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The allergic mouse model of asthma: normal smooth muscle in an abnormal lung?

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Wagers, Scott, Lennart K. A. Lundblad, Mari Ekman, Charles G. Irvin, and Jason H. T. Bates. The allergic mouse model of asthma: normal smooth muscle in an abnormal lung? *J Appl Physiol* 96: 2019–2027, 2004. First published December 5, 2003; 10.1152/japplphysiol.00924.2003.—Mice with allergically inflamed airways are widely used as animal models of asthma, but their relevance for human asthma is not understood. We, therefore, examined the time course of changes in respiratory input impedance during induced bronchoconstriction in BALB/c mice sensitized and challenged with ovalbumin. Our results indicate that bronchoconstriction in mice is accompanied by complete closure of substantial regions of the lung and that closure increases markedly when the lungs are allergically inflamed. With the aid of an anatomically accurate computational model of the mouse lung, we show that the hyperresponsiveness of mice with allergically inflamed airways can be explained entirely by a thickening of the airway mucosa and an increased propensity of the airways to close, without the involvement of any increase in the degree of airway smooth muscle shortening. This has implications for the pathophysiology of asthma and suggests that at least some types of asthma may benefit from therapies aimed at manipulating surface tension at the air-liquid interface in the lungs.

inflammation; lung impedance; resistance; elastance; mucosal thickening

RESEARCH ON ANIMAL MODELS of bronchial hyperresponsiveness is critical for investigating the pathophysiology of asthma. Mice sensitized and challenged with allergens experience a transient influx of cells into the lungs, similar to that seen in humans, and are widely used as animal models of asthma (9, 19, 28, 30, 51). Such mice also exhibit an elevated dose-response relationship to challenge with methacholine (51), another hallmark feature of asthma, yet the mechanisms responsible are not understood. The purpose of the present study was, therefore, to try and elucidate these mechanisms, as this is essential for determining how relevant the allergically inflamed mouse is as an animal model of asthma. Our approach was to examine the time course of the lung's response to a single methacholine challenge, rather than focusing on the usual dose-response relationship. Our laboratory has previously shown in dogs (1, 2, 4) and rats (5) that the temporal dynamics of bronchoconstriction reveal important physiological information not present in the dose-response curve, so we hypothesized that this approach might also lead to insights into the nature of bronchial hyperresponsiveness in the mouse.

Nevertheless, linking abnormalities in function to alterations in structure in the lung of any species is rarely straightforward. It is often possible to interpret a given set of physiological data in a number of different but apparently plausible ways when it

comes to mechanisms responsible for bronchial hyperresponsiveness (7). However, not all such mechanisms will stand up to a quantitative analysis of their consequences. Therefore, a key component of the present study was to use an anatomically accurate computational model of the mouse lung to interpret our experimental measurements of lung function during bronchoconstriction. Simulation studies with such a model allow us to test the plausibility of different potential mechanisms of hyperresponsiveness (15, 33, 50). This allows us to discard mechanisms that might sound reasonable at the outset but that are not capable of producing the levels of hyperresponsiveness that we see experimentally.

METHODS

Experimental. We studied a control group of BALB/c mice ($n = 11$) and a group sensitized to ovalbumin ($n = 7$). The sensitized animals were treated with two intraperitoneal injections of ovalbumin in alum, spaced 1 wk apart, and then studied 1 wk later. Both control and sensitized groups were challenged with ovalbumin immediately before being anesthetized for experimentation by being placed in a closed aerosolization chamber and being exposed to ovalbumin aerosol (1% in phosphate-buffered saline) for 30 min. Animals were then anesthetized (pentobarbital sodium, 90 ml/kg by intraperitoneal injection), tracheostomized, and 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 positive end-expiratory pressure of 3 cmH₂O. Animals were paralyzed with an intraperitoneal injection of pancuronium bromide (0.08 µg/kg) and then exposed to an aerosol of methacholine (12.5 mg/ml) for 40 s by directing the inspiratory line of the ventilator through the aerosolization chamber of an ultrasonic nebulizer (Porta-Sonic model 8500C, DeVillbiss Health Care, Somerset, PA) while the animals were ventilated at 30 breaths/min with a tidal volume of ~0.4 ml. At the end of the 40-s challenge, the nebulizer was immediately taken out of the inspiratory circuit, and mechanical ventilation was resumed at 200 breaths/min with a tidal volume of 0.2 ml. Every 10 s for the following 3 min, ventilation was interrupted to allow a 1-s passive expiration followed by the application of a 2-s broadband (1–19.625 Hz) volume perturbation to the lungs while the pressure required to generate the perturbations was measured (17, 51). The peak-peak excursion of the ventilator piston during delivery of these perturbations was 0.17 ml, resulting in a volume delivered to the lungs of ~0.14 ml after accounting for gas compression in the ventilator cylinder and connecting tubing. Perturbations were delivered above the end-expiratory lung volume. These studies were approved by the Institutional Animal Care and Use Committee of the University of Vermont.

