Airway and tissue mechanics in a murine model of asthma: alveolar capsule vs. forced oscillations

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ALTHOUGH ASTHMA IS TRADITIONALLY thought of as a disease of the pulmonary airways, it is becoming increasingly clear that mechanical abnormalities of the lung parenchyma are also important in this disease. The importance of the lung periphery in asthma is suggested by the finding that parenchymal lung resistance (Rl) may be significantly elevated, even in patients with spirometry within normal limits (13–15). Hyperpnea with dry cool gas has also been shown to alter parenchymal mechanical responses in asthmatic patients, which has been interpreted to be related more to parenchymal dysfunction or heterogeneous airway closure than to uniform airway narrowing (13, 14). Lung biopsies of patients with nocturnal asthma (16), as well as patients dying of status asthmaticus (1), provide compelling pathological evidence that asthmatic inflammatory processes involve the lung parenchyma. To the extent that bronchoalveolar lavage (BAL) samples the alveolar spaces, there are also ample data to show the presence of peripheral inflammatory cells and their bioactive products within parenchymal structures (38).

Despite this evidence of peripheral inflammation and mechanical dysfunction, the role of parenchymal mechanics in asthma is unclear. This state of affairs is likely due to the fact that assessing the mechanical properties of the lung parenchyma, independent of the other structures of the respiratory system, is not straightforward.

The most direct way to assess parenchymal mechanics is with the alveolar capsule (AC) technique (4), which is a highly invasive approach and limited solely to animal models. An alternative and much less invasive approach is to use the forced oscillation (FO) technique to measure mechanical impedance up to ~20 Hz and then partition it into its airway and tissue components on the basis of a simple lumped-parameter model. This approach has been used in a variety of species, including rat (9, 21, 30) and mouse (2, 5). However, the FO technique has only been validated in the dog by comparison with direct measurements made with the AC technique (7, 8, 22, 32). Unfortunately, it has proven to be difficult to produce an asthmalike state in the dog. Given that the mouse is now widely used in the exploration of mechanisms that cause inflammation and the asthma phenotype (29, 39), development of more invasive approaches in this species is desirable.

The first goal of the present study was, therefore, to establish how accurately the FO technique can measure lung parenchymal mechanics in the mouse by comparing input impedance (Z) measurements with the results of the more direct AC technique. We made this comparison during induced bronchospasm with inhaled methacholine as well as during antigen-induced inflammation. The second goal of our study was to further explore the role of lung parenchymal mechanics in the response to inflammation in a murine system of immunization and antigen challenge.

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was then punctured to a depth of 1 mm with an electrocautery instrument linear model of the lung least squares (17) from the equation of the single-compartment linear model of the lung

\[
\text{Protocol.} \quad \text{We collected 10 breaths of regular ventilation data to establish the baseline for each animal. We then assessed the response to methacholine as follows. First, we switched the animals from the flexiVent to a second volume-cycled ventilator (model 683 rodent ventilator, Harvard Apparatus, South Natick, MA) connected through a lateral port in the ventilatory circuit close to the trachea. Increasing concentrations (0.33, 1.0, 3.3, 10, 33, and 100 mg/ml) of methacholine aerosol (Aldrich Chemical, Milwaukee, WI) were delivered into the trachea at 20 breaths/min for 30 s, with the ventilator piston delivering a tidal volume of \( \sim 0.8 \) ml (the actual tidal volume reaching the lungs would have been somewhat less than this because of gas compression within the ventilator circuit, particularly after bronchoconstriction). Before each methacholine challenge, an aerosol of saline was given as a control. Before each aerosol challenge, the lungs were inflated to total lung capacity (25 cmH2O) three times to establish a standard volume history and to check for any leaks in the system. After each aerosol challenge, 10 breaths were collected for analysis of AC data at the peak of the response, as determined from real-time breath-by-breath measurements of \( R_L \).

