Intrinsic and antigen-induced airway hyperresponsiveness are the result of diverse physiological mechanisms


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Wagers SS, Haverkamp HC, Bates JHT, Norton RJ, Thompson-Figueroa JA, Sullivan MJ, Irvin CG. Intrinsic and antigen-induced airway hyperresponsiveness are the result of diverse physiological mechanisms. J Appl Physiol 102: 221–230, 2007. First published September 28, 2006; doi:10.1152/japplphysiol.01385.2005.—Airway hyperresponsiveness (AHR) is a defining feature of asthma. We have previously shown, in mice sensitized and challenged with antigen, that AHR is attributable to normal airway smooth muscle contraction with exaggerated airway closure. In the present study we sought to determine if the same was true for mice known to have intrinsic AHR, the genetic strain of mice, A/J. We found that A/J mice have AHR characterized by minimal increase in elastance following aerosolized methacholine challenge compared with mice (BALB/c) that have been antigen sensitized and challenged [concentration that evokes 50% change in elastance (PC50): 22.9 ± 5.7 mg/ml for A/J vs. 3.3 ± 0.4 mg/ml for antigen-challenged and -sensitized mice; P < 0.004]. Similar results were found when intravenous methacholine was used (PC50 0.22 ± 0.08 mg/ml for A/J vs. 0.03 ± 0.004 mg/ml for antigen-challenged and -sensitized mice). Computational model analysis revealed that the AHR in A/J mice is dominated by exaggerated airway smooth muscle contraction and that when the route of methacholine administration was changed to intravenous, central airway constriction dominates. Absorption atelectasis was used to provide evidence of the lack of airway closure in A/J mice. Bronchoconstriction during ventilation with 100% oxygen resulted in a mean 9.8% loss of visible lung area in A/J mice compared with 28% in antigen-sensitized and -challenged mice (P < 0.02). We conclude that the physiology of AHR depends on the mouse model used and the route of bronchial agonist administration.

METHODS

Animals. We studied A/J mice, untreated BALB/c mice (Jackson Laboratories, Bar Harbor, ME), and BALB/c mice with antigen-induced AHR. All mice were 6–8 wk old with an approximate average weight of 20 g. All protocols were approved by the University of Vermont Institutional Animal Care and Use Committee.

Antigen sensitization and challenge. BALB/c mice were injected intraperitoneally on days 0 and 14 with a 100-μl mixture of 50 μl ovalbumin (200 μl/ml) and 50 μl of alum (adjuvant). Beginning on day 21, the mice received daily aerosol challenges with ovalbumin for each of 3 days and were studied 48 h after the last challenge. Bronchoalveolar lavage cell counts and differentials were determined to confirm the presence or absence of inflammation.

AHR. Mice were anesthetized with an intraperitoneal injection of pentobarbital sodium (90 mg/kg), tracheostomized, and then connected to a mechanical ventilator (flexiVent, Scireq, Montreal, Canada) via an 18-gauge intratracheal cannula. The mice were ventilated at 200 breaths/min with a delivered tidal volume of 0.20 ml against a study of molecular and structural changes induced by inflammation. Because mice can be sedated and tracheostomized, eliminating the effects of voluntary skeletal muscle movement, as well as upper airway obstruction and shunting, their use allows one to conduct sensitive physiological studies (1). Using a forced oscillation technique and a computational model of the lung, we have recently shown that in a commonly used mouse model of asthma, BALB/c mice with inflammation of the airways induced by antigen sensitization and challenge, AHR can be attributed to airway wall thickening and exaggerated airway closure (45). This finding is contrary to the commonly held notion that AHR is attributable to alterations in airway smooth muscle function.

