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Transient oscillatory force-length behavior of activated airway smooth muscle

J. H. T. Bates, S. R. Bullimore, A. Z. Politi, J. Sneyd, R. C. Anafi and A. -M. Lauzon

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Computational assessment of airway wall stiffness in vivo in allergically inflamed mouse models of asthma

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Cojocaru A, Irvin CG, Haverkamp HC, Bates JH. Computational assessment of airway wall stiffness in vivo in allergically inflamed mouse models of asthma. *J Appl Physiol* 104: 1601–1610, 2008. First published April 17, 2008; doi:10.1152/jappphysiol.01207.2007.—Allergic inflammation is known to cause airway hyperresponsiveness in mice. However, it is not known whether inflammation affects the stiffness of the airway wall, which would alter the load against which the circum-scribing smooth muscle shortens when activated. Accordingly, we measured the time course of airway resistance immediately following intravenous methacholine injection in acutely and chronically allergically inflamed mice. We estimated the effective stiffness of the airway wall in these animals by fitting to the airway resistance profiles a computational model of a dynamically narrowing airway embedded in elastic parenchyma. Effective airway wall stiffness was estimated from the model fit and was found not to change from control in either the acute or chronic inflammatory groups. However, the acutely inflamed mice were hyperresponsive compared with controls, which we interpret as reflecting increased delivery of methacholine to the airway smooth muscle through a leaky pulmonary endothelium. These results support the notion that acutely inflamed BALB/c mice represent an animal model of functionally normal airway smooth muscle in a transiently abnormal lung.

airway resistance; airways hyperresponsiveness; airway smooth muscle; airway remodeling

AIRWAY REMODELING HAS BEEN shown to occur in asthma, but there is little consensus as to whether or not remodeling impacts airways hyperresponsiveness (AHR) (8, 21, 25, 33, 34). On the one hand, remodeling of the airway wall might make it stiffer than normal, which would be expected to limit the extent to which it can be narrowed by activation of airway smooth muscle (ASM). On the other hand, remodeled airway walls also tend to be thicker than normal, which could geometrically amplify the luminal narrowing caused by a given degree of smooth muscle shortening. This richness of possibilities makes the mechanical effects of airway remodeling a fruitful area for theory and speculation (1), but complicates its experimental elucidation.

Our laboratory recently developed a computational model of a single airway contracting against the elastic tethering forces of the parenchyma in which it is embedded (5). We showed that this model accurately describes the effects of positive end-expiratory pressure (PEEP) and tidal volume on airway responsiveness in normal animals and also explains much of the effect on airway resistance (R_{aw}) caused by a deep inflation in constricted mice (3), provided the model includes a parameter to account for the stiffness of the airway wall. Thus, by fitting this model to continuous measurements of R_{aw} made

at different lung volumes following a bolus injection of bronchial agonist, we can estimate the effective stiffness of the airway wall in vivo. In the present study, we use this approach to investigate whether airway wall stiffness is altered in allergically inflamed mice, a commonly used animal model of asthma.

METHODS

Animal Groups

Female BALB/c mice were obtained from Jackson Laboratories (Bar Harbor, ME) at ~8 wk of age. Our studies conformed to the National Research Council Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Vermont.

Acute allergic inflammation. Our first set of experiments was designed to determine how airway wall stiffness is affected during the acute phase of allergic inflammation. We know that BALB/c mice are hyperresponsive to methacholine during this phase, and computational modeling indicates that this is due to increased thickness of peripheral airway walls (38). The purpose of the present experiments was to determine whether the mechanical properties of the walls are also affected. To produce an acute inflammation, mice were sensitized with an intraperitoneal injection of ovalbumin (20 μ g in 2.25 mg alum) on *days 0* and *14*. They were challenged on each of *days 21*, *22*, and *23* by being placed in a compartmentalized aerosolization chamber and exposed to ovalbumin aerosol (1% in phosphate-buffered saline) for 30 min. Airway responsiveness to a bolus intravenous injection of 137 μ g/kg methacholine (0.074 mg/ml in ~40 μ l saline) was measured (see below) on *day 25*. This group of mice are referred to as the Acute Ova group ($n = 6$). The results from these animals were compared with those obtained from an age-matched Acute Control group ($n = 6$) that was prepared in the same way as the Acute Ova group, except that the control animals were not exposed to ova. We also measured airway responsiveness to three times the dose of methacholine (411 μ g/kg) in a second group of control mice, the Acute Control High-Dose group ($n = 8$), to see if our technique for estimating airway wall stiffness is affected by the degree of bronchoconstriction.

Chronic allergic inflammation. Our second set of experiments was designed to see if allergic inflammation leads to any long-term alterations in airway wall stiffness that persist, even when the acute inflammatory phase is no longer present. We, therefore, subjected BALB/c mice to a more extended ova challenge protocol and waited for the inflammation to resolve before studying their airway responsiveness. Mice were sensitized with an intraperitoneal injection of ovalbumin (20 μ g in 2.25 mg alum) on *days 0* and *14*. They were challenged with 1% aerosolized ovalbumin on *days 21*, *22*, and *23*, and then once per week for the following 3 wk. Physiological measurements were performed 4 wk after the final ovalbumin exposure. This group of mice are referred to as the Chronic Ova group ($n =$

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8). As a basis for comparison, we also studied an age-matched Chronic Control group ($n = 9$) that was not exposed to ovalbumin.

