus the Rrs and Edyn,rs spectra were analyzed, and the values of Rrs at 19.75 Hz were taken to reflect the mean Raw.

To examine the degree of airway responsiveness of each animal, the values for Raw as a function of log2 methacholine doses were plotted, and the values for reactivity and sensitivity to methacholine were estimated. The rate of increase of Raw or reactivity to methacholine was calculated by multiple linear regression analysis. The sensitivity to methacholine was reflected by the dose of methacholine that increased the value of Raw by 200% above baseline (ED200) and was calculated by linear interpolation.

**Examination of cell type in BALF.** After completion of pulmonary physiology studies, mice were killed, and then 2 ml of phosphate-buffered saline solution were injected into the trachea and gently suctioned. The total number of cells in the bronchoalveolar lavage fluid (BALF) was determined by use of a hemacytometer. Cytospin preparations of BALF were stained with Hema-3 (Fisher Scientific) and examined by use of oil-immersion microscopy. Cells were identified as eosinophils, macrophages, lymphocytes, and neutrophils by standard morphological criteria. At least 200 cells were counted per cytospin preparation, and the absolute number of each cell type was calculated on the basis of the volume of fluid recovered from lavage.

**Statistical analysis.** Results are expressed as means \( \pm \) SE. Comparisons between the two groups of mice were analyzed by using unpaired t-tests. The statistical analysis was performed by using the SPSS statistical package (SPSS, Chicago, IL). A value of \( P < 0.05 \) was considered statistically significant.

**RESULTS**

**Cellular composition of bronchoalveolar lavage fluid.** In OVA/OVA mice, the numbers of lymphocytes and eosinophils recovered in BALF were significantly increased relative to those in control animals (Table 1).

**Respiratory system mechanics under baseline conditions.** The values for Rrs decreased quasi-hyperbolically with frequency, reflecting the viscoelastic behavior of the respiratory tissues (Fig. 1A). The values for Rrs at frequencies lower than 2 Hz were significantly enhanced \((P < 0.01)\) in OVA/OVA mice relative to those in control mice. In all mice, Edyn,rs increased slightly with frequency up to 10 Hz, as a consequence of the viscoelastic properties of the respiratory tissues and asynchronous displacements of the chest wall, which is normally highly compliant in mice (13) (Fig. 1B). At frequencies higher than 10 Hz, Edyn,rs decreased, reflecting the effects of airway inertance on \( \text{Im}(\text{Zin,rs}) \). Whereas the frequency dependence of Edyn,rs was similar in both groups of mice, the values of Edyn,rs were higher in OVA/OVA mice than in control mice \((P < 0.001)\). These differences were not accounted for by disparities in the animals’ body weight, which averaged 23.5 \( \pm \) 0.3 and 22.7 \( \pm \) 0.3 g in OVA/OVA and control mice, respectively \((P = \text{not significant})\). In line with the differences in Zin,rs spectra between the two groups of mice shown in Fig. 1, the values for Gti and Hti were significantly higher in mice having pulmonary inflammation than in control animals (Table 2). The mean value for respiratory system hysteresivity was 0.13 for both groups of mice.

**Table 1. Cellular composition of bronchoalveolar lavage fluid**

<table>
<thead>
<tr>
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<th>Control ((n = 12))</th>
<th>OVA/OVA ((n = 13))</th>
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<tbody>
<tr>
<td>Eosinophil ((\times 10^4 \text{ ml}^{\text{-1}}))</td>
<td>3.6 ( \pm ) 3.1</td>
<td>300.9 ( \pm ) 85.3*</td>
</tr>
<tr>
<td>Lymphocyte ((\times 10^5 \text{ ml}^{\text{-1}}))</td>
<td>1.3 ( \pm ) 0.6</td>
<td>32.0 ( \pm ) 14.0*</td>
</tr>
<tr>
<td>Neutrophil ((\times 10^4 \text{ ml}^{\text{-1}}))</td>
<td>1.4 ( \pm ) 0.6</td>
<td>10.5 ( \pm ) 2.4</td>
</tr>
<tr>
<td>Macrophage ((\times 10^5 \text{ ml}^{\text{-1}}))</td>
<td>85.0 ( \pm ) 34.7</td>
<td>98.7 ( \pm ) 28.3</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE; \( n \) = no. of mice. OVA/OVA, ovalbumin-sensitized, ovalbumin-challenged mice. *\( P < 0.05 \).
system resistance (Rrs) and elastance (Edyn,rs) as a function of respiratory system input impedance data obtained in mice under baseline conditions (OVA)-sensitized OVA-intranasally challenged (OVA/OVA) mice (○; n = 17). Relationships of airway resistance (Raw) and Edyn,rs elastance at 0.5 Hz as a function of methacholine dose are depicted in A and B, respectively. Symbols represent mean ± SE values.