Multiple studies have been published that make the assumption that AHR is physiologically the same in different mouse models of asthma (6, 7, 11, 12, 26, 27). In the present study, we sought to apply an approach similar to the one we used previously (45) to another commonly used mouse model of asthma that is known to have intrinsic AHR, A/J mice (26). This intrinsic AHR in A/J mice has been attributed to the presence of airway smooth muscle with an increased velocity of shortening (9). We chose to study A/J mice in the absence of airway inflammation and to compare the physiology of their intrinsic AHR to that of mice that had undergone antigen sensitization and challenge. We hypothesized that AHR in the A/J mice would be physiologically distinct from the AHR in antigen-sensitized and -challenged mice.

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positive end-expiratory pressure (PEEP) of 3 cmH2O applied by a water trap. Aerosolized methacholine challenges (untreated BALB/c n = 8, antigen-sensitized and -challenged BALB/c n = 10, A/J mice n = 9) were performed by delivering three successive concentrations of methacholine: 3.125, 12.5, and 50 mg/ml. Intravenous methacholine challenges (untreated BALB/c n = 6, antigen-sensitized and -challenged BALB/c n = 7, A/J mice n = 6) were performed by intravenously delivering, through a Silastic catheter that was surgically inserted into the left external jugular vein, six successive doses of methacholine: 0.0027, 0.0082, 0.0247, 0.0741, 0.222, and 0.666 mg/ml, where the volume injected was 1.85 μl/body wt of mouse (20). Following each aerosol or intravenous methacholine challenge, each 10-s ventilation was interrupted allowing for a 1-s passive expiration followed by a 2-s broad-band (1–19.625 Hz) volume perturbation. The peak-to-peak excursion of the ventilator piston during delivery of these perturbations was 0.17 ml, resulting in a volume delivered of ~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 a complex input impedance (Zr) of the respiratory system. Using an iterative scheme (45), Zr was then fit to a uniformly ventilated model of the lung with a constant-phase tissue impedance described by (15):

\[ Z_r(f) = R_N + i2\pi f L_{aw} + \frac{G - iH}{(2\pi f)^{\alpha}} \]  

where \( R_N \) is a Newtonian resistance composed mostly of the flow resistance of the conducting pulmonary airways, \( L_{aw} \) is the inductance of the gas in the central airways, \( G \) (tissue damping) reflects dissipative energy storage in the tissues, \( f \) is frequency, \( i = \sqrt{-1} \), and \( \alpha \) couples \( G \) and \( H \). Using the frequency normalization scheme of Ito et al. (21), \( R_N \), \( G \), and \( H \) all have units of centimeters H2O times seconds per milliliter. Linear interpolation of the methacholine dose-response curve was used to calculate the concentration of methacholine that would cause a 200% increase in each \( R_N \) and \( G \) (PC200) or a 50% increase in \( H \) (PC50) for mice that received aerosolized methacholine. A 300% increase in \( R_N \) and \( G \) (PC300) and a 30% increase in \( H \) (PC30) were used for mice that received intravenous methacholine. The PC values were chosen according to the appearance of the dose-response curves, so as to provide a point of analysis that represented both the sensitivity and maximal response of the curves in question. If for a given mouse the PC value was not reached, the maximal dose given was used as the PC value.

Computational model experiments. The time course of bronchoconstriction was plotted for the 12.5 mg/ml aerosol and the 0.0247 mg/ml intravenous dose of methacholine. As previously described (45), we simulated the time course of bronchoconstriction using a computational model of the mouse lung that followed the asymmetric branching scheme of Horsfield et al. (17) with structural data for the mouse airway tree as described by Gomes and Bates (14). The airway structural 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 SDs appropriate to the airway order as per the anatomic data (14). The impedance of each airway (Zaw) in the model was calculated, assuming Poiseuille flow to determine resistance and the mass of the airway gas to determine inductance, as follows

\[ Z_{aw}(f) = \frac{8\mu L}{\pi r^4} + \frac{2\mu p}{r^2} \]  

where \( r \) is airway radius, \( L \) is airway length, \( \mu \) is gas viscosity, and \( p \) is gas density. We neglected the influence of airway wall shunting by making the airway walls rigid. Each of the most distal airways terminated in an identical viscoelastic tissue unit with impedance given by the equation