Experimental Protocol

Mice were anesthetized with pentobarbital sodium by intraperitoneal injection (90 ml/kg diluted in phosphate-buffered saline to 5 mg/ml) and tracheostomized, and an 18-gauge cannula was tied into the trachea. The mice were connected to a computer-controlled small-animal mechanical ventilator (flexiVent, SCIREQ, Montreal, Quebec) for mechanical ventilation at 200 breaths/min and a tidal volume of 0.2 ml against a PEEP of 3 cmH₂O. The animals were paralyzed with an intraperitoneal injection of pancuronium bromide (0.8 µg/kg). The experimental protocol began with the delivery of a deep breath to an airway pressure limit of 25 cmH₂O. Approximately 1 min later, the animals in all five groups were injected with a bolus of methacholine through a catheter placed in the jugular vein. The injection took ~1 s to deliver and was followed by 50-µl saline to flush the catheter. At the beginning of injection, regular mechanical ventilation was suspended, and the animals were allowed to expire passively against the external PEEP for 1 s. Immediately after this expiration, a volume perturbation was applied to the lungs by the ventilator piston for 20 s. The perturbation had a peak-to-peak amplitude of 0.1 ml and consisted of 10 repeats of a 2-s signal containing 12 sinusoids, having mutually prime frequencies from 1 to 20.5 Hz and amplitudes that decreased inversely with frequency. Regular mechanical ventilation was resumed immediately after the perturbation sequence was complete. During the application of the perturbation, the volume displacement of the ventilator piston and the pressure inside its cylinder were recorded and stored for subsequent analysis. This methacholine challenge procedure was repeated at PEEP levels of 1, 3, and 6 cmH₂O in random order, with 10 min allowed between subsequent methacholine challenges. These maneuvers required that the mice be deprived of normal ventilation for 20-s periods, which is a rather long time for a mouse. One might thus worry about changes in blood gases and possibly neural tone affecting lung mechanics by the end of the measurement period, although we have previously found neural tone to be negligible in mice (30). In any case, both effects would have been mitigated by the volume perturbations that were applied during the measurement period and that had an amplitude of about one-half that of normal tidal volume. Thus by far the major effect on lung mechanics was produced by the methacholine.

At the end of the protocol, a lung lavage was performed by instilling 1 ml of phosphate-buffered saline containing 3.2% sodium citrate into the trachea with a syringe and then withdrawing it back into the syringe (withdrawn volume being ~0.8 ml). The lavage fluid was stored on ice for later analysis of cell counts, and the mice were euthanized with an overdose of pentobarbital sodium, followed by opening of the thoracic cavity.

Calculation of Impedance

The pressure and flow data sampled at 128 Hz during application of each volume perturbation were used to calculate the complex input impedance of the respiratory system (Z_{rs}) within a 2-s sliding window that moved across the 20-s data segment in steps of 0.125 s (37) after digital removal of the mechanical effects of the ventilator circuit, as previously described (10). Each estimate of Z_{rs} was fit to the equation of a lung model consisting of a single airway serving a constant-phase viscoelastic tissue unit, the so-called constant-phase model of Z_{rs} (12), described by the equation

$$Z(f) = R + i\omega I + \frac{G - iH}{\left(\frac{\omega}{\omega_0}\right)^a} \quad (1)$$

where Z is Z_{rs} ; R is a Newtonian resistance composed mostly of the flow resistance of the conducting pulmonary airways (36); I reflects

the inertance of the gas in the central airways; G reflects viscous dissipation of energy in the respiratory tissues (tissue damping); H reflects elastic energy storage in the tissues (tissue stiffness); ω is angular frequency, $i = \sqrt{-1}$; and f is frequency. The exponent a couples G and H through the expression $a = (2/\pi)\arctan(H/G)$ (12). I has negligible effect in the mouse lung <20 Hz and so can be ignored (10). Angular frequency in Eq. 1 is normalized to $\omega_0 = 1$ rad/s, so that R , G , and H all have units of cmH₂O·s·ml⁻¹ (14). We thus obtained time courses for R , G , and H sampled at 8 Hz from 1 to 19 s after each injection of methacholine.

Model Fitting

Our computational model of a contracting airway and the method we use to fit it to experimental data have been described in detail previously (4, 5). For completeness, the following is a brief overview. We model an airway in two dimensions as a circular ring of ASM wrapped around an elastic airway wall embedded in homogeneously elastic lung parenchyma. This neglects the fact that at least some ASM cells are oriented at a slight angle to the circumferential direction (19), so that, in reality, ASM contraction may cause changes in airway length as well as radius. This is a complicated issue for which we do not know how to account precisely, so, for the present purpose, we assume that ASM contraction only decreases airway radius by pulling against the parenchymal attachments to the outside of the airway wall. This outward pull comes from two sources: 1) the transpulmonary pressure (P_{tp}) that is transmitted across the parenchyma when it is undistorted (uniform and isotropic), which is determined by lung volume under the assumption of a constant tissue elastance; and 2) the local distortion of the parenchyma caused by narrowing of the airway, which is assumed to follow the relationship identified by Lai-Fook (15). The inward recoil of the airway wall is determined by its stiffness, which is assumed to arise from a fraction $(1 - k)$ of the airway circumference that expands according to the one-third power of P_{tp} . The remaining fraction, k , of the circumference is assumed to be inextensible, where $0 < k < 1$. Once activated, the ASM follows the classic Hill force-velocity relationship that is hyperbolic when active force (F_A) is less than isometric force (F_0), and linear when $F_A \geq F_0$ with slopes matched at F_0 (11); thus

$$\begin{aligned} -2\pi \frac{dr}{dt} &= \frac{b(F_0 - F_A)}{a + F_A} \quad \text{when } F_A < F_0 \\ &= \frac{bF_0}{a + F_0} - \frac{bF_A}{a + F_0} \quad \text{when } F_A \geq F_0 \end{aligned} \quad (2)$$

where r is airway radius, t is time, and a and b are constants. Following experimental findings reported in rats (6), we set $a = F_0/4$. Equation 2 thus contains two free parameters, F_0 and b .

At any point in time, F_A is the force that adds to the outward recoil of the parenchyma and the inward recoil of the airway wall to give a net force difference of zero. The explicit expression for F_A that this produces is derived in Ref. 5 and is given by

$$\begin{aligned} F_A &= \left[r_{TLC} \left(\frac{P_{tp}}{P_{tpTLC}} \right)^{\frac{1}{3}} - 0.3r \right] \frac{P_{tp}}{0.7} \\ &\quad - r P_{tpTLC} \left[\frac{rk}{(1-k)r_{TLC} \left(\frac{P_0}{P_{tpTLC}} \right)^{\frac{1}{3}}} + \frac{r}{r_{TLC}} - \frac{k}{1-k} \right] \end{aligned} \quad (3)$$

where r_{TLC} is the radius that the virtual hole occupied by the airway would have at total lung capacity (TLC), if it expanded like the rest of the parenchyma; P_{tpTLC} is P_{tp} at TLC; and P_0 is the value of P_{tp} at which the unstricted airway induces no distortion in the parenchyma surrounding it. Note that the stiffness of the airway wall in this model does not include a contribution from the ASM itself, the

stiffness of which has been shown to increase markedly during activation (24), because we are concerned here with the stiffness seen by the ASM due to the elastic structures upon which it acts.