Fig. 2. Respiratory system responses to cholinergic stimulation in control (○; n = 21) and OVA/OVA mice (●; n = 17). A and B illustrate the mean ± SE values for respiratory system resistance (Rrs) and elastance (Edyn,rs) as a function of frequency, respectively. Inset in A shows Rrs-vs.-frequency relationships at frequencies <2 Hz. *P < 0.05 and **P < 0.01, respectively. ***P < 0.001 at frequencies <16.75 Hz.

Responsiveness to methacholine. The airway responsiveness to methacholine was significantly enhanced in OVA/OVA mice compared with that in control mice (Fig. 2A). This difference was reflected by values for reactivity to methacholine that were higher in OVA/OVA mice than in control mice (reactivity: 0.88 ± 0.011 vs. 1.47 ± 0.09 cmH2O·mI-1·s/log2 μg·kg-1·min-1 methacholine for control and OVA/OVA mice, respectively; P = 0.001) and values for ED500 that were significantly lower in OVA/OVA mice than in the control animals (log2 ED500: 8.24 ± 0.11 vs. 7.43 ± 0.10 μg·kg-1·min-1 for control and OVA/OVA mice, respectively; P < 0.001). Moreover, methacholine elicited a uniform and modest increase in Edyn,rs at 0.5 Hz in control mice, whereas it produced a biphasic response of Edyn,rs at 0.5 Hz in OVA/OVA mice (Fig. 2B).

Fig. 1. Frequency (f) dependence of respiratory system mechanics under baseline conditions in control mice (○; n = 21) and ovalbumin (OVA)-sensitized OVA-intranasally challenged (OVA/OVA) mice (●; n = 17). A and B illustrate the mean ± SE values for respiratory system resistance (Rrs) and elastance (Edyn,rs) as a function of frequency, respectively. Inset in A shows Rrs-vs.-frequency relationships at frequencies <2 Hz. *P < 0.05 and **P < 0.01, respectively. ***P < 0.001 at frequencies <16.75 Hz.

Frequency dependence of mechanics during induced constriction. The inspection of the Zin,rs spectra revealed marked disparities of frequency dependence of mechanics during induced constriction between the two groups of mice. To examine these differences, Rrs and Edyn,rs spectra obtained at similar levels of Raw were compared. The data illustrated in Fig. 3 were obtained at mean methacholine log2 doses of 8.7 and 7.8 μg·kg-1·min-1 in control and OVA/OVA mice, respectively. These doses increased the values for Raw to 17.9 ± 0.18 and 18.42 ± 0.91 cmH2O·mI-1·s in control and OVA/OVA mice, respectively (P = not significant).

In control mice, the percent increase of Raw above baseline (ΔRaw%) was greater than that of Rrs at 0.5 Hz (ΔRrs0.5Hz%) (372 ± 41 vs. 223 ± 29%; P < 0.01). Similar changes were observed in OVA/OVA mice, although the differences between ΔRaw% and ΔRrs0.5Hz% were not statistically significant (415 ± 70 vs. 265 ± 57%; P = not significant). In addition, the magnitude of Rrs at 0.5 Hz tended to be greater in OVA/OVA mice than in controls (P = 0.05) (Fig. 3A).

These Rrs spectra showing increases of Raw that are

Table 2. Average ± SE values of constants in Eq. 2 obtained by fitting the constant-phase model to the respiratory system input impedance data obtained in mice under baseline conditions

<table>
<thead>
<tr>
<th>Group</th>
<th>Raw, cmH2O·mI-1·s</th>
<th>Gti, cmH2O·s·(mg·kg)-1·mI-1</th>
<th>Hti, cmH2O·s·(mg·kg)-1·mI-1</th>
<th>α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 21)</td>
<td>0.37 ± 0.01</td>
<td>2.52 ± 0.04</td>
<td>18.42 ± 0.03</td>
<td>0.91 ± 0.00</td>
</tr>
<tr>
<td>OVA/OVA (n = 17)</td>
<td>0.40 ± 0.01</td>
<td>2.91 ± 0.10*</td>
<td>21.63 ± 0.82*</td>
<td>0.91 ± 0.00</td>
</tr>
</tbody>
</table>

Raw, airway resistance plus Newtonian chest wall resistance; Gti, respiratory tissue damping; Hti, respiratory tissue elastance; α = (2/π) arctan (Hti/Gti). *P < 0.001.

