EXAGGERATED AIRWAY NARROWING IN MICE TREATED WITH

INTRA-TRACHEAL CATIONIC PROTEIN


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Running title: Airway narrowing in mice treated with cationic protein

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ABSTRACT

Airway hyperresponsiveness in mice with allergic airway inflammation can be attributed entirely to exaggerated closure of peripheral airways (J Appl Physiol 96: 2019-27, 2004). However, clinical asthma can be characterized by hyperresponsiveness of the central airways as well as the lung periphery. We therefore sought to establish a complementary model of hyperresponsiveness in the mouse due to excessive narrowing of the airways. We treated mice with a tracheal instillation of the cationic protein poly-L-lysine (PLL), hypothesizing that this would reduce the barrier function of the epithelium and thereby render the underlying airway smooth muscle more accessible to aerosolized methacholine. The PLL-treated animals were hypersensitive to methacholine: they exhibited an exaggerated response to sub-maximal doses but had a maximal response that was similar to controls. With the aid of a computational model of the mouse lung we conclude that the methacholine responsiveness of PLL-treated mice is fundamentally different in nature to the hyperresponsiveness we found previously in mice with allergically inflamed lungs.

Key words: respiratory impedance, computational model, airway hyperresponsiveness, asthma
INTRODUCTION

Airway hyperresponsiveness is a characteristic feature of asthma (25), and is thought to involve exaggerated narrowing of both central and peripheral airways (19-21). We recently reported, however, that hyperresponsiveness in the most commonly used animal model of asthma, the mouse with allergic airway inflammation, can be attributed entirely to accentuated airway closure without any evidence to support an increase in the degree of airway smooth muscle shortening (29). The allergically inflamed mouse thus appears to manifest only a limited set of the various functional abnormalities that may be present in clinical asthma. Indeed, asthma appears to be a particularly human disease, and attempts to re-create all its attributes in a single animal model have been rather unsuccessful despite many attempts (2, 5, 15, 30).

It may thus serve us better to pursue the development of a set of animal models that collectively encompass the full spectrum of abnormalities seen in asthma, but which individually represent only part of this spectrum. This would allow us to examine the effects of each abnormality individually, and perhaps even to study the interactions between abnormalities if more than one can be implemented simultaneously in the same animal. In this sense, the acutely allergically inflamed mouse is a useful representation of one kind of hyperresponsiveness, namely that which occurs when an inflamed mucosa encroaches into the airway lumen. Such encroachment amplifies the increases in airway resistance and airway closure caused by a normal degree of smooth muscle shortening. This is by no means the only mechanism for producing hyperresponsiveness, however, and significant gaps remain in the currently available set of animal models embodying other mechanisms. The goal of the present study was therefore to establish a mouse model, complementary to the allergically inflamed preparation, in which hyperresponsiveness is due to enhanced narrowing of the conducting airways.
On the basis of previous work, we hypothesized that increased airway narrowing would occur in mice treated with poly-L-lysine (PLL), an artificial analog of native cationic protein. We have previously shown that PLL permeabilizes epithelial cells (28), and that rats treated with intra-tracheal PLL are hyperresponsive to methacholine that is delivered as an aerosol but not that is injected intravenously (16). These observations support the notion that PLL increases bronchial responsiveness by an epithelium-dependent mechanism, apparently making the underlying smooth muscle more accessible to an inhaled agonist. Such an effect would be expected to lead to increased contraction of the smooth muscle, and hence exaggerated airway narrowing, when the doses of methacholine are sub-maximal. By contrast, when the methacholine dose is supra-maximal the airway smooth muscle should contract maximally regardless of how quickly the agonist is able to traverse the epithelium. Furthermore, at all doses we would expect airway narrowing to be more rapid than normal, due to enhanced penetration of agonist to the underlying smooth muscle. The PLL-treated mouse should therefore constitute a model of airways hypersensitivity. To test these notions, we examined the time-course of respiratory impedance following administration of increasing doses of methacholine aerosol to mice pretreated with intra-tracheal PLL. As in our previous study in allergically inflamed mice (29), we interpreted the results by performing equivalent virtual experiments on an anatomically-based computational model of the mouse lung.