Immediately after collecting these data, we switched the animals back to the flexiVent and applied a 16-s V perturbation for the calculation of Z by the FO technique. The applied V perturbation signal consisted of the superposition of 12 sinusoidal components having mutually prime frequencies from 0.25 to 19.625 Hz with approximately hyperbolically decreasing amplitudes. Before each animal was prepared for experimentation, the flexiVent was dynamically calibrated with the endotracheal cannula in place as follows. First, a closed calibration was done by completely blocking the open end of the cannula while oscillating the piston of the flexiVent with the V perturbation. Then the cannula was un-blocked, and the piston was again oscillated with the V perturbation. The signals of pressure and volume displacement within the flexiVent cylinder (\( P_{cyl} \) and \( V_{cyl} \), respectively) were recorded for subsequent data analysis (9, 35). These calibration signals allow the physical properties of the flexiVent itself (essentially, gas compression within the ventilator cylinder and flow resistance through the connecting tubing) to be removed from the measurements of Z, allowing calculation of the impedance of the animal alone.

Data analysis. The analysis of data for the calculation of airflow and tissue mechanics using the AC was achieved with a custom-developed computer program written in Labview 3.01 (National Instruments, Austin, TX) (10).

\( R_L \) and lung elastance (\( E_L \)) were calculated by recursive least squares (17) from the equation of the single-compartment linear model of the lung

\[
P_{tr}(t) = R_L \dot{V}(t) + E_L \ddot{V}(t) + P_1 \tag{1}
\]

where \( P_1 \) is an estimate of PEEP and \( t \) is time. Tissue resistance (\( R_{ti} \)) and elastance (\( E_{ti} \)) were calculated similarly from the corresponding equation for the lung tissue

\[
P_{a}(t) = R_L \dot{V}(t) + E_L \ddot{V}(t) + P_2 \tag{2}
\]

where \( P_2 \) is another estimate of PEEP. Airway resistance (Raw) determined by the AC (Raw\(_{AC}\)) was calculated by subtraction; thus

\[
\text{Raw}_{AC} = R_L - R_{ti} \tag{3}
\]

Pressure-volume and resistive pressure-flow relationships were observed in real time on the computer screen during each experiment. The values of \( R_L \) and \( R_{ti} \) were accepted only when \( J \) the magnitudes and phases of the swings in \( P_a \) and

\[
J \text{ Apfl Physiolog * VOL 90 * JULY 2002 www.japj.org}
\]
where $i$ is the square root of $-1$, $\text{Raw}_{FO}$, $I$, $G_{ti}$, and $H_{ti}$ are adjustable parameters, and

$$ a = \left( \frac{2}{\pi} \right) \arctan \left( \frac{H_{ti}}{G_{ti}} \right) $$

This model has been shown previously to fit extremely well to $Z$ obtained in a variety of species (5, 8, 9, 21, 22, 30, 32). The parameters $\text{Raw}_{FO}$ and $I$ correspond to the usual notions of Raw and airway inertance, respectively. The other two parameters, $G_{ti}$ and $H_{ti}$, although related to $R_{ti}$ and $E_{ti}$, respectively, are not precisely the same as these more well-known quantities. From Eq. 4, $G_{ti}$ and $H_{ti}$ can be seen to have the same units of cmH$_2$O$^{-1}$s$^{-\alpha}$-ml$^{-1}$. They characterize the dissipative (resistive) and conservative (elastic) mechanical properties of the lung tissue, respectively.

Assessment of inflammation. In different groups of NIM and OVA/OVA mice, we lavaged the lungs with 1.0 ml of sterile saline warmed to 37°C. The volume of BAL fluid collected was $\sim$0.7 ml. The number of leukocytes in the BAL fluid was counted with a hemocytometer (Reichert, Buffalo, NY), and the leukocyte differential was determined from cytocentrifuged slide preparations with a Lab-count Denominator (Denominator, Woodbury, CT), as previously described (12).

Statistical analysis. Values are means ± SE. Statistical significance of changes in the various physiological variables as a function of methacholine dose was determined by two-factor analysis of variance. Statistical significance of differences in any variable between the two groups of mice was determined with the Mann-Whitney $U$-test. The association of corresponding physiological parameters obtained by using the AC and FO techniques was evaluated with the Pearson correlation coefficient. Significance was taken as $P < 0.05$.

RESULTS

Figure 1 shows an example of the fit of Eq. 4 to $Z$ from NIM and OVA/OVA groups at 100 mg/ml of methacholine. Even in inflamed lungs and at a high dose of methacholine, the constant-phase model fit well, although there are slight deviations between data and fit at the lower frequencies.

Table 1 presents the cellular analysis of the BAL fluid from the two groups of mice. The OVA/OVA group had higher numbers of cells than the NIM group in terms of total cells and individual cell types. This indicates the presence of a substantial inflammatory response in the lungs of the OVA/OVA mice at the time of the mechanical assessment.