We used the above equations to calculate how r varies with time when the model was driven with a prescribed volume signal that, when multiplied by lung elastance, produces a time-varying $P_{tp}(t)$ signal. Initially, the ASM was relaxed so that $F_A = 0$ and r were determined by the force balance between the inward recoil of the airway wall and the outward recoil of the surrounding parenchyma. Once the ASM in the model was activated, F_A was given by Eq. 3. A constant level of activation was then assumed so that, at each time step of 0.0625 s, $P_{tp}(t)$ was used in Eq. 3 to determine F_A . The result was substituted into Eq. 2 to provide dr/dt , which was then used to determine r at the next time step using first-order Euler integration. This new value of r was then used in Eq. 3 again to determine the next value for F_A , and so on, until a complete time profile of r was produced. Finally, invoking the assumption of Poiseuille flow through the airway, a normalized Raw profile was calculated by raising r_{TLC}/r to the fourth power.

The model was driven by a volume signal that varied sinusoidally at a frequency of 1 Hz above the lung volume set by PEEP. The amplitude of the sinusoid was chosen so that it produced simulated excursions in P_{tp} comparable to the peak-peak pressure excursions measured experimentally in the mice. This neglects any loss of ventilator volume due to gas compression in the ventilator circuit, but, as lung elastance was ~ 20 cmH₂O/ml (see below) and the elastance of the gas in the ventilator circuit was ~ 140 cmH₂O/ml, this amounts to a volume of loss of $\sim 15\%$, which is unlikely to have a significant bearing on our conclusions. The inflation pressures in the airway at the start of each simulation matched the experimental PEEP levels. Each model simulation was generated by choosing values for the parameters b and F_0 in Eq. 2 and k in Eq. 3, and then generating R signals at each of the three PEEP levels of 1, 3, and 6 cmH₂O. The model was thus fit simultaneously to the data obtained at each of the three different PEEP levels. The resulting R signals were scaled by a single factor so that they matched, in a least squares sense, the corresponding experimental R signals. The model thus has four free parameters: the scale factor just described together with b , F_0 , and k . We did not have to include the other wall stiffness parameter (P_0 in Eq. 3) as an additional free parameter because, although this parameter is required for the derivation of the model, our laboratory has found previously (5) that the quality of the model fit is very insensitive to its value. Accordingly, we fixed the value of P_0 at 10 cmH₂O. The best fit values of the four free parameters were found using a grid search, as previously described (5).

Sensitivity Analysis and Statistics

We determined the sensitivity of each fitted parameter to the data by keeping the other parameters fixed at their best fit values while adjusting the parameter in question on either side of its best fit value until the root-mean-squared residual increased 5% above its minimum value. Comparisons of model parameter values between study groups were made on the basis of overlap between the confidence intervals calculated, as described above.

We also fit the model to the data from each individual animal in each group to make a statistical comparison between parameter values from different groups. Comparison of parameter values between each group and the Acute Control group was performed by unpaired t -test. Statistical significance was taken as $P < 0.05$.

RESULTS

Figure 1 shows the cell counts obtained from those animals whose bronchoalveolar lavage fluid was of sufficient quality for the cells to be clearly seen under the microscope. There are three expected differences in cellular differentials between both

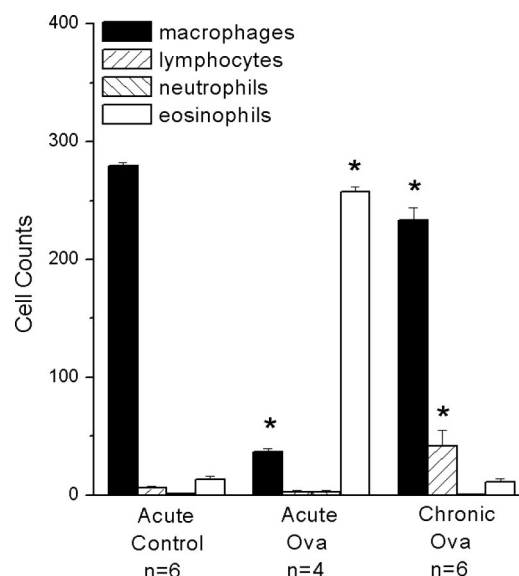


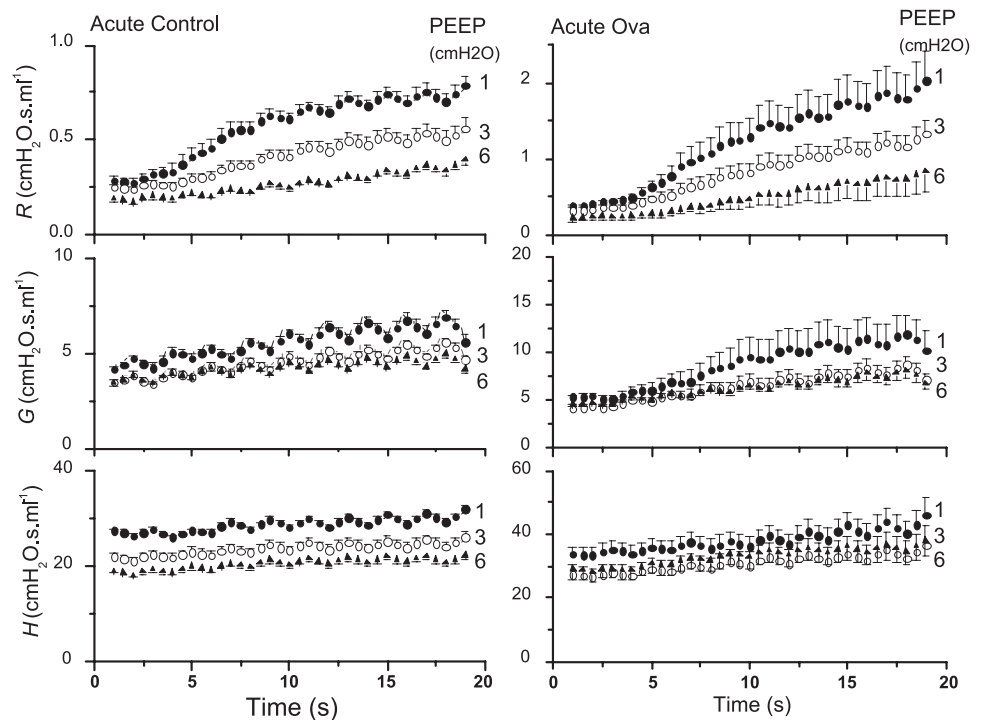
Fig. 1. Cell counts in bronchoalveolar lavage fluid obtained in three of the study groups. See METHODS for explanation of groups. Values are means $\pm$ SE. *Significant differences between cell counts compared with the Acute Control group (unpaired t -test, $P < 0.05$).