METHODS

Animal experiments: All protocols were approved by the University of Vermont Institutional Animal Care and Use Committee. We studied a group of 6-10 week old BALB/c (Jackson Laboratories, Bar Harbor) mice (n = 8, 20.8 ± 2.2 g) treated with intra-tracheal PLL, and a corresponding control group of mice (n=7, 20.2 ± 0.8 g) treated with intra-tracheal phosphate-
buffered saline (PBS). A preliminary analysis of the data showed that one animal in each group had poor impedance spectra that were not fit well by a model of impedance (see below). One additional animal in the PLL group had a dose-response curve that was substantially different to the others in the group. The remaining animals (n = 6 in each group) were tightly grouped, and were selected for consideration in this study. The mice were anesthetized with an intraperitoneal injection of sodium pentobarbital at a dose of 90mg/kg and then tracheostomized. A length of polyethylene tubing was passed into the trachea to allow instillation of 50 µl of PLL in PBS at a concentration of 2 mg/ml, followed by two or three 0.3 ml aliquots of air in order to push the PLL into the lung, as previously described (6, 16). Next, a 19-gauge metal cannula was secured in the tracheal opening and connected to a mechanical ventilator (*flexiVent*, Scireq, Montreal). Mechanical ventilation was administered for 30 min prior to the start of the experiment, at which point ¼ of the original dose of pentobarbital was given. Baseline mechanical ventilation was applied at 180 breaths/minute with a tidal volume of 0.25 ml against a positive end-expiratory pressure (PEEP) of 3 cmH$_2$O applied by a water trap.

The experimental protocol began with the normalization of lung volume history by the delivery of two deep lung inflations of 1.0 ml followed by 2 min of regular ventilation. Mice were then challenged with an aerosol of PBS for 40s, achieved by channeling the inspiratory flow from the ventilator through an ultrasonic nebulizer containing PBS. During the challenge the piston of the ventilator was programmed to deliver a tidal volume of 0.8 ml at a rate of 30 breaths/min. Due to the shunt compliance of the nebulizer chamber, however, the tidal volume actually delivered to the lungs was approximately 0.6 ml. Following cessation of aerosol delivery, the ventilatory rate and tidal volume were returned to baseline, and measurements of the complex input impedance ($Z_{rs}$) of the respiratory system were made at regular intervals for the next three minutes. Finally,
two more deep lung inflations were given. The above protocol was then repeated three more times with aerosols containing methacholine in PBS at sequentially increasing concentrations of 3.125, 12.5 and 50 mg/ml. Responses were obtained for all concentrations in all animals studied, with the exception of one of the animals in the Saline group from which data at 50 mg/ml were not obtained.

During the 3 minutes following delivery of each aerosol challenge, every 10 seconds of regular ventilation was terminated in a 1s passive expiration followed by a 2 second broad-band (1-19.625 Hz) volume perturbation, after which ventilation was immediately resumed. The peak-to-peak excursion of the ventilator piston during delivery of these perturbations was 0.17 ml above functional residual capacity, resulting in a volume delivered of about 0.14 ml after accounting for gas compression in the ventilator cylinder and connecting tubing. The pressure and flow data obtained during application of the volume perturbations were used to calculate $Z_{rs}$, which was then fit to a model of lung mechanics using an iterative scheme described previously (29). The model consists of a single airway having a Newtonian resistance ($R_N$) serving a uniformly ventilated tissue compartment having a constant-phase impedance. The model is described by the equation (14)

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

where $I_{aw}$ is the inertance of the gas in the central airways, $G$ reflects viscous dissipation of energy in the respiratory tissues, $H$ reflects elastic energy storage in the tissues, $f$ is frequency, $i = \sqrt{-1}$, and $\alpha$ couples $G$ and $H$. Following the approach of Ito et al. (18), we consider $f$ in the above equation to be normalized to the frequency at which $2\pi f = 1$, so that $G$ and $H$ both have
the same units as $R_N$, namely cmH$_2$O.s.ml$^{-1}$. This model has been shown to accurately describe $Z_{rs}$ between 0 and 20 Hz under control conditions and during mild bronchoconstriction (12, 13, 24). We have found previously (29) that $I_{aw}$ has negligible effect in the mouse lung below 20 Hz, no doubt because the mass of the gas in the mouse trachea is so small. Also, the tracheal cannula bypasses a significant fraction of the trachea, and the impedance of the cannula itself is removed in the calculation of $Z_{rs}$. Consequently, $I_{aw}$ cannot be estimated reliably from $Z_{rs}$ below 20 Hz and so will not be considered further.