The pressure and flow data obtained during application of each volume perturbation were used to calculate a complex input imped-

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ance of the respiratory system (Z_{rs}), as previously described (17). Z_{rs} was then fit to a model consisting of a single airway serving a constant-phase viscoelastic tissue unit. The model equation is (20)

$$Z_{rs}(f) = R_N + i2\pi f I_{aw} + \frac{G - iH}{(2\pi f)^\alpha} \quad (1)$$

where R_N is a Newtonian resistance composed mostly of the flow resistance of the conducting pulmonary airways (51), I_{aw} reflects the inertance of the gas in the central airways, G reflects viscous dissipation of energy in the respiratory tissues (tissue resistance), H reflects elastic energy storage in the tissues (tissue stiffness), f is frequency, $i = \sqrt{-1}$, and α couples G and H (20). Equation 1 was fit to measured values of $Z_{rs}(f)$ as follows. First, α was given a value 1.0, and R_N and G were obtained by fitting the real parts of $Z_{rs}(f)$ to the real terms of Eq. 1 by using multiple linear regression to minimize the unweighted residuals between model and data. I_{aw} and H were similarly determined by using the imaginary terms. The α was then reevaluated from the expression

$$\alpha = \frac{2}{\pi} \tan^{-1} \frac{H}{G} \quad (2)$$

and the parameters R_N , G , I_{aw} , and H were refit, again by multiple linear regression. The α was reevaluated from the new values of G and H , and the process was repeated 10 times. We have found that the parameter values determined using this iterative approach typically converge to four significant figures in three or four iterations.

This model has been shown to accurately describe respiratory impedance between 0 and 20 Hz under control conditions and during mild bronchoconstriction (17, 20, 46). We thus obtained a time course of the parameters R_N , I_{aw} , G , and H for the 3 min following bronchial challenge in the mice. I_{aw} has a negligible effect in the mouse lung <20 Hz and so can be ignored (17). Therefore, we confine our attention to the parameters R_N , G , and H .

Lung sections were prepared in two additional animals, one control and one allergically inflamed. The lungs were fixed in formalin at 30 cmH₂O, embedded in paraffin, cut in 8- μ m sections, and stained with picosirius red.

Computational model. We constructed a computational model of the mechanics of the mouse lung following the asymmetric branching scheme of Horsfield (21) using the airway structural data determined for the mouse by Gomes and Bates (16). These data include the diameters and lengths of 19 airway orders, with a Horsfield delta value of 6 for orders 1–12 and delta values decreasing stepwise to zero for subsequent orders. Random values were assigned to the baseline (unconstricted) radii of the airways in the model, according to Gaussian distributions, having means and standard deviations appropriate to the airway order as per the data tabulated for the mouse lung by Gomes and Bates. The impedance of each airway (Z_{aw}) in the model was calculated, assuming Poiseuille flow to determine resistance and the mass of the airway gas to determine inertance, as follows

$$Z_{aw}(f) = \frac{8\mu L}{\pi r^4} + i \frac{2fL\rho}{r^2} \quad (3)$$

where r is airway radius, L is airway length, μ is gas viscosity, and ρ is gas density. This assumes that the airways are rigid in terms of their influence on impedance, that is, we neglect the influence of airway wall shunting.

Each of the most distal airways terminated in an identical tissue unit with impedance given, in analogy with Eq. 1, by

$$Z_{ti}(f) = \frac{G_{ti} - iH_{ti}}{(2\pi f)^\alpha} \quad (4)$$

where Z_{ti} is tissue impedance, G_{ti} is tissue G , and H_{ti} is tissue H , with the ratio of G_{ti} to H_{ti} (G_{ti}/H_{ti}) being assigned a value of 0.1. The total impedance of the model (Z_{mod}) was calculated by adding the indi-

vidual Z_{aw} and Z_{ti} in series or parallel, as appropriate. Z_{mod} was calculated at each of the frequencies in the volume perturbation signal used to obtain Z_{rs} experimentally in the mice.

The Monte-Carlo approach was used to obtain a set of different Z_{mod} by running the computational model multiple times, each time using a different statistical realization of the airway radii values. Simulations of the time course of bronchoconstriction were achieved by having the model airways transiently decrease their radii according to a prescribed function of time (see below).

RESULTS

Experimental results. Figure 1 shows Z_{rs} obtained from the control mice under baseline (unconstricted) conditions. The values of R_N , G , and H obtained by fitting Eq. 1 to these data were 0.29, 3.0, and 20.4 cmH₂O·s·ml⁻¹, respectively. The baseline parameter values from the allergically inflamed mice were only slightly elevated at $R_N = 0.34$, $G = 3.5$, and $H = 23.4$ cmH₂O·s·ml⁻¹.

Figure 2 shows the resulting time courses of R_N , G , and H , expressed as percent changes from baseline (prechallenge) values in both the control and inflamed mice. Delivery of methacholine aerosol was completed at time = 0. Both control and inflamed animals demonstrated initial transient increases in ΔR_N and ΔG (where Δ is change) that peaked at 30–50 s, followed by elevated plateaus (Fig. 2, top and middle). By contrast, ΔH (Fig. 2, bottom) was devoid of an initial peak and only exhibited a final elevated plateau in both control and inflamed mice. ΔR_N , ΔG , and ΔH were all returned from their plateau values to near baseline by administration of two deep inflations to 25 cmH₂O.

Lung inflammation induced by antigen challenge had a modest effect on the time course of ΔR_N ; the peak was elevated by ~70% compared with control, with a similar relative elevation in the plateau level (Fig. 2, top). In contrast, the plateau in ΔH in the inflamed animals was about five times as high as in the controls (Fig. 2, bottom). The effect of inflammation on ΔG (Fig. 2, middle) was intermediate between those on ΔR_N and ΔH .