of the ova-treated groups compared with control, but the overriding picture that emerges is a major difference in cellularity (macrophages are decreased and eosinophils are increased) in the Acute Ova group compared with the others. This indicates the presence of acute inflammation in the Acute Ova group that had largely resolved in the chronically treated mice.

Figure 2 shows the time courses for R, G, and H from the Acute Control and Acute Ova groups at the three different PEEP levels. The Acute Ova animals were substantially more responsive to intravenous methacholine than were the Acute Control animals, as evidenced by the relative rates at which R increased throughout the duration of the measurements (note the different scales on the vertical axes in the left and right panels in Fig. 2). In both cases, however, modest increases in PEEP had a major mitigating effect on responsiveness in R (Fig. 2, top). These results are mirrored to some extent in G (Fig. 2, middle). Of particular note, however, is the fact that H increased very little during bronchoconstriction, and those changes that did occur were similar at all PEEP levels (Fig. 2, bottom). These relative changes in R, G, and H are typical of all five study groups. At PEEP 1 cmH₂O, the average increase in mean H between 1 and 19 s for the five study groups was 18% (SD 4%). At PEEP 3 and 6 cmH₂O, the mean (SD) increases were 23% (4%) and 22% (5%), respectively.

Figure 3 shows the SE ranges for the experimental measurements of R, together with the corresponding computational model fits for all five study groups. The top left panel of Fig. 3 compares the Acute Control and Acute Control High Dose groups, from which it is clear that tripling the dose of methacholine caused, as would be expected, a substantial increase in the rate of rise of Raw at all PEEP levels. By contrast, the Acute Ova group exhibited responses in R (Fig. 3, bottom left) that were clearly augmented compared with those of the control group receiving the same dose of methacholine. The Chronic Control group (Fig. 3, top right) behaved very simi-

Fig. 2. Impedance parameters Newtonian resistance (R), viscous dissipation of energy in the respiratory tissues (tissue damping) (G), and elastic energy storage in the tissues (tissue stiffness) (H) following methacholine injection in Acute Control and Acute Ova groups of mice at three different positive end-expiratory pressure (PEEP) levels, as indicated to the *right* of each tracing. Values are means $\pm$ SE.



larly to the Acute Control group that received the same methacholine dose. The responsiveness of the Chronic Ova group (Fig. 3, *bottom right*) was perhaps slightly elevated compared with its Chronic Control, but was also much less than that of the Acute Ova group. Also shown in Fig. 3 are the fits provided by the computational airway model to the mean data in each

group. In each case, the model fits follow the temporal trends in the data and their dependencies on PEEP accurately. The values of the best fit model parameters obtained with the mean data sets are listed in Table 1, along with their sensitivity ranges (see METHODS) and the mean squared residual between each set of fitted curves and their corresponding data points.

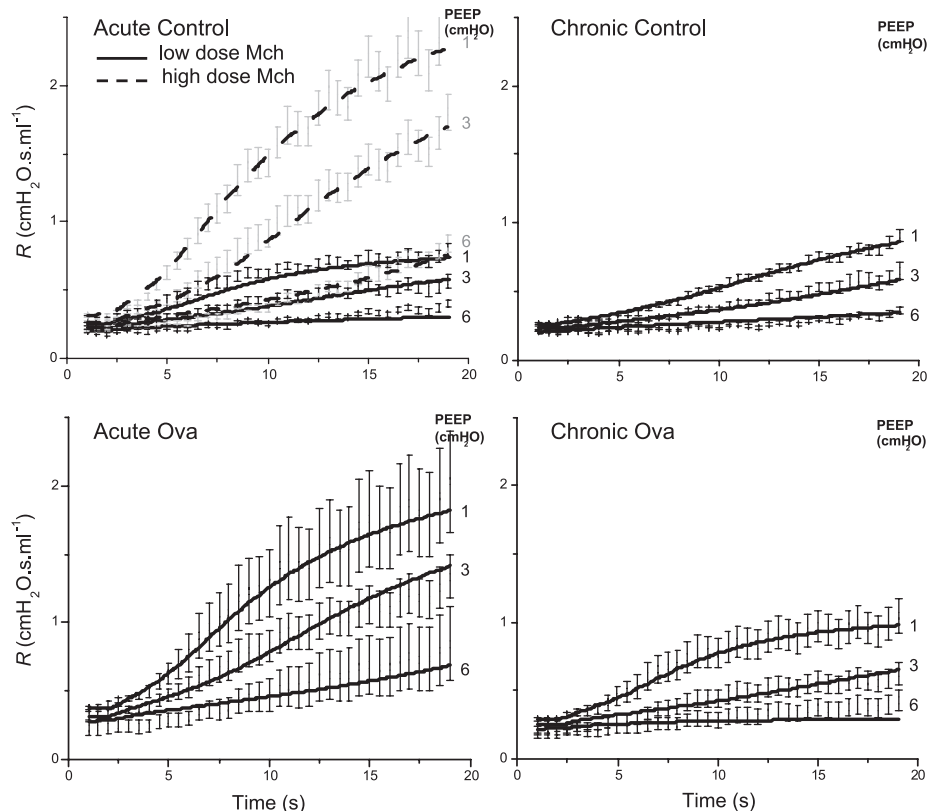


Fig. 3. Time courses of R in the various experimental groups of mice following methacholine (MCh) injection at three different PEEP levels, as indicated to the *right* of each tracing, together with the fits provided by the computational model of a contracting airway embedded in elastic parenchyma (solid curves). Values are means $\pm$ SE.