\[ Z_r(f) = \frac{G - H}{(2\pi f)^{\alpha}} \]  

where \( Z_r \) is tissue impedance, \( G \) is tissue damping \( G \), and \( H \) is tissue elasticity, \( H \), with the ratio of \( G \) to \( H \) (\( G/H \)) being assigned a value of 0.1. The total impedance of the model (Zmod) was calculated by adding the individual Zaw and Zr in series or parallel, as appropriate. Zmod was calculated at each of the frequencies in the volume perturbation signal used to obtain Zaw experimentally in the mice. The Monte-Carlo approach was used to obtain a set of different Zmod 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.

The time-course of fractional change in airway radius necessary to have the model simulate transient bronchoconstriction was determined as follows. First, the mean profile of the measured ΔR was added to unity, inverted, and the fourth root taken, as if to assume Poiseuille flow in the airways. Then, to force a good match between the simulated and measured ΔR profiles, the time course of fractional change in airway radius had to be further scaled by the empirically determined factor of 1.3 presumably because of the inherent heterogeneity in the model and the structural differences between a cast of an inflated fixed specimen and the lung of a living animal.

Visualization of airway closure. A mixed gas that is trapped behind a closed airway eventually will have all of the oxygen present extracted. With ambient air as the respired gas, this has little effect as oxygen comprises only 21% of ambient air. In contrast, if the fraction of inspired oxygen is increased to 100%, almost all the gas trapped behind closed airways will be extracted, over time resulting in collapse of all the alveoli distal to the closed airways with a consequent reduction in lung volume. This is a well-recognized phenomenon known as absorption atelectasis (13). In alveoli served by airways that remain open, the oxygen will be constantly replaced, and little absorption atelectasis will occur. Separate groups of mice that had undergone antigen sensitization and challenge were studied during ventilation with either humidified room air (n = 4) or humidified 100% oxygen (n = 4). For comparison, a group (n = 4) of A/J mice was studied during ventilation with humidified 100% oxygen. Once placed on the ventilator, as above, the anterior portion of the chest wall was removed. A single 12.5 mg/ml dose of aerosolized methacholine was delivered as above. At baseline and following methacholine, a series of high-resolution digital images of the lungs was captured each minute using a 1-megapixel black-and-white charge-coupled device camera. These images were taken after 1 s of passive expiration against 3 cmH2O PEEP and immediately before the start of the volume perturbations that were used to determine lung mechanics. The area of the visible lung was determined at baseline and 8 min following methacholine by tracing around the lung border using image analysis software (Image J, NIH, Bethesda, MD) (Fig. 1). The area of visible lung for each mouse and at each time point was measured independently by two observers blinded to the treatment and strain of mouse.

RESULTS

Antigen sensitization and challenge was successful in all mice studied. The average cell count for those studied using aerosol methacholine was 591,000 cells/ml, and for those in which intravenous methacholine was used was 625,000 cells/ml. Naive mice and A/J mice groups had average cell counts of 77,000 and 88,000 cells/ml, respectively.
Baseline values for all three parameters were similar for all six groups of mice (Table 1). Compared with controls, A/J mice and BALB/c mice that had undergone antigen sensitization and challenge were indeed hyperresponsive whether challenged with aerosol or intravenous methacholine (Figs. 2 and 3). However, while the antigen-sensitized and -challenged mice demonstrated AHR in all three parameters, i.e., $R_N$, $G$, and $H$, A/J mice exhibited AHR that was similar in magnitude but limited to the parameters $R_N$ and $G$ (Figs. 2 and 3).

Figure 4 shows the time courses of $R_N$, $G$, and $H$ expressed as fractional changes from baseline following methacholine challenge for all groups of mice. Time 0 corresponds to the point of cessation of aerosol delivery. The time course of $R_N$ in antigen-sensitized and -challenged mice demonstrates a plateau that was not present when intravenous methacholine was used.