Table 1. Total cell counts and cellular composition in BAL fluid

<table>
<thead>
<tr>
<th></th>
<th>NIM</th>
<th>OVA/OVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cell count, $\times 10^4$</td>
<td>9.3 ± 1.8</td>
<td>37.8 ± 5.0$^*$</td>
</tr>
<tr>
<td>Macrophage, %</td>
<td>96.8 ± 1.2</td>
<td>50.6 ± 4.7$^*$</td>
</tr>
<tr>
<td>Eosinophil, %</td>
<td>1.1 ± 0.8</td>
<td>43.9 ± 4.0$^*$</td>
</tr>
<tr>
<td>Neutrophil, %</td>
<td>0.5 ± 0.2</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td>Lymphocyte, %</td>
<td>1.9 ± 0.7</td>
<td>4.8 ± 1.0$^*$</td>
</tr>
</tbody>
</table>

Values are means ± SE. BAL, bronchoalveolar lavage; NIM, non-immunized control mice; OVA/OVA, mice immunized and challenged with ovalbumin. *$P < 0.05$. 

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The baseline lung mechanics data as determined with the AC method were not different between the two groups, despite the presence of lung inflammation (Table 2). The baseline mechanical parameters obtained with the FO technique are shown in Table 3. There are no significant differences between the NIM and OVA/OVA groups in any parameter measured with either technique, indicating that the lung inflammation induced by OVA treatment was not severe enough to cause a measurable change in baseline lung function. In contrast, the inflammation was sufficient to affect bronchial responsiveness, as indicated by Fig. 2, which shows the Rt dose-response curves to methacholine challenge. The OVA/OVA group was significantly more responsive than the NIM group (ANOVA, P = 0.013).

The partitioning of the methacholine dose-response curve into its airway and tissue contributions by the AC and FO techniques is shown in Fig. 3. For both techniques, although the corresponding estimates of Raw (RawAC and RawFO) appear to be higher at a given methacholine dose for the OVA/OVA group than for the NIM group, the differences were not statistically significant. In contrast, the corresponding measures of tissue mechanics (Gi and Hti) were significantly more responsive in the OVA/OVA group. This indicates that the effect of allergic inflammation in the lung is more pronounced in the lung periphery (thereby affecting lung tissue mechanics) than in the conducting airways.

We compared the results of the AC and FO techniques by correlating their respective measures of Raw. Figure 4 shows RawFO vs. RawAC obtained from both groups of mice under baseline and bronchoconstricted conditions. The two quantities are significantly correlated (r = 0.764, P < 0.0001, n = 112). Figure 5 shows that the corresponding estimates of Eti obtained from the two techniques (i.e., El vs. Hti) are also significantly correlated (r = 0.88, P < 0.0001). We did not pursue the values of I returned by the constant-phase model (Eq. 4), because they were not sufficiently strongly determined by the data to be reliable. As in our previous studies in mice (5, 9), we found that the effects of I were negligible at <20 Hz.

**DISCUSSION**

The results of the present study address two distinct issues. The first is a methodological issue concerning the validation of the FO technique for assessing the mechanical properties of pulmonary airways and parenchyma. The second is a pathophysiological issue dealing with the effects of allergic inflammation on lung mechanical properties and the responses to induced bronchoconstriction in an animal model with considerable relevance to the investigation of asthma.

The relatively equivalent results provided by the AC and FO techniques under baseline conditions and after methacholine challenge (Figs. 3 and 4) suggest that the two techniques provide similar information about airway and tissue mechanics. Although this might seem expected in light of the common but simplistic view of the lung as a homogeneously ventilated unit on the end of a flow-resistant conduit, other considerations make this outcome less than obvious. The lung is not perfectly homogeneous, even under baseline conditions but, rather, exhibits a substantial variation in pathway length from trachea to individual alveoli with additional regional variation in airway dimensions (33) and tissue elasticity (34). This would be expected to lead to variation in regional Pa, which has been demonstrated in other species, especially after induced bronchoconstriction (3, 18, 23). However, if the AC technique samples alveoli randomly in a manner that is not biased toward any particular part of the heterogeneity spectrum, then one would expect regional dif-

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**Table 2. Baseline lung mechanics parameters obtained with the AC technique in mice**