Table 1. Best fit model parameter values for the computational model fits shown in Fig. 3

Study Group	F_0 , cm/cmH ₂ O	b , cm/s	k	bF_0 , cm ² ·cmH ₂ O·s ⁻¹	RMSR, cmH ₂ O·s·ml ⁻¹
Acute Control	2.7 (3.0, 2.5)	0.030 (0.033, 0.027)	0.80 (0.81, 0.79)	0.081 (0.099, 0.068)	0.030
Acute Ova	5.2 (5.7, 4.7)	0.030 (0.034, 0.027)	0.76 (0.77, 0.75)	0.156 (0.194, 0.127)	0.072
Acute Control High Dose	4.8 (5.1, 4.6)	0.042 (0.045, 0.040)	0.73 (0.73, 0.72)	0.202 (0.230, 0.184)	0.060
Chronic Control	4.5 (4.8, 4.3)	0.018 (0.019, 0.017)	0.78 (0.78, 0.77)	0.081 (0.091, 0.073)	0.022
Chronic Ova	2.3 (2.4, 2.1)	0.037 (0.041, 0.033)	0.76 (0.77, 0.76)	0.085 (0.098, 0.069)	0.043

See METHODS for definition of terms and groups. The quantities in brackets are confidence intervals calculated by varying the value of each parameter in turn, either side of its best fit value, until the root mean squared residual (RMSR) increase by 5% above its minimum value. The value of the parameter a in Eq. 2 is forced equal to $F_0/4$. The upper and lower confidence intervals for bF_0 were calculated from the products of the corresponding upper and lower limits of b and F_0 .

Interestingly, the variability in R is greatest for the Acute Ova group, possibly because of the additional variability of inflammation level in this group.

The parameters F_0 and b are measures of, respectively, the maximum force-generating capacity and the maximum shortening velocity of the ASM in the model. These parameters, therefore, reflect the contractility of the ASM. However, we found that the individual values of F_0 and b tended to vary rather widely, probably because they can compensate for each other by moving in opposite directions. That is, one parameter can increase and the other decrease with relatively little effect on the quality of the model fit, as explained in the APPENDIX. These relative variations are canceled when the two quantities are multiplied together, making the product bF_0 more robust than either quantity on its own. Furthermore, bF_0 is a measure of the power output of the ASM as it contracts from the unloaded to the isometric state, and, therefore, reflects its overall contractile capacity (see APPENDIX). Figure 4A shows that bF_0 was significantly greater in the Acute Control High Dose group than that in the Acute Control group, not surprisingly given that the former received three times the methacholine dose of the latter and so presumably exhibited a correspondingly greater ASM power output. The mean value of bF_0 in the Acute Ova group was also greater than that of the Acute Control group, although this was not quite statistically significant due to the large variability among the animals of the Acute Ova group ($P = 0.089$ for a one-tailed t -test of the hypothesis that bF_0 was greater in Acute Ova vs. Control). On the other hand, the Raw time courses in Fig. 3 show a clear elevation in the responsiveness of the Acute Ova group. Also, the confidence intervals about bF_0 for the mean data from the Acute Control and Acute Ova groups (Table 1) do not overlap, whereas the intervals for the Acute Ova and Acute Control High Dose groups do overlap. In other words, the Acute Ova mice responded to a given dose of methacholine more vigorously than control animals, thus behaving more like control animals receiving a higher dose of methacholine.

Finally, Fig. 4B shows results pertaining to our original question about the role of airway wall stiffening on airway responsiveness, as evidenced by the parameter k . We had expected that airway wall stiffness, and hence the value of k , might be increased in allergic inflammation. However, there was no difference in k between either Ova group and its respective control, indicating that neither acute allergic inflammation nor its long-term sequelae lead to a functional change in airway wall stiffness, at least from the perspective of the contracting ASM. There was a small but significant reduction in k in the Acute Control High Dose group.

DISCUSSION

The principal goal of our study was to determine whether the stiffness of the airway wall is affected by allergic inflammation in BALB/c mice. We were motivated to pursue this goal by reported histological evidence of structural changes in the lungs of inflamed mice. In particular, acute sensitization and challenge with ovalbumin in BALB/c mice leads to a physical

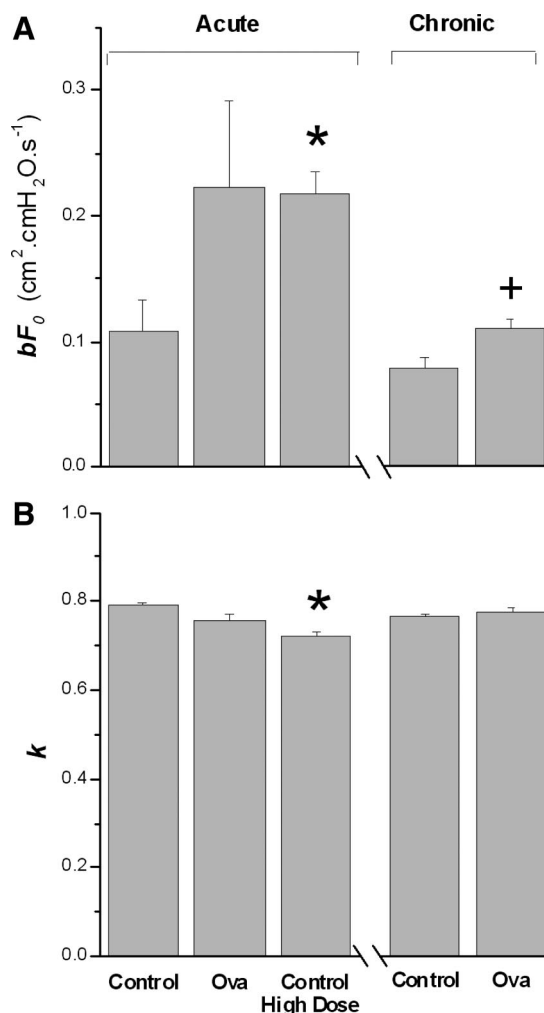


Fig. 4. A: values of bF_0 (where b is a constant and F_0 is isometric force) obtained from the computational model fits to the data from the various experimental groups. B: values of fraction of the circumference (k). Values are means ± SE. *Significant difference from Acute Control group. +Significant difference from Chronic Control group.