Computational results. Figure 3 shows Z_{rs} obtained from the control mice under baseline conditions, together with the

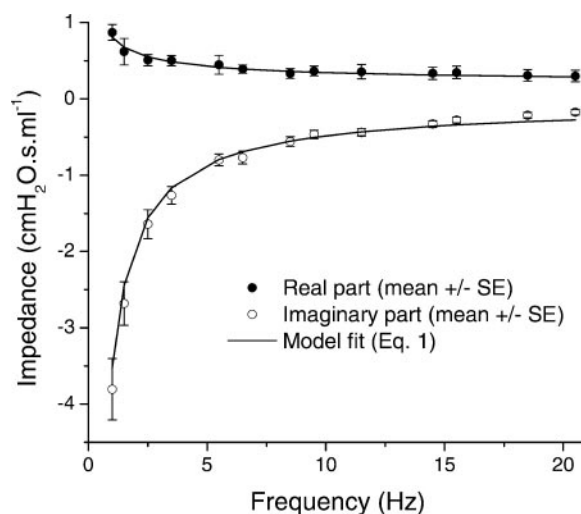


Fig. 1. Fit of Eq. 1 (solid lines) to impedance of the respiratory system (Z_{rs}) from normal mice ($n = 11$) under baseline conditions.

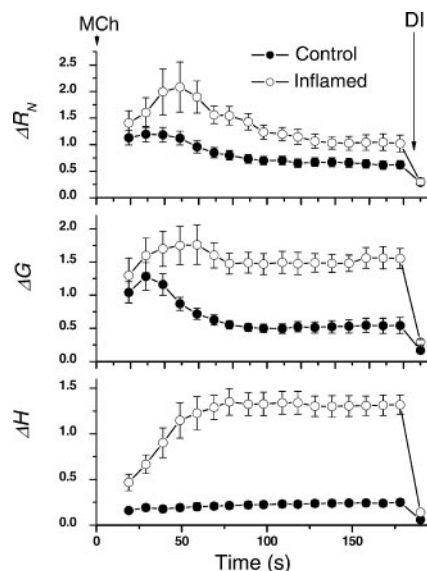


Fig. 2. The parameters of respiratory input impedance [Newtonian resistance (R_N), viscous dissipation of energy in respiratory tissues (G), and elastic energy storage in tissues (H)] expressed as fractional changes above baseline (ΔR_N , ΔG , and ΔH , respectively) in control and allergically inflamed mice following a 40-s challenge with an aerosol of methacholine. Values are means $\pm$ SE. MCh, completion time of methacholine delivery. DI, delivery time of two deep lung inflations to 25 cmH₂O.

real and imaginary parts of Z_{mod} obtained from 11 Monte-Carlo simulations under baseline conditions when the values of the tissue parameters for each terminal unit in the computational model (i.e., G_{ti} and H_{ti} in Eq. 4) were given values of 180 and 1,800 cmH₂O·s·ml⁻¹, respectively, and when the mouse airway radii reported by Gomes and Bates (16) were divided by a factor of 1.2. When the constant-phase model (Eq. 1) was fit to Z_{mod} , the values of the impedance parameters obtained were model resistance (R_{mod}) = 0.032 ± 0.01 (SE), model G (G_{mod}) = 3.5 ± 0.1 , and model H (H_{mod}) = 20.8 ± 0.1 cmH₂O·s·ml⁻¹. These values are close to the values for R_N , G , and H reported above for the control mice under baseline conditions.

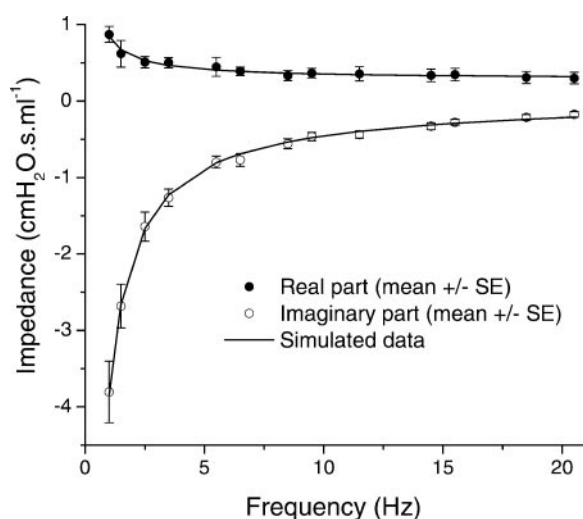


Fig. 3. Computer-simulated (mean of 11 Monte-Carlo simulations) and experimental values of Z_{rs} from normal mice under baseline conditions.

To determine the transient fractional decrease in airway diameters needed to create a time course for ΔR_{mod} matching that observed in ΔR_N , the mean profile of ΔR_N from the control mice (Fig. 2, top, solid circles) was added to unity and inverted, and the fourth root of the result was taken (22). This gave a signal expected to produce the experimentally observed fractional increase in airway resistance under the assumption of Poiseuille flow. The simulated increase in ΔR_{mod} obtained with this signal, however, was not quite large enough to match the experimental observations. When the signal was scaled by a factor of 1.3 (Fig. 4, solid circles), the time course of ΔR_{mod} matched that of ΔR_N from the control animals closely (Fig. 5A, top).

The time courses of ΔG_{mod} and ΔG profiles also matched well during the plateau phase, but ΔG_{mod} did not achieve the initial peak of ΔG (Fig. 5A, middle). Most notable, however, is that the simulated ΔH_{mod} was unable to achieve the plateau exhibited by the experimental ΔH (Fig. 5A, bottom).