<table>
<thead>
<tr>
<th></th>
<th>NIM (n = 8)</th>
<th>OVA/OVA (n = 8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rt</td>
<td>0.86 ± 0.02</td>
<td>0.85 ± 0.04</td>
<td>0.495</td>
</tr>
<tr>
<td>Gi</td>
<td>0.63 ± 0.02</td>
<td>0.61 ± 0.03</td>
<td>0.753</td>
</tr>
<tr>
<td>Raw</td>
<td>0.23 ± 0.03</td>
<td>0.22 ± 0.02</td>
<td>0.564</td>
</tr>
<tr>
<td>El</td>
<td>13.7 ± 1.0</td>
<td>17.1 ± 2.0</td>
<td>0.462</td>
</tr>
</tbody>
</table>

Values are means ± SE. AC, alveolar capsule; Rt, lung resistance; Gi, tissue resistance; Raw, airway resistance; El, lung elastance.

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**Table 3. Baseline lung mechanics parameters obtained with the FO technique in mice**

<table>
<thead>
<tr>
<th></th>
<th>NIM (n = 8)</th>
<th>OVA/OVA (n = 8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>0.28 ± 0.04</td>
<td>0.31 ± 0.02</td>
<td>0.462</td>
</tr>
<tr>
<td>Gi</td>
<td>2.81 ± 0.16</td>
<td>2.99 ± 0.36</td>
<td>0.655</td>
</tr>
<tr>
<td>Hti</td>
<td>17.7 ± 1.2</td>
<td>20.4 ± 1.8</td>
<td>0.231</td>
</tr>
</tbody>
</table>

Values are means ± SE. FO, forced oscillation; Gi and Hti, parameters related to tissue resistance and elastance, respectively.
ferences in Pa to average out over some number of AC measurements (19, 20, 25–27). If this reasoning applies to the present study, then RawAC and Rti should, on average, represent the overall resistances of the airways and tissues, respectively. The FO technique, on the other hand, produces an average measure of airway and tissue properties, because it obtains data representative of the entire lung, rather than a single site. Thus it is to be expected that any individual estimate of airway and tissue mechanics provided by the AC method would show considerable variation about corresponding quantities obtained from the FO technique. This is indeed what we found. RawAC and RawFO are strongly correlated and do not show any obvious bias away from the line of identity (Fig. 4), as is the case for Ef and Hti (Fig. 5). We thus conclude that the two techniques provide, on average, similar mechanical information from an inhomogeneous lung, whether caused by methacholine or enhanced by the presence of an inflammatory event. We do not show corresponding plots for the AC and FO parameters pertaining to tissue resistive properties (Rti and Gti),

Fig. 3. Dose-response relationship between NIM (●, n = 8) and OVA/OVA (○, n = 8) groups showing responses of lung mechanics parameters to methacholine obtained from the forced oscillations (FO) technique (A, C, and E) and the alveolar capsule (AC) method (B, D, and F). Values are means ± SE. RawFO, airway resistance measured by the FO technique; RawAC, airway resistance measured by the AC method; Gti and Hti, parameters related to tissue resistance and elastance, respectively; Rti, tissue resistance; Eti, lung elastance.
because their comparison is not quite so straightforward. That is, whereas RawFO vs. RawAC purport to measure precisely the same quantity (i.e., the flow resistance of the airways), Rti and Gti do not measure the same quantity. Rti is a measure of tissue resistance in the conventional sense made at a single ventilation frequency, whereas Gti is a parameter that characterizes dissipation of energy in the tissues but is frequency independent and does not have the units of resistance. EL and Hti are not comparable in general either, for the same reason, but they become formally identical at a frequency of 1/2π. Therefore, it makes more sense to compare them with each other than to compare Rti and Gti.

Although the AC technique has been applied in numerous studies in larger species (3, 4, 7, 8, 19, 20, 22, 25, 27, 32), the only previous study using the technique in mice is that by Nagase et al. (26), who studied the ICR strain of mice. These workers found that, at baseline, the contribution of Rti to RL was ~50%, which is smaller than the 70% we found in the present study. Possible reasons for this difference include the fact that the relative contribution of Rti to Rl depends markedly on ventilation frequency (6), the applied PEEP (9, 36), and the fact that the ICR mouse is substantially larger than the BALB/c mouse. Indeed, in preliminary studies in older animals (data not shown), we have also found Rti to contribute ~50% to Rl, suggesting that the difference is likely due to lung size or age. Nagase et al. (26) administered methacholine to the lungs via intravenous injection and found increases in Rl that were similar to our own NIM group, despite the different route of agonist administration. The tissue response in our study was larger, however, which is perhaps not surprising, inasmuch as Nagase et al. (25) also showed in rats that methacholine administered via aerosol induced greater tissue distortion in the lung than that administered via intravenous injection. This finding is thought to be due to inhomogeneous deposition of agonist.