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equal to or greater than those of Rrs at low frequencies are consistent with the Rrs spectra predicted by a model of homogeneous airway narrowing of human airways of \( \frac{H}{11021} \) 2 mm in diameter (5, 14). The Edyn,rs spectra obtained in the two groups of mice were markedly different (Fig. 3B). The percent increase of Edyn,rs at 0.5 Hz above baseline (\( \frac{H}{9004} \) \( \text{Edyn,rs}_{0.5 \text{Hz}} \% \)) was 17\( \pm \) 3 and 46\( \pm \) 9\% for control and OVA/OVA mice, respectively (\( P < 0.001 \)) (Fig. 2B). The relative increase of Edyn,rs from 0.5 to 10 Hz was similar in the two groups of mice; at frequencies greater than 10 Hz, the values for Edyn,rs remained elevated in OVA/OVA mice, whereas they decreased progressively in control mice.

To further analyze the frequency dependence of mechanics during progressively increased airway narrowing, the average ratios of Rrs at 0.5 Hz to Raw (Rrs0.5Hz/Raw), of Edyn,rs at 9.25 Hz to Edyn,rs at 0.5 Hz were obtained at methacholine mean log2 doses of 10.220.33.1 on April 7, 2017 http://jap.physiology.org/ Downloaded from 10.8\( g \) kg\(^{-1}\) min\(^{-1}\) in both sets of mice, at which the values of Raw increased to 3.93\( \pm \) 0.42 and 3.38\( \pm \) 0.36 cmH\(_2\)O m\(^{-1}\) l\(^{-1}\) s in control and OVA/OVA mice, respectively (\( P = \text{not significant} \)). In control mice, the frequency-dependent changes of Rrs were similar to those in Fig. 3A, in that \( \Delta \text{Rrs}_{0.5 \text{Hz}} \% \) was greater than \( \Delta \text{Rrs}_{0.5 \text{Hz}} \% \) (928\( \pm \) 83 vs. 495\( \pm \) 45\%, \( P < 0.01 \)). In contrast, OVA/OVA mice exhibited Rrs spectra characterized by increases in Rrs at 0.5 Hz (1,792\( \pm \) 153\%) that were significantly greater (\( P < 0.001 \)) than those of Raw (774\( \pm \) 86\%). The magnitude of Rrs, which at 0.5 Hz was markedly enhanced in the experimental animals relative to controls (22.95\( \pm \) 4.15 vs. 6.98\( \pm \) 0.55 cmH\(_2\)O m\(^{-1}\) l\(^{-1}\) s, \( P < 0.001 \)), showed a pronounced frequency-dependent decrease in OVA/OVA mice. The features of these Rrs spectra are consistent with those predicted by a model of severe and heterogeneous airway narrowing (5, 14).

The differences in Edyn,rs spectra between the two groups of mice shown in Fig. 3B became more pronounced at a greater level of constriction (Fig. 4B). The values for \( \Delta \text{Edyn,rs}_{0.5 \text{Hz}} \% \) increased to 33\( \pm \) 3 and 390\( \pm \) 67\% in control and experimental mice, respectively (\( P < 0.001 \)) (Fig. 2B). The relative increase of Edyn,rs from 0.5 to 10 Hz was similar in the two groups of mice; at frequencies greater than 10 Hz, the values for Edyn,rs remained elevated in OVA/OVA mice, whereas they decreased progressively in control mice.

In control mice, the frequency-dependent changes of Rrs were similar to those in Fig. 3A, in that \( \Delta \text{Rrs}_{0.5 \text{Hz}} \% \) was greater than \( \Delta \text{Rrs}_{0.5 \text{Hz}} \% \) (928\( \pm \) 83 vs. 495\( \pm \) 45\%, \( P < 0.01 \)). In contrast, OVA/OVA mice exhibited Rrs spectra characterized by increases in Rrs at 0.5 Hz (1,792\( \pm \) 153\%) that were significantly greater (\( P < 0.001 \)) than those of Raw (774\( \pm \) 86\%). The magnitude of Rrs, which at 0.5 Hz was markedly enhanced in the experimental animals relative to controls (22.95\( \pm \) 4.15 vs. 6.98\( \pm \) 0.55 cmH\(_2\)O m\(^{-1}\) l\(^{-1}\) s, \( P < 0.001 \)), showed a pronounced frequency-dependent decrease in OVA/OVA mice. The features of these Rrs spectra are consistent with those predicted by a model of severe and heterogeneous airway narrowing (5, 14).