Figure 5 shows the experimental data obtained from the A/J mice that received aerosol methacholine together with the first attempt to simulate the time course of bronchoconstriction using the computational model. The minimum airway radii achieved during this narrowing process were 67% of baseline. The remaining characteristics of the computational model were identical to those we found in our previous study and accurately reproduced the time course of bronchoconstriction in normal BALB/c mice (45). As the time course of fractional change in airway radius in the model is based on the measured $\Delta R_N$, the simulated $\Delta R_N$ was virtually identical to the measured. The $\Delta G$ simulated time course profile is also close to that of the measured $\Delta G$ time course profile. However, the simulated and computational time course profiles for $\Delta H$ are highly discordant (Fig. 5A). Thus, in the next simulation, we reduced the degree of airway closure by changing the closure threshold from 38 $\mu$m, as was used in the previous study (45), to 28 $\mu$m. With this modification we obtained an excellent match between computational and experimental time courses of $\Delta R_N$, $\Delta G$, and $\Delta H$ (Fig. 5B).

Figure 6A shows the experimental data obtained from mice with antigen-induced AHR that received intravenous methacholine together with the first attempt to simulate the time course of bronchoconstriction using the computational model. The same model that was used in our previous study to fit experimental data from mice with antigen-induced AHR that received aerosol methacholine with thickened epithelium and an elevated threshold of airway closure radius was used. When this model was modified to only constrict the larger or central airways, radius $> 150$ $\mu$m, an improved match was obtained with the experimental data (Fig. 6B).

Figure 7 shows the experimental data obtained from the A/J mice that received intravenous methacholine together with simulations generated from the computational model. The computational model that was used in the second simulation for A/J mice that received aerosol methacholine was used (airway closure threshold radius 28 $\mu$m). Once again the simulated time course of $\Delta H$ is highly discordant with that of the experimental time course (Fig. 7A). When the model was further modified by constricting only the airways with a radius of $150$ $\mu$m or greater and then further rescaling the fourth root of the experimental $R_N$ by a factor of 1.5 instead of 1.3, we achieved an improved fit (Fig. 7B).

Figure 8 shows representative images from antigen-sensitized and -challenged BALB/c mice ($n = 4$) and A/J mice ($n = 4$) at baseline and 8 min after methacholine while being ventilated with 100% oxygen. Patches of atelectasis are visible on the surface of the lungs of mice that had been antigen sensitized and challenged, while minimal areas of atelectasis were seen in A/J mice. Antigen-sensitized and -challenged mice had a 28% reduction in visible lung area after methacholine when 100% oxygen was administered. This was a larger

Table 1. Baseline impedance parameter values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BALB/c</th>
<th>BALB/c + antigen</th>
<th>A/J</th>
<th>BALB/c</th>
<th>BALB/c + antigen</th>
<th>A/J</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_N$ cmH$_2$O·s·ml$^{-1}$</td>
<td>0.20±0.01</td>
<td>0.23±0.02</td>
<td>0.24±0.01</td>
<td>0.34±0.03</td>
<td>0.29±0.02</td>
<td>0.24±0.02</td>
</tr>
<tr>
<td>$G$ cmH$_2$O·s·ml$^{-1}$</td>
<td>2.12±0.2</td>
<td>1.45±0.24</td>
<td>2.8±0.07</td>
<td>3.33±0.23</td>
<td>2.85±0.09</td>
<td>3.23±0.18</td>
</tr>
<tr>
<td>$H$ cmH$_2$O·s·ml$^{-1}$</td>
<td>17.3±0.64</td>
<td>13.8±0.49</td>
<td>20.4±0.64</td>
<td>24.2±1.9</td>
<td>21.0±0.8</td>
<td>21.5±1.53</td>
</tr>
</tbody>
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Values are means ± SE. For aerosolized methacholine challenge, $n = 8$ untreated BALB/c, 10 antigen sensitized and challenged (BALB/c + antigen), and 9 A/J mice. For intravenous methacholine challenge, $n = 6$ untreated BALB/c, 7 BALB/c + antigen, and 6 A/J mice. $R_N$, Newtonian resistance; $G$, tissue damping; $H$, elastance. See text for further description of each parameter.
reduction than was seen when air was used (13.7%). By contrast, the A/J mice had only minimal reduction in visible lung area at 8 min after methacholine (9.8%), which was significantly less \((P < 0.02)\) than the lung area reduction seen in mice with antigen-induced AHR (Fig. 8).