We next included a mechanism for airway closure in the computational model by following the diameters of all airways as bronchoconstriction proceeded. Whenever an airway narrowed to a critical radius, it immediately closed and remained closed for the duration of the simulation. We found, by trial and error, that a critical radius of 38 μ m produced a plateau in ΔH_{mod} similar to that seen experimentally, while hardly affecting ΔR_{mod} , but at the same time it worsened the match between ΔG_{mod} and ΔG (Fig. 5B). The match between ΔG_{mod} and ΔG was improved, however, when we allowed G_{ti}/H_{ti} , which had an initial value of 0.1, to increase during bronchoconstriction, as has been observed in vitro (10). We do not know exactly how to do this, so we adopted the ad hoc approach of scaling this ratio inversely with the degree of fractional airway narrowing (i.e., by the inverse of the upper curve shown in Fig. 4). These modifications to the model caused the simulated and experimental parameters to match closely in most respects over the course of the data (Fig. 5C).

Having thus calibrated the computational model to the time course data from the control animals, we turned our attention to

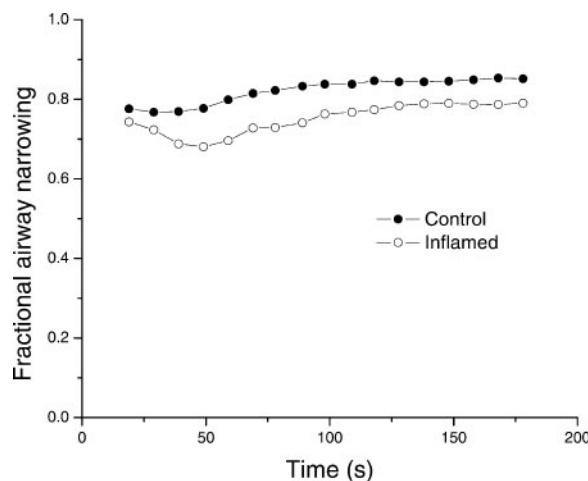


Fig. 4. Time courses of fractional airway radius used to simulate transient bronchoconstriction in the computational model of control mice and in the model of inflamed mice, assuming increased shortening of the airway smooth muscle.

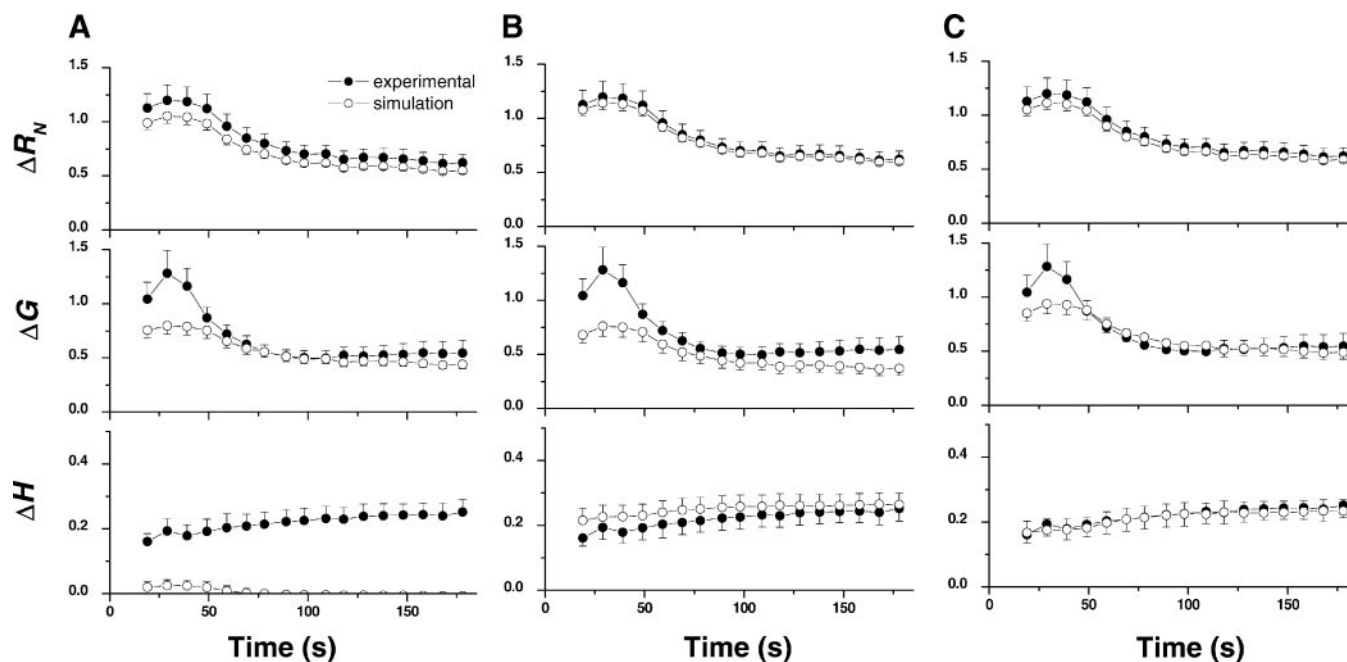


Fig. 5. Simulations of fractional changes above baseline of impedance parameters during bronchoconstriction in a normal mouse. A: with airway narrowing only. B: with the addition of airway closure at a threshold radius of 38 μm . C: with the further addition of a hysteresivity that increases with the degree of airway narrowing. Experimental values are mean $\pm$ SE; $n = 11$. Simulated values are means $\pm$ SE of 11 Monte-Carlo simulations.