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To further analyze the frequency dependence of mechanics during progressively increased airway narrowing, the average ratios of Rrs at 0.5 Hz to Raw (Rrs0.5Hz/Raw), of Edyn,rs at 9.25 Hz to Edyn,rs at 0.5 Hz

Fig. 3. Comparison of Rrs (A) and Edyn,rs (B) spectra at similar levels of Raw in control mice (\( \bullet ; n = 21 \)) and OVA/OVA mice (\( \circ ; n = 17 \)). Data were obtained at methacholine mean log2 doses of 8.7 and 7.8\( \mu \)g·kg\(^{-1}\)·min\(^{-1}\) in control and OVA/OVA mice, respectively. Symbols represent mean ± SE values.

Fig. 4. Comparison of Rrs (A) and Edyn,rs (B) spectra at similar levels of Raw in control mice (\( \bullet ; n = 21 \)) and OVA/OVA mice (\( \circ ; n = 17 \)). Data were obtained at methacholine mean log2 doses of 10.8\( g \) kg\(^{-1}\) min\(^{-1}\) in 2 two groups of mice. Symbols represent mean ± SE values.
Hz $\text{Edyn,rs}_{9.25Hz}/\text{Edyn,rs}_{0.5Hz}$, and of $\text{Edyn,rs}$ at 19.75 Hz to $\text{Edyn,rs}$ at 0.5 Hz ($\text{Edyn,rs}_{19.75Hz}/\text{Edyn,rs}_{0.5Hz}$) were plotted as a function of both methacholine dose and degree of mean airway constriction (Fig. 5). In control mice, the mechanical responses showed a monotonic behavior, which was characterized by a progressive decrease of $\text{Rrs}_{0.5Hz}/\text{Raw}$ (Fig. 5, A and D) and progressive increases of $\text{Edyn,rs}$ at 0.5 Hz (Fig. 2B) and of $\text{Edyn,rs}_{9.25Hz}/\text{Edyn,rs}_{0.5Hz}$ (Figs. 5, B and E). The $\text{Edyn,rs}_{19.75Hz}/\text{Edyn,rs}_{0.5Hz}$ did not change significantly in control mice. Taken together, these data indicate that control mice experienced homogeneous airway narrowing throughout the methacholine challenge. In contrast, responses of OVA/OVA mice demonstrated a distinct biphasic behavior. During the first phase, which took place during the first three doses of methacholine administered, the frequency dependence of $\text{Rrs}$ was similar to that in control mice and $\text{Edyn,rs}$ at 0.5 Hz increased by a small amount above its baseline values (Fig. 2B). Whereas the magnitude of $\text{Edyn,rs}_{9.25Hz}/\text{Edyn,rs}_{0.5Hz}$ and $\text{Edyn,rs}_{19.75Hz}/\text{Edyn,rs}_{0.5Hz}$ were similar in the two groups of mice during the first two doses of methacholine, those ratios were significantly increased at the third dose of methacholine in OVA/OVA mice compared with control mice. The second phase of the constrictor response in OVA/OVA mice came about during the last three doses of methacholine and was characterized by sudden and marked elevations of $\text{Edyn,rs}$ at 0.5 Hz (Fig. 2B) and $\text{Rrs}$ at low frequencies, the latter being reflected by significant increases in $\text{Rrs}_{0.5Hz}/\text{Raw}$ (Fig. 5, A and D). In addition, there was a marked frequency-dependent increase of $\text{Edyn,rs}$, which was reflected by significant elevations in $\text{Edyn,rs}_{9.25Hz}/\text{Edyn,rs}_{0.5Hz}$ and $\text{Edyn,rs}_{19.75Hz}/\text{Edyn,rs}_{0.5Hz}$ relative to those obtained in control mice (Fig. 5). Increases in the former ratio reflected the effects of parallel time-constant mechanical heterogeneity (21), whereas those in the latter ratio reflected the effect of airway shunt associated with severe narrowing and/or closure of airways (16).