**Virtual experiments:** As previously described (29), we also performed virtual experiments using a computational model of the mechanics of the mouse lung based on the asymmetrical airway branching scheme of Horsfield (11). We assumed Poiseuille flow in each airway in order to calculate its flow resistance. Together with the mass of gas contained in the lumen, this gives airway impedance ($Z_{aw}$) as:

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

(2)

where $r$ is airway radius, $L$ is airway length, $\mu$ is the viscosity of air, and $\rho$ is the density of air. We neglected the influence of airway wall shunting on $Z_{rs}$ by assuming that the airways are rigid during the application of the oscillatory volume perturbations used to determine $Z_{rs}$.

As in our previously study (29), each of the most distal airways terminated in an identical tissue unit with impedance $Z_{ti}$, given in analogy to Eq. 1 by

$$Z_{ti}(f) = \frac{G_t - iH_t}{(2\pi f)\alpha}$$

(3)
where $H_{ti}$ is the constant-phase elastic parameter for each individual tissue unit, and $G_{ti}$ is the corresponding dissipative tissue parameter. $G_{ti}$ is equal to the product of $H_{ti}$ and the unit hysteresivity, $\eta$ (8).

The total impedance of the model, $Z_{mod}$, was calculated by adding the individual $Z_{aw}$ and $Z_{ti}$ in series or parallel, as appropriate, at each of the frequencies used to obtain $Z_{rs}$ experimentally. The following Monte-Carlo procedure was used. Sixteen independent determines of $Z_{mod}$ were made, with the individual values of $r$ in each case being made by random selection from a Gaussian distribution having mean and standard deviation appropriate for the airway order in question, as determined by Gomes and Bates (11). The final $Z_{mod}$ was the average of the 16 individual $Z_{mod}$.

$Z_{mod}$ is thus constrained by the airway tree structure defined by Gomes and Bates (11) and by the forms of Eqs. 2 and 3. We adjusted $Z_{mod}$ to match a given set of experimental data by choosing the values of only three parameters; $H_{ti}$ and $\eta$, and a scaling factor $\beta$ which was simultaneously applied to all values of $r$ in order to achieve a uniform relative narrowing or dilatation of all the model airways.

Once the computational model was adjusted so that $Z_{mod}$ matched baseline $Z_{rs}$, we made the model bronchoconstrict with a time-course similar to a set of experimental data by having the radii of all airways in the model assume a time-varying fraction of their respective baseline values. This fractional time-course was calculated as described below. The model also incorporated our previously described mechanism for lung derecruitment (29), whereby any airway narrowing to a specified threshold radius would be closed completely for the remainder of the simulation.
Statistical analysis: Differences in the magnitude or timing of responses to methacholine between the Saline and PLL groups were compared using unpaired t-tests. Differences in the coefficient of determination (a measure of goodness-of-fit of Eq. 1 to experimental data) between two points along the time-course of the methacholine response within a group of animals (either Saline or PLL) were compared using paired t-tests. Differences in the coefficient of determination at the same time point between the two groups were compared using unpaired t-tests. Statistical significance was taken as p < 0.05. We used the coefficient of determination, a standard measure of goodness-of-fit, to gauge how well Eq. 1 fit the experimental measurements of \( Z_{rs} \). The coefficient of determination is the fraction of the variance of a data set that is accounted for by the model.