thickening of the airway epithelium, which our laboratory has previously shown (38) can be held accountable for the increased responsiveness to aerosolized methacholine seen in these animals. More chronic ovalbumin treatment in mice has also been reported to cause some degree of subepithelial fibrosis (41) and altered ASM morphology (26). While it is easy to speculate that any or all of these histological changes might affect the stiffness of the airway wall, obtaining experimental evidence of this is complicated by the difficulty of assessing airway wall stiffness *in situ*. To do this, we used an indirect approach in which a parameter reflecting airway wall stiffness (k in Eq. 3) is estimated by fitting a computational model of a contracting airway to dynamic measurements of Raw. The k is defined such that, at a transmural pressure of 30 cmH₂O (nominal TLC), the airway wall behaves as if a fraction k of its circumference is completely rigid, while the remaining fraction ($1 - k$) expands in the same way as the parenchyma. Of course, this is not to say that the wall circumference is physically divided into two domains with these respective properties; k merely serves to empirically quantify the specific stiffness of the airway wall relative to that of the parenchyma.

Our data also allow us to make an independent assessment of airway wall stiffness by examining how R changes with PEEP at baseline before activation of the ASM by methacholine, as follows. If we assume lung elastance to be constant, then r increases to the one-third power of Ptp, provided the airways behave exactly like the parenchyma. If we further assume that the airways expand isotropically, then R is proportional to the inverse third power of r (an inverse fourth power dependence on r coupled with a linear dependence on airway length). Of course, when the airways are stiffer than the parenchyma, the pressure acting to expand the airway, transmural pressure, is not exactly equal to Ptp because of local parenchymal distortion around the airway. Nevertheless, if we assume these two pressures are equal, and that transmural pressure is reflected in the PEEP applied to the lungs, then PEEP is proportional both to the inverse of R and to the cube root of r . Fitting a line to all the baseline values of R in Fig. 3 vs. their respective levels of PEEP, we obtained the linear relationship $1/R = 3.1 + 0.31 \times \text{PEEP}$. This equation predicts that the value of r at a PEEP of 0 cmH₂O should be 63% of its value at a PEEP of 30 cmH₂O. In other words, k is estimated by this method to be 0.63. By contrast, the values of k estimated by fitting the airway model to the entire time courses of bronchoconstriction lie in the range 0.7 to 0.8 (Fig. 4B). These values are not too dissimilar, however, which is interesting in view of the fact that there is no reason to suspect that they should be the same. The value of k estimate from the R -PEEP relationship reflects airway wall stiffness in expansion when the ASM is relaxed, whereas the active contraction of ASM is opposed by the compressive elasticity of the wall. Importantly, the tensile and compressive moduli of the airway wall are by no means automatically the same, so this issue applies to any method for assessing wall stiffness in expansion, such as one based on directly imaging the airways (9). This issue of tensile vs. compressive elastic modulus has also bedeviled attempts to understand how mucosal buckling opposes smooth muscle shortening (40). Nevertheless, our estimates of k by two different methods suggest that the tensile and compressive moduli of the airway wall in mice are fairly similar.

To the extent that this computational model captures the essential aspects of reality, our results are clear: allergic inflammation, either acute or chronic, does not change the effective stiffness of the airway wall in BALB/c mice (Fig. 4B). Interestingly, we did find a small but still statistically significant change in k compared with control values when triple the dose of methacholine was given to normal mice (Fig. 4B). As both Acute Control groups of mice received identical treatments before methacholine challenge, this difference in k between the low and high doses of methacholine likely reflects nonlinear effects. In particular, as r decreases, the wall tension required to induce further narrowing also decreases as a consequence of the Laplace law (5), all other things being equal. This could make it appear as if wall stiffness decreases with increasing levels of bronchoconstriction, which would explain why k was slightly smaller in the control mice receiving the higher dose of methacholine. This may also explain the trend for k to be lower in the Acute Ova group than in control ($P = 0.085$), with the degree of constriction again being greater in the former group. In any case, the roughly 10% decrease in k that we found in the Acute Control High Dose group (Fig. 4B) is likely of minor importance physiologically compared with the changes in mechanical load that even small changes in lung volume would present to the ASM.

The most notable consequence of ova treatment observed in the present study was a marked AHR in the acutely inflamed animals (Fig. 3), as has been reported previously (33). The value of bF_0 was substantially elevated relative to controls (Table 1 and Fig. 4). Furthermore, the methacholine responses we observed in the Acute Ova group were similar in magnitude to those in the Acute Control High Dose group (Figs. 3 and 4), suggesting an elevated level of ASM activation in the Acute Ova group. However, although the responses in R were robust, particularly at low PEEPs, H increased very little over the 20-s measurement period, even in the inflamed animals (Fig. 2, bottom). We have previously found a similar lack of effect of intravenous methacholine on H in BALB/c mice (39), which stands in marked contrast to what happens when the mice are challenged with an aerosol of methacholine. With aerosol, H increases substantially, even in control animals, and in allergically inflamed mice the hyperresponsiveness in H is proportionally greater than that in either R or G , even when the fractional increases in R are not as great as in the present study (38). We have shown that these increases in H are due mainly to closure of small airways in the lung periphery (22, 38). Interestingly, H was clearly somewhat elevated in the acutely inflamed animals (Fig. 2, compare bottom panels), suggesting that these animals had either some degree of baseline airway closure (22, 38), or alterations in the intrinsic elastic properties of the parenchyma secondary to the distortion caused by airway constriction (32). However, H increased only minimally following intravenous challenge, suggesting that few additional lung units were de-recruited during the ensuing bronchoconstriction. The modest rises in G in Fig. 2 (middle) thus likely reflect heterogeneity of airway narrowing (2, 23). We, therefore, conclude that the effects of methacholine injection in both normal and allergically inflamed BALB/c mice are largely limited to narrowing the conducting airways, while causing essentially no closure of peripheral airways (22). Why this should be, when aerosol delivery of methacholine is clearly so effective at causing airway closure, is not entirely clear. Per-