**DISCUSSION**

Our results indicate that, in addition to airway hyperresponsiveness, mice induced to have eosinophilic pulmonary inflammation exhibit frequency-dependent respiratory system mechanics characteristics both under baseline conditions and in response to cholinergic stimulation that are significantly different from those observed in control mice. Under baseline conditions, our control mice exhibited values for $\text{Raw}$, respiratory $\text{Gti}$, and $\text{Hti}$ that were similar to those reported by Tomioka et al. (28) in open-chest, nonimmunized mice under baseline conditions. Moreover, they reported no differences in the lung tissue properties between their murine model of asthma and control mice, whereas the baseline values for $\text{Gti}$ and $\text{Hti}$ in our mice with pulmonary eosinophilic inflammation were significantly increased relative to those in control mice (Table 2). The magnitude of hysteresivity was identical in both groups of animals, which indicates that the increase in energy dissipation ($\text{Gti}$) within the lung tissues of experimental mice was associated with a matched increase in energy storage ($\text{Hti}$) (2). Although the mech-

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**Fig. 5.** Frequency-dependent changes of respiratory system mechanics during induced constriction in control mice ($\bigcirc; n = 21$) and OVA/OVA mice ($\bullet; n = 17$). Ratios of $\text{Rrs}$ at 0.5 Hz to $\text{Raw}$ as a function of methacholine dose and $\text{Raw}$ are shown in A and D, respectively. Ratios of $\text{Edyn,rs}$ at 9.25 Hz to $\text{Edyn,rs}$ at 0.5 Hz as a function of methacholine dose and $\text{Raw}$ are shown in B and E, respectively. Ratios of $\text{Edyn,rs}$ at 19.75 Hz to $\text{Edyn,rs}$ at 0.5 Hz as a function of methacholine dose and $\text{Raw}$ are shown in C and F, respectively. Symbols represent mean ± SE values. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.
anism of this interesting finding is presently unknown, it is possible that the elevations of Gt and Ht in OVA/OVA mice reflect either opening and closing of airways, as suggested by the features of recently reported quasi-static pressure-volume relationships in mice with airway inflammation (29) or an increased number of cells within the lung tissue. Under baseline conditions, Edyn,rs showed a mild frequency dependence, the degree of which was similar in the two groups of animals (Fig. 1), suggesting that the distribution of ventilation under baseline conditions was not altered by airway inflammation. This finding is at variance with the marked frequency dependence of Edyn,rs reported in asthmatic subjects during periods of clinical stability (12), which suggests that asthmatic airways exhibit a substantial parallel-pathways time-constant heterogeneity (9, 21).

In line with the lung impedance spectra obtained in healthy subjects during induced constriction (11, 12), responses to methacholine in control mice were characterized by increases of airway resistance that were consistently greater than of Rrs at low frequencies (Figs. 3–5). In addition, it caused mild increases of Edyn,rs at 0.5 Hz (Fig. 2B) and modest frequency-dependent elevations of Edyn,rs at frequencies <10 Hz (Figs. 3–5). These changes were similar to those anticipated by a model analysis of homogeneous narrowing of human airways <2 mm in diameter (5, 14).