**DISCUSSION**

In this study we found that AHR in A/J mice is physiologically distinct from that induced by antigen sensitization and challenge of BALB/c mice. This conclusion is supported by...
three different sets of experimental evidence: 1) dose-response curves, 2) computational model simulations, and 3) direct observation. AJ mice have AHR that is limited to the impedance model parameters $R_N$ and $G$, while mice with antigen-induced AHR have a response in all three parameters, but most strikingly there is an accentuated rise in $H$ (Fig. 3). While this difference is less evident when intravenous methacholine is administered, it is still present at the highest doses. $R_N$ has been shown to represent narrowing of the central airways (15, 42). The parameter $G$ will increase if the resistive properties of the lung tissue increase (15), or if there is increased heterogeneity of airway narrowing (28). Therefore, $G$ can increase with either changes in the parenchymal tissue properties or narrowing of the airways. Hence, the rise in $G$ in the AJ mice during bronchoconstriction without an accompanying rise in $H$ suggests that the airway narrowing induced by methacholine is heterogeneous in AJ mice. The parameter $H$ represents lung elastance (15). If airways close, the effective elastance of the lung becomes the sum of that of the regions remaining open and $H$ will therefore increase; however, a rise in $H$ could also be the result of the stimulation of contractile elements in the lung parenchyma (8). In our previous study (45), where we also employed a computational modeling approach, we demonstrated that exaggerated airway closure can account for the exaggerated rise of $H$ induced by aerosolized methacholine in antigen-sensitized and -challenged BALB/c mice. In addition,
we showed that the rise in $R_N$ in antigen-sensitized and -challenged mice was not explained by an exaggeration of airway smooth muscle shortening but rather was due to the combined effect of normal smooth muscle shortening, accentuated airway closure, and a thickened airway wall. In the present study, we found that we could simulate the time course of bronchoconstriction observed in A/J mice (Fig. 6) by reducing the amount of airway closure in the computational model that we used in our previous study to simulate the time course of bronchoconstriction in antigen-sensitized and -challenged mice (45), thus suggesting that airway closure does not occur to a significant degree in A/J mice, a conclusion that was further supported by the significant attenuation of the loss of visible lung area in A/J mice ventilated with 100% oxygen compared with antigen-sensitized and -challenged mice. Thereby, we have demonstrated that physiology of AHR in A/J mice is distinct from that in antigen-sensitized and -challenged mice, particularly when one uses aerosolized methacholine.

Our study has certain limitations. The conclusions made herein are based on mathematical models of the lung, which embody assumptions that are always open to discussion. In the case of the model used to fit the impedance data (Eq. 1), the assumption of a particularly simple lung structure is necessary in order for the variables of the model to be uniquely identifiable from the data (15). Furthermore, the computational model (Eq. 2) is based on the airway tree structure of a strain of mouse (14) that is different from the strains used in the present study. Therefore, the accuracy of our computational model depends on the assumption that such scaling is valid. We are also assuming that it is appropriate to narrow all

Fig. 5. Computational simulations: A/J mice with aerosol methacholine. Time courses of bronchoconstriction for simulated and measured experimental data for A/J mice are depicted. Computer simulations are represented by dotted lines and experimental data by solid lines. A: simulation 1 is with model parameters equal to those used to fit naive BALB/c mice in our previous study (45). B: simulation 2 is when the threshold radius of airway closure is reduced from 38 to 28 μm.