RESULTS

The coefficient of determination obtained from fitting Eq. 1 to the experimental measurements of \( Z_{rs} \) had values at baseline of 0.749 ± 0.356 for the Saline group (the relatively large variation being due to a single animal that had an anomalously small value) and 0.890 ± 0.003 for the PLL group. These values were not significantly different. The between group values were also not different at the peak of the response to 50 mg/ml methacholine, and at the final plateau. However, the coefficient of determination decreased slightly but significantly at the peak to 0.729 ± 0.299 and 0.839 ± 0.064 in the Saline and PLL groups, respectively, and were still significantly depressed at the plateau at 0.738 ± 0.286 and 0.850 ± 0.073, respectively.

Figure 1 shows the time-courses of \( R_N \), \( G \) and \( H \) following challenge with the three increasing concentrations of methacholine aerosol (the responses are plotted sequentially for ease of comparison, but there was actually a small period of time between the individual challenges,
including the 40 s required for aerosol delivery). In both Saline and PLL groups, \( R_N \) shows progressively increasing peaks with each challenge that descend to plateau levels only slightly above baseline (Fig. 1, top panel). The heights of the peaks in \( R_N \) are significantly elevated in the PLL group relative to controls at the intermediate concentrations of 3.125 and 12.5 mg/ml, but at the highest concentration of 50 mg/ml both groups responded similarly. PLL treatment reduced the time to reach peak response; the maximum value of \( R_N \) in the PLL group was achieved significantly earlier than in the Saline group at methacholine concentrations of 3.125 and 12.5 mg/ml and just failed to reach significance (\( p = 0.081 \)) at 50 mg/ml. These various observations are mirrored closely in the parameter \( G \) (Fig. 1, middle panel); the peak in each \( G \) response tended to be higher in the PLL group compared to the Saline group (although not reaching statistical significance), and occurred earlier (significant except for the peak 12.5 mg/ml methacholine which was just not significant at \( p = 0.59 \)). \( H \), on the other hand, showed no initial peak at all in most cases, and a very modest peak in others, leading to slightly elevated plateaus (Fig. 1, lower panel). The plateau in the PLL group was significantly higher compared to the Saline group at 12.5 mg/ml, and just failed to be significantly higher (\( p = 0.067 \)) at 50 mg/ml. The plateaus in \( R_N \), \( G \) and \( H \) were all abolished by a deep inflation.

The computational model was used to simulate the time-courses of \( R_N \), \( G \) and \( H \) to each concentration of methacholine. The fractional time-course of airway radii necessary to have the model simulate transient bronchoconstriction was determined using the same approach as in our previous study (29). Specifically, the mean profiles of the measured \( R_N \) (Fig. 1, top panel) were normalized to their baseline values measured prior to the first methacholine challenge, inverted, and the fourth root taken (i.e. assuming Poiseuille flow in the airways). These fractional time-courses were then further scaled by the empirically determined factor of 1.3, as this was required
to improve the match between the simulated and measured $R_N$ profiles, as was the case in our previous study (29). The resulting fractional airway narrowing profiles for the Saline and PLL groups are shown in Fig. 2.

In our previous study (29) in which we initially established the computational model of the mouse lung and applied it to data from control and allergically inflamed BALB/c mice, we achieved a good fit to baseline data from normal animals with values for the three adjustable parameters $\beta$, $H_{ti}$ and $\eta$ of 0.83, 1800 cmH$_2$O.s.ml$^{-1}$, and 0.1, respectively. In order to match the data from the present control animals at baseline as closely as possible the values of $\beta$ and $H_{ti}$ had to be changed slightly from those used in our previous study, possibly reflecting subtle differences in animal size or developmental history; $\beta$ was increased by 9% and $H_{ti}$ was decreased by 11%. We also found that the simulated values of $G$ were too high if we retained the value of 0.1 for $\eta$ that we used in our previous study (29). However, this value of $\eta$ was taken from a published study of dog lung strips that used single-frequency length oscillations, because data from mouse lung strips were not available at the time of our previous study. Recently, Fust et al. (9) fit the model in Eq. 1 to the mechanical impedance of isolated strips of mouse lung tissue subjected to uniaxial broad-band length oscillations up to 20 Hz, and found a value for $\eta$ of 0.042. We therefore used this value of $\eta$ in the present computational model and obtained simulated times-courses for $R_N$, $G$ and $H$ that reproduce the main features of the experimental measurements in both the Saline group (Fig. 3) and the PLL group (Fig. 4).