In contrast to the monotonic responses observed in control mice, mice having pulmonary eosinophilic inflammation responded to cholinergic stimulation in a biphasic fashion (Fig. 5, A and D). The first phase was characterized by small elevations of Edyn,rs at 0.5 Hz (Figs. 2B and 3) and increases of Rrs that exhibited a frequency dependence similar to that in control mice (Fig. 5, A and D). This initial phase likely reflected the effects of a moderately severe homogeneous airway narrowing in the murine model (5, 14). The second phase was characterized by a sudden and dramatic elevations of both Edyn,rs at 0.5 Hz (Fig. 2B) and Rrs at low frequencies (Figs. 3 and 4), the latter being reflected by pronounced increases in Rrs0.5Hz/Raw (Fig. 5, A and D). Furthermore, the second phase of the constrictor response was associated with a marked frequency-dependent increase of Edyn,rs (Figs. 3–5). The increases in the values of Edyn,rs9.25Hz/Edyn,rs0.5Hz and Edyn,rs19.75Hz/Edyn,rs0.5Hz likely reflected the effects of parallel time-constant mechanical heterogeneity (21) and airway shunt associated with severe narrowing and/or closure of peripheral airways (16), respectively. It is remarkable that the Rrs and Edyn,rs spectra obtained during the second phase of induced constriction in the murine model of asthma were similar to those reported in clinically stable asthmatic subjects (11, 12) and in subjects with mild or moderately severe asthma during induced constriction (15). Recently, Tomioka et al. (28) have investigated the pulmonary mechanical responses to methacholine in mice by using both the forced oscillation technique and the alveolar capsule technique. The methacholine dose-response relationships reported by these investigators in mice with pulmonary inflammation showed biphasic increases in Ht and Gt, which were qualitatively similar to that shown for Edyn,rs at 0.5 Hz in our OVA/OVA mice (Fig. 2B). The Gt and Ht are frequency-independent parameters that reflect, respectively, the dissipative (resistive) and conservative (elastic) mechanical properties of the lung tissue (28). However, differences in the experimental conditions between the two studies make direct comparisons difficult. Specifically, Tomiako and co-workers aerosolized methacholine to open-chest mice, whereas in the present study methacholine was delivered intravenously to closed-chest animals. Suki et al. (27) have reported that, in open-chest dogs, the pulmonary constrictor responses elicited by methacholine were diminished relative to those obtained in the same animals in closed-chest conditions. Peták et al. (22) have shown that the aerosol administration of methacholine produced greater elevations of Ht and Gt than those caused by the intravenous administration of methacholine. On histology, methacholine delivered as an aerosol has been shown to produce more heterogeneous reductions in airway diameters and greater distortion of the lung parenchyma compared with those associated with the intravenous administration of methacholine.

Several mechanisms have been invoked to explain the occurrence of exaggerated increases of Rrs at low frequencies. First, the analysis of constrictor responses in a human lung model by Lutchen and colleagues (5, 14) predicts that heterogeneous narrowing of airways <2 mm in diameter causes a pronounced increase in Rrs at low frequencies and a marked frequency-dependent decrease of Rrs as a result of substantial parallel (21) and serial mechanical heterogeneity (16, 22). The close similarity between the Rrs spectra predicted by the human model and those obtained in our murine model of asthma during high levels of constriction supports our hypothesis that mice with airway inflammation experience heterogeneous airway narrowing during airway smooth muscle activation. Unfortunately, the lack of morphometry data on murine airway trees makes it impossible to know which airways in the lung periphery underwent the heterogeneous decreases in airway diameter expected to occur in human airways of <2 mm in diameter. Second, increases in lung tissue resistance (Rti) will increase the magnitude of Rrs at low frequency. The magnitude of Rti in our OVA/OVA mice could have been increased as a consequence of agonist-induced activation of cellular elements within the lung parenchyma (3, 25), distortion of lung tissue (19, 20), and/or closure of airways. The latter can be accounted for by the non-Newtonian behavior of Rti. Thus an excessive expansion of the lung parenchyma that remains in communication with the airway opening will increase the magnitude of Rti. The presence of widespread closure of airways in our mice with pulmonary inflammation is supported by the sudden and marked increase of Edyn,rs at 0.5 Hz, the magnitude of Edyn,rs at 0.5 Hz at high levels of constriction being 10-fold greater than those in control mice (Fig. 2B). In addition to airway closure, other
mechanisms could have contributed to the observed increase in Edyn,rs at 0.5 Hz in OVA/OVA mice, such as changes in the viscoelastic properties of the lung tissues (2, 3), parallel time-constant heterogeneity (9, 21), and radial (17) and axial components (26) of active airway smooth muscle tension during airway smooth muscle activation.

It is remarkable that the two groups of mice exhibited significant differences in the frequency dependence of mechanics at similar levels of mean airway constriction (Fig. 5), suggesting that heterogeneously narrowed airways and closure of airways were associated with airway inflammation. Altered airway elastance, changes in the composition of airway surface fluid (30), and increased amount of mucus and debris within the airway lumen may have contributed to cause heterogeneous airway narrowing and airway closure. Presently, the relative importance of these mechanisms remains to be determined. Airway closure is a major abnormality in patients with severe asthma, as indicated by the reported lung impedance spectra (15) and the elevated values of closing volume (10).

In summary, airway dysfunction in mice with eosinophilic pulmonary inflammation is characterized by hyperresponsiveness and a frequency dependence of mechanics during induced constriction that reflects heterogeneous airway narrowing and closure of airways. This murine model, which resembles clinically significant features of human asthma, is expected to be an important tool to elucidate the mechanisms that cause heterogeneous narrowing and closure of airways in asthmatic subjects.

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