The practical use of the AC in mice is limited by several factors. First, because of the animals’ small size, it is technically more difficult to fix capsules to the lung. Second, the AC requires invasive surgery that is technically demanding and very stressful to the animal. Third, the pathway from AC chamber to trachea may become totally blocked with blood or secretions, rendering the AC pressure valueless. One may discard data that are obviously affected in this way, as we did according to the criteria listed in METHODS. However, the decision to discard can never be entirely objective; therefore, one runs the risk of biasing the results by arbitrarily including only data from open airways. This would underestimate the effects of an intervention. For these reasons, the AC technique will never be widely used and is clearly unsuitable for studies (e.g., transgenic phenotype assessments) in which a large number of animals are required.

By contrast, the FO technique is experimentally much easier than the AC method and does not suffer from the obvious limitation of the AC method in terms of number of sampling sites. We, therefore, were interested to see if the FO technique gave the same mechanical information as the AC method in mice. The only similar comparisons of which we are aware are studies in dogs during induced bronchoconstriction (8, 32), which showed excellent agreement between the tissue parameters provided by the two techniques but, interestingly, not between the airway parameters. One explanation for this is that they were able to measure simultaneously from multiple ACs and average the results. This probably yielded a lower bias than we were able to achieve in the present study with only a single AC per animal. Consequently, they may have uncovered a subtle systematic difference between RawAC and RawFO that was obscured by the noise in our study (Fig. 4); indeed, there is a tendency for the RawFO response to be larger than the RawAC response (Fig. 3). In any case, our results indicate that the FO technique does, in fact, usefully partition Z into its separate airway and tissue components. This makes
the FO technique an extremely useful tool for physiological investigation, because it means that interventions can be conveniently investigated for their differential effects on airways and tissues.

We must bear in mind, however, that the distinction between airways and tissues is made on the basis of a particular model idealization of $Z$: the serial airway and constant-phase tissue model of Eq. 4. This model makes the assumption that the lungs are homogeneously ventilated, which is not entirely true, even in healthy lungs (11) and certainly not in diseased or bronchoconstricted lungs (3, 29). Nevertheless, our data suggest that this assumption is, on average, justified. Thus, in using this model, we are essentially performing the same kind of averaging that is made with multiple ACs. There is good evidence to suggest that, under control conditions (6, 8, 21, 30, 32) and under moderate bronchoconstriction (8, 21), this leads to a useful characterization of the overall properties of airway and tissues. When bronchoconstriction becomes severe, a significant blurring of the functional contributions from tissues and airways may occur, because the low-frequency portion of $Z$ becomes substantially affected by ventilation inhomogeneity as well as by the complex rheology of the tissues (7, 21, 35). Thus it is possible that inflammation produced subtle geometric changes in the lung periphery that were undetectable at baseline but became amplified with bronchoconstriction as a result of the highly nonlinear relationship between the degree of smooth muscle shortening and the resistance of an airway (24). A similar response also occurs in patients with asthma (13–15). Alternatively, the inflammation could have involved local release of mediators, making the peripheral smooth muscle or other reactive structures more responsive.

In summary, we studied airway and tissue mechanics at baseline and after challenge with methacholine aerosol in control mice and in an allergic murine model that simulates some of the features of asthma. The AC technique and the calculation of $Z$ by the FO technique yielded equivalent results. The FO technique has substantial practical advantages over the AC method. Accordingly, these data validate the further use of these techniques to localize the effects of various interventions in the lung. Our results also show that allergic inflammation in the mouse causes a significant increase in the responsiveness of the lung periphery to methacholine challenge. We conclude that the FO technique constitutes a valid and useful approach to the detailed study of murine lung function and that allergic inflammation in mice leads to the kind of peripheral lung hyperresponsiveness observed in patients with asthma.

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REFERENCES