Fig. 6. Computational simulations: antigen-sensitized and -challenged mice with intravenous methacholine. Time courses of bronchoconstriction for simulated and measured experimental data for antigen-sensitized mice are depicted. Computer simulations are represented by dotted lines and experimental data by solid lines. A: simulation 1 is with model parameters equal to those used to fit antigen-sensitized and -challenged mice in our previous study (45). B: simulation 2 is when bronchoconstriction is limited to only those airways with a radius of >150 μm.
airways in the computational model by the same fraction to simulate bronchoconstriction, whereas in reality airways narrow heterogeneously (2, 28, 42), and heterogeneous narrowing has been used in a computational model of the lung (41). However, heterogeneity is incorporated in our model as the starting airway radii are randomly chosen. Nonetheless, because of the complex and interdependent nature of lung structure and function, isolating single components of the physiological response to methacholine is difficult to do without significantly altering their behavior, making studying components of bronchoconstriction in isolation nearly impossible (1). Therefore, we are left with having to infer the nature of the complete system on the basis of the extent to which we can reproduce its behavior using an anatomically based computational model. It has been recently pointed out that the inferences made with this approach are not unlike the inferences made in genomics (40).

Another limitation is that we assumed that measuring the visible surface area of the lung was a reasonable surrogate for total lung volume. It may be that areas that were not visible on the surface collapsed more. Nonetheless, at the very least we can conclude that the visible areas of the lung did or did not experience a reduction in volume. The removal of the chest wall could potentially reduce the outward recoil forces on the lung, thereby facilitating collapse of airways and alveoli. However, as it is known that the volume dependence of lung mechanics in mice is chest wall independent (39), a reasonable assumption is that the outward recoil of the chest wall is negligible in mice. Additionally, all mice in this arm of the study had the chest wall removed, making the comparison between groups valid. The strength of this approach is that it allows for direct visual confirmation of what was being inferred from the measurement of impedance and the computational modeling. In contrast to histological assessment of atelectasis, this technique allows for in vivo assessment that is not limited by fixation artifacts.

AHR in A/J mice has been studied previously. Quantitative trait locus mapping studies (6, 11, 12, 26) have linked genes to AHR when comparisons were made between A/J mice and a hyporesponsive strain of mice, C3HeJ. However, when antigen-sensitized and -challenged mice were used, different genetic loci were identified (11), which is perhaps not surprising in light of the results of the present study. Duguet et al. (9) also compared A/J mice to mouse strains with minimal and no AHR and found no apparent morphological difference between the strains. There was, however, a difference in the functional properties of the airway smooth muscle in that it had an increased velocity of shortening.

The physiological difference between A/J and antigen-sensitized and -challenged mice is likely the consequence of airway instability and closure. Airway closure will occur when small airways narrow to the point of complete apposition of the airway walls (41) or narrow enough that liquid bridging occurs across the airway lumen (29). If the exaggerated H response was purely a consequence of airway narrowing, we would expect to see it also in the A/J mice as the rise in $R_N$, when aerosolized methacholine is used, is similar to, if not greater than, the rise in $R_N$ in antigen-sensitized and -challenged mice. This of course could also be the result of regional variation in aerosol deposition; however, when intravenous methacholine was administered the difference persisted, albeit at doses of methacholine that resulted in a greater response in $\Delta R_N$ compared with that which was seen with aerosolized methacholine. As there is no systemic circulation in the peripheral bronchioles of mice (32), a fact that is consistent with our computational model results, the exaggerated rise in $H$ when intravenous methacholine is administered is either the result of large airways constricting to the point that they close, or an instability in the peripheral airways such that the diminished airflow secondary to large airway narrowing leads to their collapse. Even if the former is true, our results still indicate that there is increased airway instability in antigen-challenged and -sensitized mice, whether in large or small airways.

There is reason to believe that the inflamed airways of the antigen-sensitized and -challenged mice become unstable. Airway inflammation results in leakage of plasma proteins onto
the airway surface (49), and plasma proteins are known to increase airway surface tension, thereby decreasing airway stability (10, 16). Furthermore, we have shown previously that the formation of extravascular fibrin on the luminal surface of the lung can induce AHR with a prominent $H$ component similar to what we observed in antigen-sensitized and -challenged mice (46).