The close matches between the experimental and simulated data shown in Figs. 3 and 4 demonstrate that the computational model of the normal BALB/c mouse we developed in our previous study for a single concentration of methacholine aerosol (29), with some minor
adjustments to its parameter values, also accurately describes the time-course of bronchoconstriction to a sequence of increasing methacholine concentrations. Furthermore, no alteration to the structure of this model is required to account for bronchoconstriction in PLL-treated mice. However, this does not prove that another model would not also describe the PLL data. Obviously, we cannot test every possibility, but we can determine how the simulated time-courses of $R_N$, $G$ and $H$ are affected when we adjust the model to correspond to the allergically inflamed mouse as in our previous study (29). In that study, we obtained an accurate reproduction of the parameter time-courses measured in inflamed animals by keeping the degree of smooth muscle shortening the same as for control animals, while adjusting the model in two ways to account for inflammatory changes: 1) all the airways of the model were lined with a uniform internal layer 18 microns in thickness, and 2) the threshold radius for airway closure was increased from 38 to 45 microns. We therefore applied these two modifications to the model and attempted to reproduce the parameter time-courses of the PLL mice. In order to achieve a good match between simulated and measured $R_N$ time-courses with these modification, we had to reduce the degree of fractional airway narrowing to 60% of that used in the above PLL simulations. However, this resulted in the simulated $G$ and $H$ profiles being substantially elevated above their respective measured values (Fig. 4), indicating that the inflamed lung model is not appropriate for describing bronchoconstriction in the PLL-treated mouse.

**DISCUSSION**

Interpreting the results of our study first requires an understanding of what the impedance parameters $R_N$, $G$ and $H$ mean physiologically. Previous studies have shown that $R_N$ reflects the overall resistance of the airway tree (27). $G$ represents tissue viscance, a measure of energy dissipation within the tissue, but also seems to increase with regional heterogeneities throughout
the lung (22). An increase in $R_N$ would thus be expected to occur when the conducting airways narrow, while the inevitable heterogeneities that accompany such narrowing would be expected to simultaneously elevate $G$. Regional heterogeneities also cause $H$ to increase, but to a lesser extent than $G$ (22, 29). Both $G$ and $H$, on the other hand, are equally sensitive to changes in intrinsic tissue rheology and to derecruitment of lung units (19, 22, 29), whereas $R_N$ is much less affected by such events because of its central airways component which always contributes regardless of what happens in the lung periphery.

In the present study, we made a number of findings that are relevant to the foregoing. First, we found that intra-tracheal PLL caused elevations in $R_N$ and $G$, but only at the low and intermediate concentrations of methacholine; at the highest concentration of 50 mg/ml the responses of $R_N$ and $G$ in the PLL and Saline groups were similar (Fig. 1, top and middle panels). In other words, the dose-response curves for $R_N$ and $G$ were shifted to the left, indicating an increase in the sensitivity of the airways to methacholine, but not in their peak responsiveness. A second and contrasting observation is that although PLL also increased the methacholine responsiveness of $H$ (Fig. 1, bottom panel), the effect became progressively more pronounced with increasing methacholine concentration. Thus, our data indicate that between 12.5 and 50 ml/ml methacholine a progressively increasing degree of peripheral airway closure took place in the PLL group without a corresponding increase in the degree of airway narrowing. A possible explanation is that incomplete re-opening of closed airways between challenges and the accumulation of airway secretions may have caused closure to increase over time. Indeed, the inability of agonist aerosol to fully penetrate a lung partially obstructed from a previous challenge has been postulated to account for the existence of a plateau in the methacholine responsiveness of a normal lung (3).
The above results are compatible with the notion that intra-tracheal PLL causes airway hypersensitivity by compromising the barrier function of the epithelium, an ability shared by other cationic proteins (6, 7). We introduced this notion previously to explain why intra-tracheal PLL causes exaggerated increases in lung resistance and elastance measured in rats at a single frequency when methacholine is delivered as an aerosol, but not when it is injected intravenously (16). PLL has been previously shown to lower the electrical conductivity of epithelial cell layers (28), which presumably reflects damage either to the cell membrane or to the tight junctions between cells. A permeabilized epithelium presumably allows more lumenally applied agonist to make its way to the smooth muscle before being cleared or degraded, resulting in enhanced muscle shortening, excessive airway narrowing, and increased airway resistance. This notion is further supported by our finding that the peaks in $R_N$ and $G$ from the PLL group occurred earlier than the corresponding peaks in the Saline group (Fig. 1). Such an effect would be expected if the challenging agonist were able to reach the airway smooth muscle more easily, and hence more quickly, than normal. By contrast, we have previously argued (29) that the relatively delayed peak in $R_N$ seen in inflamed BALB/c mice reflects the increased transit time required for an agonist to cross a swollen, thickened epithelium. An effect of PLL solely on the epithelium, and not on the airway smooth muscle itself, would also mean that the maximum ability of the muscle to narrow the airways would be unchanged, which is indeed what we observed; the response of the PLL-treated animals to 50 mg/ml methacholine, which likely approached a supra-maximumal dose, was similar to the saline-treated controls.