haps one possibility is that the saline carrier in the aerosol adds to the fluid layer lining the small airways, leading to enhanced liquid bridge formation. In any case, our results agree with those of Nagase et al. (27), who found that methacholine was more evenly distributed and caused fewer effects on tissue viscance when delivered intravenously than by aerosol in rats.

We also found a significantly increased central airway responsiveness in the Chronic Ova group (Figs. 3), although the effect was not nearly as pronounced as in the acutely inflamed animals (Fig. 4). We suspect that this reflects the fact that, in the Chronic animals, the acute inflammatory process induced by ova treatment was well on the way to being resolved, as evidenced by the return of the cell counts toward control levels (Fig. 1). Of course, to be sure of this, we would have to perform a more complete time course study, and also possibly examine the airway wall for histological evidence of remodeling. Those issues aside, however, our data suggest that the hyperresponsiveness we observed in the Acute Ova animals was related to the presence of active inflammation in the lungs rather than the progressive accrual of any permanent structural changes.

Taken at face value, the findings of the present study might seem to suggest that allergic inflammation merely induces a transient hyperresponsiveness of the ASM, without significantly affecting any other mechanical aspects of the lung. However, these results stand in marked contrast to our previous finding that H increases proportionately more than R in allergically inflamed mice when methacholine is delivered as an inhaled aerosol. Using an anatomically based computational model of the mouse lung, we showed that these earlier findings can be ascribed entirely to an increase in the number of small peripheral airways that close during bronchoconstriction in the inflamed animals as a result of a thickened epithelium and increased secretions (22, 38). In other words, the increased R response seen in inflamed mice caused in response to methacholine aerosol can be explained entirely by the geometrical amplification that occurs when the airway walls become thickened, so that a given degree of smooth muscle shortening leads to an increased amount of small airway closure. In other words, the ASM itself in the allergic animals appears to respond normally (38). How, then, do we reconcile this apparent dichotomy between airway responsiveness measured using aerosol vs. intravenous challenge in inflamed mice? Our laboratory's previous study (38) shows that the ASM in inflamed mice seems to contract normally in response to an aerosol challenge, while, in the present study, it seems that intravenous challenge causes ASM contraction to be excessive compared with control mice (Fig. 3), even when there is almost no evidence of airway closure (Fig. 2).

A possible clue to the resolution of the above conundrum is suggested by the fact that the responsiveness in R seen in the Acute Ova group (Fig. 3B) is similar to that observed in the Acute Control High Dose group (Fig. 3A). Thus, even though the Acute Control High Dose group received three times the dose of intravenous methacholine as the Acute Ova group, it is as if the amount of methacholine that actually reached the ASM in each case was similar. In other words, our results are compatible with more methacholine having reached the ASM in the Acute Ova mice compared with the Acute Control mice, despite both groups having received the same injected dose (Fig. 3A). Of course, we did not measure vascular leak in our

study. However, in a previous study, Lee et al. (18) found a marked increase in plasma extravasation into the lung, as quantified by Evan's blue dye, in the same acute allergic mouse model as we used in the present study. It is thus plausible that increased delivery of methacholine to the ASM of the Acute Ova animals could have occurred as a result of their general inflammatory state, because endothelial leak is a known consequence of inflammation (28). That is, if the pulmonary vascular endothelium in the inflamed mice was more leaky than normal, then more of the injected methacholine could have passed into the interstitium of the lung, and thence to the ASM, compared with what would have occurred in the less permeable control animals. If this is true, then the hyperresponsiveness of the acutely inflamed mice could be a reflection of increased delivery of agonist to the ASM, rather than having anything to do with the responsiveness of the ASM itself. Interestingly, Larson et al. (16) showed that the methacholine responsiveness of isolated ASM from ovalbumin-exposed BLAB/c mice is not different from normal animals, which also fits with our laboratory's previously advanced notion (38) that acute allergic inflammation in the BALB/c mice represents an animal model of normal ASM in an abnormal lung. On the other hand, increased ASM mass has been reported in animal models of asthma (13), and this might be accompanied by a simultaneous decrease in contractile proteins (26), perhaps as a result of the ASM cells assuming a more secretory phenotype (29). This combination of factors could leave the overall contractile ability of the ASM essentially unaltered from normal, even though the ASM itself would be quite abnormal.

We can formalize the above notion about differences in methacholine delivery by drawing on a theoretical model that our laboratory developed previously to account for differences in bronchoconstriction dynamics in dogs subjected to aerosolized vs. injected bronchial agonists (17). In that study, we observed that the onset and decay of bronchoconstriction were relatively delayed following aerosol delivery. By simulating the delivery of agonist via the aerosolized and injected routes in terms of passage through various compartments, we were able to accurately model the relative time courses of bronchoconstriction resulting from the two modes of delivery. In particular, we estimated that the time constant of diffusion of agonist across the airway wall in dogs is in the order of 60 s. In the mouse, the transfer rate across the airway wall would presumably be faster, because the relevant tissues are thinner and the corresponding diffusion distances shorter. Nevertheless, assuming that the same model structure applies in mice, our previous studies with aerosolized methacholine (22, 38) and the results of the present study together suggest that acute allergic inflammation affects airway responsiveness in mice by modifying the accessibility of the ASM to methacholine. In particular, the present study suggests that, in inflammation, there may be a decreased barrier presented to injected methacholine by a leaky capillary wall, while our laboratory's previous study with aerosol challenge (38) indicates that inflammation may actually increased the barrier to methacholine presented by a thickened airway wall.