In addition to the difference we observed between the two mouse models, there were differences between the response to aerosolized and the response to intravenous methacholine. Intravenous methacholine resulted in a greater $R_N$ response in both models and a minimal response in $H$, and this is best explained by the fact that there is no systemic circulation in the peripheral airways of mice (32). Close examination of the time course of bronchoconstriction also reveals that there was less of a plateau in the $\Delta R_N$ response in the antigen-sensitized and -challenged mice when intravenous methacholine is administered (Fig. 4). This difference could represent the consequence of methacholine-induced mucus secretion and consequent luminal narrowing, or it could be that the smooth muscle response to aerosolized methacholine is different from that of intravenous methacholine. Perhaps aerosolized methacholine is not cleared as quickly as intravenous methacholine, or perhaps the airway epithelium has an effect on how aerosolized methacholine interacts with the airway smooth muscle. Such an aerosol-epithelial-airway smooth muscle interaction has been previously shown in AHR induced by cationic proteins (3, 5). However, it is important to note that when a deep inhalation is given at the end of the time course this residual elevation in $\Delta R_N$ is reversed. Deep inhalations are thought to relax airway smooth muscle and thereby reduce AHR (38). Residual tone and stretching and subsequent relaxation of the airway smooth muscle could explain this reversible plateau we see in the antigen-sensitized and -challenged mice, but if this were the cause it would be difficult to explain why there was not a similar plateau observed when intravenous methacholine was administered. A more parsimonious explanation is that the plateau is the result of mucus secretion that is stimulated more by aerosolized than by intravenous methacholine. Antigen-sensitized and -challenged mice have excess mucus production (36, 43), and it has been shown that mucus secretion is stimulated by aerosolized methacholine (37). Increased mucus in the airway lumen could result in effective airway closure and likely contributes to the rise in $H$ observed in the antigen-

![Antigen sensitized and challenged BALB/c mice](image1)

![A/J mice](image2)

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Fig. 8. Absorption atelectasis with fraction of inspired oxygen at 100% during bronchoconstriction. Top, left and right: digital images of the same mouse with antigen-induced AHR at baseline and 8 min after methacholine administration (12.5 mg/ml). The lung is visibly smaller and has developed patches of atelectasis. Bottom, left and right: A/J mouse at baseline and also 8 min after receiving methacholine. Some A/J mice (data not shown) did develop small areas of atelectasis.
challenged and -sensitized mice following methacholine administration, which would be reversible by deep inhalation.

Is there a preferred mouse model that should be used to study the pathophysiology of asthma? It is obvious that the response to methacholine involves smooth muscle contraction; nonetheless, exaggerated airway narrowing is not the only possible physiological derangement that can contribute to what is measured as AHR. Physiological and recent imaging studies demonstrate that functional airway closure is not only present in asthmatics, it is inducible by methacholine, and reversible by bronchodilators (18, 24, 25, 35, 44, 47, 48). It has recently been shown, using a computational modeling approach, that the heterogeneous ventilation defects observed in asthmatic patients can be attributed to narrowing and subsequent closure of small airways (41). This suggests that physiology in antigen-sensitized and -challenged BALB/c mice more closely mimics that seen in asthmatic individuals. As such, other mouse models, like A/J mice, that have more of an exaggerated central airway response to methacholine seem less relevant. However, the phenotype of asthma is highly variable (31), and there is evidence that some patients have more or less of a central airway response especially during an exacerbation (18, 22). Therefore, the choice of mouse model and route of methacholine delivery should depend on the asthma phenotype of interest. Furthermore, while it is obvious that no mouse model is a perfect representation of clinical asthma, the physiological variability demonstrated in the present study suggests that mouse models may nonetheless provide an opportunity to study the various phenotypes of asthma in relative isolation.

GRANTS

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