To put the above interpretation in the context of our current understanding of lung structure, we now examine the data in light of our computational model of mouse lung mechanics (29). This model serves as a virtual laboratory for reproducing experiments in silico, and allows us to
determine the consequences of particular hypotheses about mechanisms of hyperresponsiveness. To use the computational model effectively we must simulate data under conditions as close to those used experimentally as possible, because Eq. 1 does not fit the experimental spectra of \( Z_{rs}(f) \) perfectly; small systematic deviations between \( Z_{rs}(f) \) and the fit provided by Eq. 1 attest to the obvious fact that the lung is a much more complex system than that represented by Eq. 1. Furthermore, as bronchoconstriction develops, the adequacy of Eq. 1 as a description of lung mechanics is likely to change. However, although the goodness-of-fit provided by Eq. 1, as measured by the coefficient of determination, was significantly lower in both groups at the peak and plateau of the response to 50 mg/ml methacholine compared to baseline, the differences were relatively small. This suggests that Eq. 1 continued to describe the experimental data adequately even during bronchoconstriction. Furthermore, by generating \( Z_{rs}(f) \) data using the computational model at the same values of \( f \) as used experimentally we are able, at least insofar as the model is a good representation of the real lung, to reproduce any variations in parameter values due to variations in the ability of Eq. 1 to describe the experimental data.

Using the computational model described above, we were able to accurately reproduce the data from both the Saline (Fig. 3) and PLL (Fig. 4) groups, with the fractional airway narrowing in the model being determined in each case (Fig. 2) by the corresponding experimental values of \( R_N \). In other words, exactly the same model structure accounted for both data sets, implying that the only effect of PLL treatment was to cause a greater degree of smooth muscle shortening at intermediate doses of methacholine. By contrast, the model representing allergically inflamed mice, with an 18 micron lining inside the airways and an 18% increase in the closure threshold radius (29), did a poor job of accounting for the experimental data from the PLL mice (Fig. 4) in two critical ways. First, in order to get the simulated \( R_N \) from the inflamed model to match the
experimental values in the PLL mice, we had to reduce the degree of airway narrowing to 60% of that used with the control model (Fig. 2). There is no plausible explanation for why a substantially reduced degree of smooth muscle shortening should occur in PLL-treated mice, particularly as our previous studies have indicated that precisely the opposite is what actually occurs. Second, we found that even with this reduced airway narrowing, the simulated $H$ profiles were elevated substantially above those observed experimentally (Fig. 4). We therefore conclude that the computational model which we used previously to account for the methacholine responsiveness of allergically inflamed mice has no relevance for the PLL-treated animals of the present study. Taken together, the above simulation results support the conclusion that greater airway narrowing occurred in the PLL mice than in controls at methacholine concentrations of 3.125 and 12.5 mg/ml, but that the PLL mice behaved similarly to controls at baseline and at 50 mg/ml methacholine.