the question of mimicking the data from the allergically inflamed animals. In particular, we sought to find a mechanism for hyperresponsiveness that, when applied to the control model, would make it behave like the allergically inflamed animals. We first tried simply increasing the amount of airway narrowing, by augmenting the transient fractional decrease in

radius (Fig. 4, open circles), while keeping all other aspects of the model identical to those used to simulate the control data shown in Fig. 5C. The results are presented in Fig. 6A and show that that ΔR_{mod} matches ΔR_N , but both ΔG_{mod} and ΔH_{mod} fall short of their respective experimental counterparts. This result indicates that the allergically inflamed lung is not

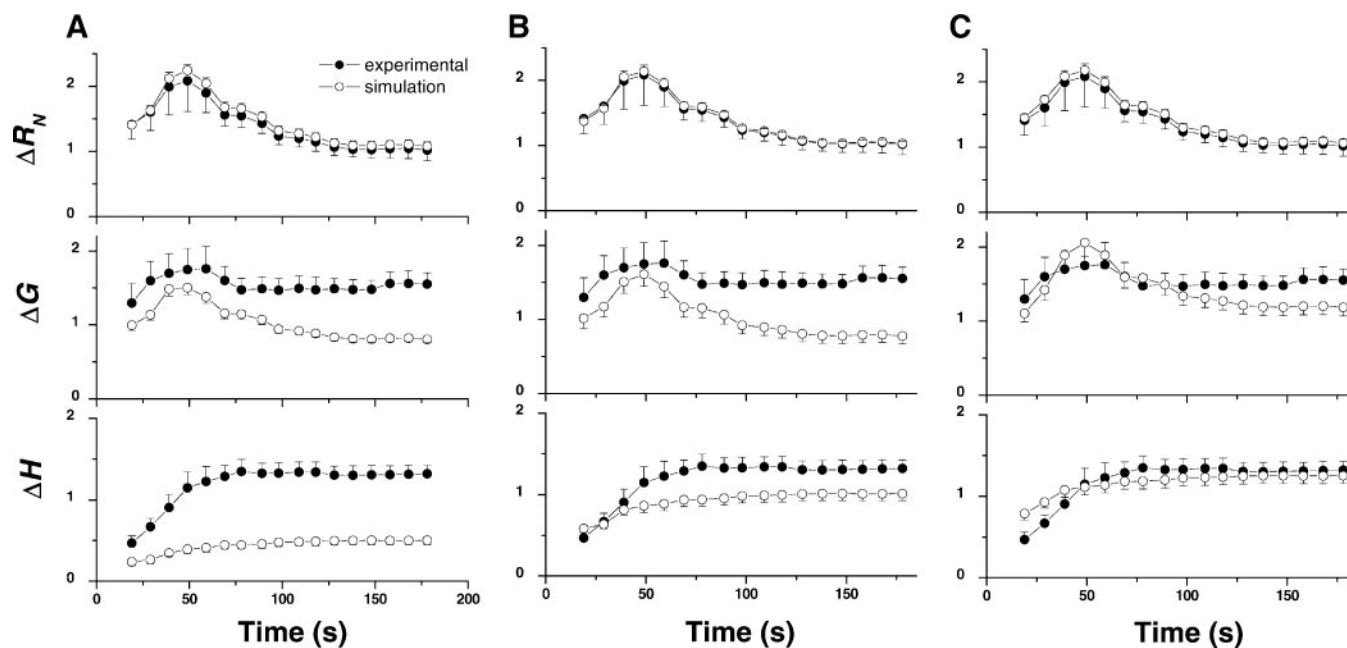


Fig. 6. Simulations of the bronchoconstriction time course in allergically inflamed mice. A: same conditions as the control simulations shown in Fig. 5C but with increased airway narrowing. B: same maximal airway narrowing as control simulations but with addition of an inner lining to the airway wall having a thickness of 18 μm . C: same as B except with a 15% increase in the airway closure threshold radius. Experimental values are means $\pm$ SE; $n = 7$. Simulated values are means $\pm$ SE of 7 Monte-Carlo simulations.

hyperresponsive simply because its airway smooth muscle responds excessively to a given methacholine stimulus.

To test an alternative mechanism for hyperresponsiveness, we adjusted the fractional degree of airway narrowing so that it had the same shape as before (i.e., as the open circles in Fig. 4) but so that its minimum value matched that of the control profile (i.e., the solid circles in Fig. 4). At the same time, the airway walls were all imbued with a lining of thickness of 18 μm , which is typical of the epithelial thickening seen histologically in allergically inflamed mice (Fig. 7). This gave a profile for R_N (Fig. 6B) that was virtually identical to that produced by the increased degree of narrowing (Fig. 6A). It also produced a substantially elevated plateau in ΔH_{mod} , although still not to the level seen in ΔH in the allergically inflamed mice. The baseline parameter values obtained by fitting Eq. 1 to the data simulated with the thickened airway wall, before constriction, were $R_{\text{mod}} = 0.46 \pm 0.02$ (SE), $G_{\text{mod}} = 4.9 \pm 0.3$, and $H_{\text{mod}} = 27.4 \pm 0.1 \text{ cmH}_2\text{O} \cdot \text{s} \cdot \text{ml}^{-1}$. These values are slightly elevated compared with the values for R_N , G , and H reported above for the inflamed mice under baseline conditions, but the differences are small compared with the changes induced by methacholine. Thus thickening the airway walls had an effect on lung mechanics that was relatively small under baseline conditions, but became substantially magnified with bronchoconstriction.