Our study has a number of limitations that must be considered. First and foremost, the inferences we have made about airway wall stiffness are based on a structurally very simple model of a single airway embedded in uniform elastic parenchyma. This neglects all of the heterogeneity among airways of

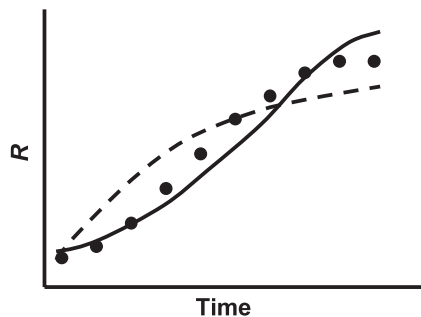


Fig. 5. Schematic representation of R time course data (●), together with two stylized model curves, both of which fit the data equally well. The solid curve has a low value of b and a high value of F_0 , whereas the dashed curve has a high value of b and a low value of F_0 .

different sizes and generations that is known to characterize the lung, and it makes numerous simplifying assumptions about the dynamics of ASM contraction and parenchymal mechanics (5). It also assumes a particularly simple mathematical form for the stiffness of the airway wall (35), which is certain to be a gross oversimplification of reality. Indeed, our laboratory recently showed that, even though this model accounts for much of the transient dynamics in R following a deep lung inflation in constricted mice (3), there appears to be significant effects due to tissue viscoelasticity in these dynamics for which the model does not account. Nevertheless, this model, with only four free parameters, is able to describe the dynamics of onset of bronchoconstriction for the entire lung at three different PEEP levels simultaneously, and under a variety of different conditions (Fig. 3). We, therefore, suspect that, had there been an important change in the effective stiffness of the airway wall in any of our study groups, we should have picked up at least some change in the value of the parameter k .

The other major limitation of our study concerns the nature of the inflammatory mouse models we studied. Even the chronic model was developed over a very short time scale, even compared with the lifetime of a mouse, let alone a human, and, therefore, is assailable on many fronts in terms of relevance to human disease. However, our purpose here is not to defend these preparations as valid models of asthma, but rather to investigate them in their own right, because allergically inflamed mice are widely used in studies of AHR (7). On the other hand, there is an essentially limitless number of sensitization and challenge protocols to which one could expose a mouse to generate inflammation, and we may have by no means chosen the best examples. This applies particularly to the chronic model we used here. Whereas the acute protocol has been well established by us and other groups, our particular choice of chronic exposure protocol was more arbitrary, and different protocols have been used by other groups. Inman and colleagues (20, 33), in particular, have been able to demonstrate sustained changes in lung function following chronic ovalbumin exposure in mice, so applying the methods of the present study to these mouse models could be a productive area for future research. It would also be interesting to apply our model-fitting approach to other situations in which altered airway wall stiffness might be expected, such as the decorin-deficient mice recently shown to have an abnormal responsiveness to PEEP (31). The bottom line is that, just because we failed to show functional evidence of a change in airway wall

stiffness in the particular chronic model we investigated, this in no way means that we would not find such evidence in a different model. By the same token, our results do not mean that increased airway wall stiffness is not a common feature in human asthma.

In conclusion, we measured the time course of R_{aw} immediately following intravenous methacholine injection in acutely and chronically inflamed mice. We estimated the effective stiffness of the airway wall in these animals by fitting to the R_{aw} profiles a computational model of a dynamically narrowing airway embedded in elastic parenchyma. Effective airway wall stiffness was estimated from the model fit and found not to change from control in either the acute or chronic inflammatory groups. The chronically inflamed animals responded to intravenous methacholine almost identically to controls. The acutely inflamed mice, however, were hyperresponsive in terms of R_{aw} , which we interpret as reflecting increased delivery of methacholine to the ASM through a leaky pulmonary endothelium. These results further support the notion that acutely inflamed BALB/c mice represent an animal model of functionally normal ASM in an abnormal lung.

APPENDIX

Figure 5 shows a representation of stylized time course data for R , together with two possible stylized model fits, both of which describe the data equally well in terms of least squares goodness of fit. One of the curves rises too slowly, but reaches a peak value that is too high and is characterized by a value of b (proportional to peak contraction velocity) that is too small and a value of F_0 that is too large. The other curve rises too quickly and peaks too low and is characterized by a value of b that is too large and a value of F_0 that is too small. However, the product bF_0 is similar in both cases.

Figure 6 shows a plot of the classic Hill relationship that we used to describe the force-velocity (F - v) for ASM. The area (A) under this curve from $F = 0$ to $F = F_0$ is given by the integral of Eq. 1 thus:

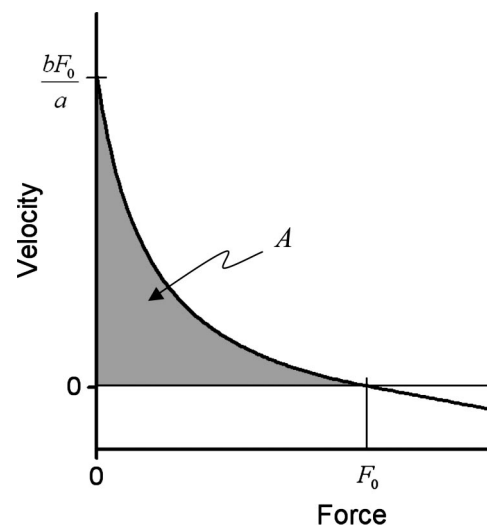


Fig. 6. Force-velocity curve described by Eq. 2. The shaded area (A) is proportional to bF_0 and is a measure of the power output of the airway smooth muscle as it shortens from the initially unloaded state to the point of isometric contraction.

$$\begin{aligned}
 A &= \int_0^{F_0} v dF \\
 &= \int_0^{F_0} \frac{b(F_0 - F)}{a + F} dF \\
 &= b(F_0 + a) \ln \left(\frac{F_0}{a} + 1 \right) - bF_0 \\
 &= 0.207bF_0
 \end{aligned} \tag{A1}$$

where we have used the fact that $F_0 = 4a$.

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