Thus, our results indicate that the BALB/c mouse treated with intra-tracheal PLL is a model of enhanced sensitivity of the airways to methacholine, as we originally hypothesized. This stands in stark contrast to our previous findings in mice with allergic airway inflammation that exhibited hyperresponsiveness predominately in $H$, indicating an effect confined to the lung periphery (29). However, our conclusions are based on mathematical models of the lung, which embody assumptions that are always open to question. The model (Eq. 1) that we fit to measurements of $Z_{rs}$ assumes a particularly simple lung structure, but this is necessary in order for the parameters of the model to be uniquely identifiable from the data. Accordingly, this model assigns a central role to $R_N$ and peripheral roles to $G$ and $H$. A key component of our study was then to further refine these roles using virtual experimentation with an anatomically-based computational model. Nevertheless, we must bear in mind that the strength of these
interpretations rests on the many assumptions inherent in the computational model. For example, the model is based on the airway tree structure of a strain of mouse (11) that is different to that used in the present study. We assumed the airways are rigid which neglects their ability to change volume in response to applied variations in pressure (10, 26). We assumed Poiseuille flow in the airways which neglects the possible presence of entrance effects, flow unsteadiness and turbulence (17, 23). We also assume that it is appropriate to narrow all airways in the model by the same fraction in order to simulate bronchoconstriction, whereas in reality airways narrow heterogeneously (1, 22, 27). Testing these assumptions remains an important area for future research.

In summary, we have studied the time-course of induced bronchoconstriction in BALB/c mice treated with intra-tracheal PLL. We found an increased response in the central airways at sub-maximal doses of methacholine aerosol, which we were able to accurately reproduce in an anatomically-based computational model of the mouse lung simply by increasing the degree of airway narrowing. Moreover, we interpret these results as reflecting a reduced barrier function of the epithelium caused by the PLL, allowing for easier and more rapid access of aerosolized agonist to the underlying smooth muscle. This represents a completely different manifestation of enhanced bronchoconstriction to that found in the allergically inflamed mouse (29), underscoring the fact that airways hyperresponsiveness can occur by a variety of mechanisms (4). Which of these mechanisms has the most relevance for human asthma remains an open question, but studying different animal models that embody the various possibilities may help in finding the answer.
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FIGURE CAPTIONS

Figure 1: The time-course of bronchoconstriction (mean + SEM) in BALB/c mice following aerosolization of 3.125, 12.5 and 50 mg/ml methacholine. The open circles are saline-treated animals (n=6) and the closed circles are animals treated with PLL (n=6). The three time-courses are shown consecutively to save space, but there was actually a 40 s aerosol delivery period prior to each peak in the responses shown. The vertical dotted lines bracket parameter values obtained following deep lung inflations given in order to re-establish baseline conditions. * indicates significant differences in parameter values between groups at time-points indicated. + indicates significant differences in timing of the indicated response peaks between the two groups.

Figure 2: The time profiles of fractional airway narrowing used to simulate the time-courses of bronchoconstriction. The computational model representing normal BALB/c mice was used to simulate both the Saline and PLL data. The model representing allergically inflamed BALB/c mice (29) was also used to simulate the PLL data.

Figure 3: Mean time-courses of $R_N$, $G$ and $H$ from the Saline group, together with the parameter profiles simulated by the computational model of the normal mouse lung. The experimental values represented by the closed symbols are the same as the open symbols shown in Fig. 1.

Figure 4: Mean time-courses of $R_N$, $G$ and $H$ from the PLL group, together with the parameter profiles simulated by the computational models of the normal mouse lung and of the lung of the allergically inflamed mouse (29). The experimental values represented by the closed symbols are the same as the closed symbols shown in Fig. 1.
Figure 1
Figure 2

Fraction of baseline airway radius

Time (s)

- Saline
- PLL
- Inflamed

3.125 mg/ml 12.5 mg/ml 50 mg/ml
Figure 3
Figure 4