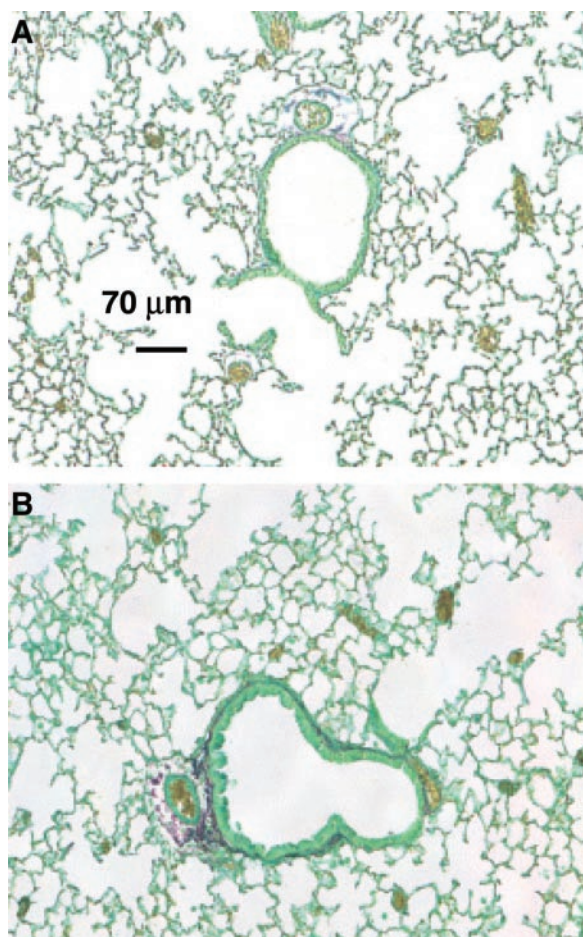


Fig. 7. Lung section from a control BALB/c mice (A) and an acutely inflamed mouse (B) (2 ova immunizations, 3 daily ova challenges, studied 48 h postfinal challenge. Magnification = $\times 100$).

Finally, we found that a further improvement in the matching of the simulated data to the experimental data from the inflamed mice could be achieved by increasing the critical closure radius in the model by 18%, from 38 to 45 μm . This represents a somewhat increased propensity for closure that an inflamed lung might have due to increased airway fluid and inflammatory debris and had virtually no effect on R_N but gave a substantially better fit for both G and H (Fig. 6C).

DISCUSSION

There are numerous ways in which a lung can potentially become hyperresponsive to bronchial challenge (7). These include mechanisms whereby the effect of a given degree of airway smooth muscle shortening is amplified via geometric effects (3, 40) and mechanisms involving increased muscle shortening (36, 37, 41, 52). Much focus has been placed recently on the notion that the primary defect in asthma is excessive shortening of the airway smooth muscle, either because of an enhanced responsiveness of the muscle itself (11, 12, 18) or because of changes in the stiffness and thickness of the airway wall (40, 41, 43). However, there is growing evidence that airway narrowing is not the only process at play in asthma. Recent imaging studies (26, 47), bronchoscopic investigations (23–25), and older spirometric studies (38, 48) indicate that airway closure contributes significantly to the asthma phenotype. Our results indicate that the airway closure is also a central phenomenon in the hyperresponsiveness of the allergically inflamed mouse, supporting the relevance of this commonly used animal model of asthma. Our results also suggest that the enhanced airway closure caused by airway wall thickening is the major factor responsible for bronchial hyperresponsiveness in allergically inflamed mice.

We arrived at these conclusions by comparing our experimental data with simulated data produced by an anatomically based computational model of the mouse lung. This is an investigational paradigm borrowed from the physical sciences, whereby mathematical and computer models are used both to interpret experimental data and to test specific hypotheses about the link between structure and function. For example, an apparently simple structural alteration, such as thickening the airway wall, can have a complicated effect on Zrs, causing R_N , G , and H to change to different degrees. Simulation studies with a computational model allow the quantitative nature of these various effects to be investigated and so related in a meaningful way to experimental measurements. This approach has been used successfully in previous respiratory investigations in dogs (50) and in humans (14, 15, 33), and we believe that it is the only way of gaining a better understanding of the mechanisms underlying a phenomenon as complex as bronchial hyperresponsiveness. However, for this approach to be successful, it is important that the model be as realistic as possible and also that it be employed under conditions that mimic those of the experiment. Thus we calculated the model impedance and fit it to the constant-phase model (Eq. 1) at exactly the same frequencies as were used to measure Zrs in the mice. This is important because the constant-phase model does not describe either the real data or the simulated data perfectly. It is, therefore, crucial that any systematic model errors incurred with the experimental data be reproduced with the simulated data. We also found that, to have the ratio of

Gmod to Hmod turn out similarly to that observed experimentally (~ 0.17), it was necessary to use values for Gti and Hti in the individual model tissue units that had a ratio of 0.1, no doubt again because of the heterogeneities inherent in the model. Interestingly, the ratio of G to H (known as hysteresivity) obtained in isolated lung tissue strips is closer to 0.1 than 0.17 (31).

The parameter R_N has been shown in mice to provide a good measure of the overall resistance of the conducting airway tree (51). Thus the initial peak in ΔR_N (Fig. 2, top) presumably represents the airway narrowing that occurs as methacholine aerosol reaches the airway smooth muscle and causes it to contract. The peak in ΔR_N is not sustained because methacholine is removed by the circulation as well as being enzymatically degraded in situ (29). Removal of methacholine, however, returns ΔR_N , not to baseline, but instead to an elevated plateau (Fig. 2, top). One possible cause of this plateau is that, once a contractile agonist has been removed, airway smooth muscle does not fully relax back to its initial configuration unless it is physically stretched. Another explanation is that bronchoconstriction is accompanied by complete closure of air spaces in the lung, as suggested by Evans et al. (9). Such air spaces might not open spontaneously when the smooth muscle relaxes but could do so when a deep inflation is administered (13, 42, 45) and could account for the normalization of R_N , G, and H that we observed following a deep inflation (Fig. 2). This raises the question of exactly which structures in the lung are closing. We have previously shown that functional residual capacity in mice increases in a dose-dependent fashion with methacholine administration (32), indicative of gas trapping in the lung. This suggests that the structures that are closing are airways rather than alveoli. Interestingly, we observed that Zrs returned close to baseline following a deep inspiration (DI) in both normal and inflamed mice, in contrast to the situation in asthmatic humans in whom a DI is often largely ineffectual at permanently reversing bronchoconstriction (6, 22).

The closure hypothesis is also supported by the time course of ΔH (Fig. 2, bottom). Closure of airways causes H to increase, because the remaining open regions constitute an effectively smaller lung with a commensurately greater overall elastance. Furthermore, closure of airways would be expected to accrue as airway narrowing develops and to persist until the high pressures accompanying a deep lung inflation manage to overcome surface tension forces at the site of closure (13, 42). ΔH could also increase due to an increase in intrinsic tissue stiffness, which could result from the distortion of the parenchyma produced as the airways narrow (2). However, this effect should be maximal when airway narrowing is maximal, so the absence of a peak in ΔH to match that in ΔR_N argues against this possibility. Another way that tissue stiffness can increase is through the development of regional heterogeneities throughout the lung (2, 34, 49). Again, however, we would expect this to be maximal when ΔR_N is maximal because heterogeneity in the narrowing of airways always seems to develop commensurately with the degree of mean airway constriction (1, 39), presumably because bronchoconstriction tends to amplify any existing heterogeneities than might exist under baseline conditions. This makes airway closure the most likely candidate to explain the elevated plateau in ΔH . Closure also might explain part of the plateau in ΔR_N . Note, however, that the plateau in ΔR_N (Fig. 2, top) is proportionately much

greater than the plateau in ΔH (Fig. 2, bottom). If the plateau in ΔR_N were also due to closure, we would expect it to be relatively no greater than that in ΔH , because the serial structure of the airway tree would cause the fractional loss of lung tissue through closure to be at least as great as fractional loss of airways. This suggests, therefore, that most of the plateau in ΔR_N is not due to closure but rather to residual narrowing of the remaining open airways. The primary effect of a deep inflation on ΔR_N , then, is to stretch the airway smooth muscle.

G purportedly reflects viscous energy dissipation in the respiratory tissues, something that is often closely allied with changes in airway resistance (2, 34), perhaps due to the parenchymal distortion that occurs when the airways constrict. However, G has also been shown to increase with increases in the degree of regional airflow heterogeneity throughout the lung (34, 35). As bronchoconstriction seems to be an inherently heterogeneous phenomenon (39), this may explain why the time course of ΔG is similar to that of ΔR_N , but with a more pronounced peak.

We implemented a transient bronchoconstriction in the model by narrowing its airways according to an appropriate time course (Fig. 4), which makes the assumption that both the peak and plateau in ΔR_N (Fig. 3) reflect only the degree of airway narrowing. However, this was not sufficient to accurately simulate the responses in ΔG and ΔH . Our initial thought was that the responses in G and H might simply reflect heterogeneities in the responses of the airways to agonist, as heterogeneities have been demonstrated previously in rats (35). In fact, the bronchoconstriction simulated by our model is automatically heterogeneous because of the random selection of the baseline airway radii, even when all of these radii are reduced by the same fraction. Indeed, such heterogeneity is essential because, otherwise, all airways of a particular order would reach the critical closure radius simultaneously, at which point the model would suddenly close completely. However, when we increased the heterogeneity of airway narrowing by varying the fractional amount by which the individual radii were reduced (results not shown), we found we could never achieve plateaus in ΔH mod without also producing large initial peaks in ΔH mod, contrary to the observed changes in ΔH (Fig. 2). On the other hand, we were able to produce persistent plateaus in ΔH mod in the model by irreversibly closing any airway that narrowed to a certain critical radius. This produced a heterogeneous process that mimics the process of sudden liquid bridge formation, which is thought to be a key mechanism for airway closure (44). Our critical radius of 38 μm was chosen because it gave a good fit between data and model simulation and is slightly less than the smallest airways in the model under normal unconstricted conditions. We also increased Gti/Hti slightly, as bronchoconstriction developed in accord with measurements on isolated strips of lung parenchyma (10). The final result was a good match between the measured and simulated impedance parameters (Fig. 5C), apart from some discrepancies around the initial peak between ΔG and ΔG mod. These differences could be due to a change in the dissipative properties of the parenchyma under conditions of airway smooth muscle activation.

We thus succeeded in constructing a model of the normal mouse lung that satisfies the major anatomic constraints and that mimics the essential features of the time course of Zrs seen in acute bronchoconstriction. However, this is essentially noth-

ing more than curve fitting, because one could always argue that some other plausible combination of features might produce an equally good fit. The real utility of the model is in exploring what it now takes to make it behave like the allergically inflamed mice. In this regard, our results indicate that a mere increase in the degree of airway smooth muscle shortening cannot explain the hyperresponsiveness of the allergically inflamed mouse (Fig. 6A). On the other hand, when the maximal fractional airway narrowing in the model was limited to that of the control simulations (Fig. 4, solid circles) and each airway in the model was imbued with an inner lining of thickness of 18 μm , an accurate simulation of ΔR_N from the inflamed animals was obtained (Fig. 6B). The thickness value of 18 μm was obtained empirically but is similar to the thickening of the epithelium seen in slices of allergically inflamed mouse lung (Fig. 7). In other words, the geometric amplification of the effect of a given degree of smooth muscle shortening by the wall thickening was sufficient to produce all of the observed hyperresponsiveness in ΔR_N seen in the inflamed animals.

The airway wall thickening also increased the amount of airway closure, causing ΔH_{mod} to be closer to experimental observation than that caused merely by increasing the amount of muscle shortening. However, the match between experimental and simulated data was still not perfect. We found that a further improvement in fit could be achieved by increasing the critical closure radius by 18% so that airways closed somewhat earlier than under control conditions (Fig. 6C). An increased propensity for closure is to be expected in inflamed lungs because airway secretions would accentuate the tendency for liquid bridging to take place as airways narrow. By increasing airway wall thickness, we would also increase the heterogeneity in the onset of closure throughout the lung, as previously suggested (14, 15).

Even better fits to the experimental data can be achieved by including various other mechanisms in the model. For example, when fixed volume perturbations are applied to the lungs in the presence of significant airway closure, the remaining open-lung regions may become distended, as has been observed directly in constricted rat lungs using magnetic resonance imaging of hyperpolarized helium (8). When we incorporated this effect in the model by preventing any airways that remained open from having radii $<150\%$ of the closure radius, we obtained even closer matches between model and data than those seen in Fig. 6C. Nevertheless, such speculative model features are difficult to validate.

A final interesting point about our simulation study concerns the standard error bars shown in Figs. 5 and 6. These arose from the Monte-Carlo simulation and reflect the variation between different sets of simulated data obtained by using different random draws for the airway radii in the model. Although the error bars around the experimental data are larger than those in the simulated data near the peak in the response, they are of a similar magnitude along the plateau. This suggests that much of the variation between different mice can be explained in the same way as we explain the variation in our simulation results. In other words, different animals of a given pure-bred strain may be a reflection of nature's own Monte-Carlo process in which the airway tree in any individual mouse is a particular random realization of a fixed genetic plan.

Our simulation results thus point to the central roles played by epithelial thickening and increased secretions in the hyperresponsiveness of the allergically inflamed mouse. These insights, however, are contingent on the physiological relevance and accuracy of our computational model, and as such are always open to question. For example, we based our model on the reported airway structure of the Harvest mouse (16), which is a much smaller animal than the BALB/C mice in which we measured Zrs. Curiously, we had to decrease the radii measured in the Harvest mouse to reproduce the impedance measured in the BALB/c mouse. However, our model calculations assumed fully developed Poiseuille flow in the airways, which would likely underestimate true resistance. Also, the morphometric data from the Harvest mouse were obtained from analysis of a lung cast, which may have endured substantial preparation artifacts. We also chose other critical features in the computational model, such as the critical closure airway radius and the thickness of the epithelial lining, on the basis of the accuracy of the simulations they produced. Although we have defended our choice of these quantities as being biologically appropriate, additional morphometric studies of inflamed vs. control lungs would be valuable in confirming our assumptions. There are many other ways in which the properties of the computational model could be adjusted to mimic the process of bronchoconstriction, such as having airways of different sizes constrict by different relative amounts, or by having a critical closure threshold that varies with baseline airway radius. At present, there is little experimental evidence to guide our choices in these matters, which is not to say they are not important or relevant.

In conclusion, we have shown that the time course of bronchoconstriction in an anatomically accurate computer model of the normal mouse lung can be made to closely resemble that of an allergically inflamed mouse simply by adding a thickened airway wall and slightly increasing the propensity of the airways to close. This was achieved without any change in the degree of airway smooth muscle shortening over that occurring in control animals. Indeed, when increased smooth muscle shortening was invoked in the model without airway thickening, the simulations produced a much poorer representation of the data. This suggests that the hyperresponsiveness of acute allergic inflammation in the mouse is due to normal airway smooth muscle acting on a structurally abnormal lung, which contrasts (30) with the widely held view that the bronchial hyperresponsiveness of asthma involves an abnormality of the smooth muscle itself (12). Indeed, the inability of a DI to reverse bronchoconstriction in humans (22) is suggestive of pathology at the level of the smooth muscle, something that was evidently absent in our allergic mice. Of course, our results only pertain to a very acute course of inflammation. Mice receiving more chronic antigen treatment exhibit evidence of airway remodeling that might well involve additional mechanisms of hyperresponsiveness, perhaps involving the airway smooth muscle. Nevertheless, our results raise the possibility that, insofar as the allergic mouse is a germane model of human asthma, airway closure may be the principle mechanism behind the bronchial hyperresponsiveness seen in human asthma, especially those forms with a state of active inflammation (23, 27). Such forms of asthma might benefit more from therapies aimed at opening closed air spaces

rather than relaxing airway smooth muscle. Identifying those individuals who could benefit from such therapies would then become an important goal.

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