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Effects of corticosteroid treatment on airway inflammation, mechanics, and hyperpolarized ^3He magnetic resonance imaging in an allergic mouse model

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Thomas AC, Kaushik SS, Nouls J, Potts EN, Slipetz DM, Foster WM, Driehuys B. Effects of corticosteroid treatment on airway inflammation, mechanics, and hyperpolarized ^3He magnetic resonance imaging in an allergic mouse model. *J Appl Physiol* 112: 1437–1444, 2012. First published January 12, 2012; doi:10.1152/jappphysiol.01293.2011.—The purpose of this study was to assess the effects of corticosteroid therapy on a murine model of allergic asthma using hyperpolarized ^3He magnetic resonance imaging (MRI) and respiratory mechanics measurements before, during, and after methacholine (MCh) challenge. Three groups of mice were prepared, consisting of ovalbumin sensitized/ovalbumin challenged (Ova/Ova, $n = 5$), Ova/Ova challenged but treated with the corticosteroid dexamethasone (Ova/Ova+Dex, $n = 3$), and ovalbumin-sensitized/saline-challenged (Ova/PBS, $n = 4$) control animals. All mice underwent baseline 3D ^3He MRI, then received a MCh challenge while 10 2D ^3He MR images were acquired for 2 min, followed by post-MCh 3D ^3He MRI. Identically treated groups underwent respiratory mechanics evaluation ($n = 4$ /group) and inflammatory cell counts ($n = 4$ /group). Ova/Ova animals exhibited predominantly large whole lobar defects at baseline, with significantly higher ventilation defect percentage (VDP = $19 \pm 4\%$) than Ova/PBS ($+2 \pm 1\%$, $P = 0.01$) animals. Such baseline defects were suppressed by dexamethasone (0% , $P = 0.009$). In the Ova/Ova group, MCh challenge increased VDP on both 2D ($+30 \pm 8\%$) and 3D MRI scans ($+14 \pm 2\%$). MCh-induced VDP changes were diminished in Ova/Ova+Dex animals on both 2D ($+21 \pm 9\%$, $P = 0.63$) and 3D scans ($+7 \pm 2\%$, $P = 0.11$) and also in Ova/PBS animals on 2D ($+6 \pm 3\%$, $P = 0.07$) and 3D ($+4 \pm 1\%$, $P = 0.01$) scans. Because MCh challenge caused near complete cessation of ventilation in four of five Ova/Ova animals, even as large airways remained patent, this implies that small airway ($<188 \mu\text{m}$) obstruction predominates in this model. This corresponds with respiratory mechanics observations that MCh challenge significantly increases elastance and tissue damping but only modestly affects Newtonian airway resistance.

ventilation defect; dexamethasone; ovalbumin; bronchoalveolar lavage

IMAGING RESEARCH ON ALLERGIC asthma has revealed that the disease causes heterogeneous ventilation, with some areas of markedly decreased ventilation, named “ventilation defects” (1, 5). Although ventilation defects have been noted by positron emission tomography (PET) and computed tomography (CT) imaging techniques (10, 18, 20), the majority of studies has employed hyperpolarized ^3He magnetic resonance imaging (MRI) (13). ^3He MRI regionally visualizes the distribution of inhaled gas in the airways and airspaces of the lung with high

resolution and without ionizing radiation. ^3He MRI has been applied to human asthmatics and has shown that ventilation defects correlate with the severity of the disease (4) and are worsened with bronchoconstrictive challenge (16).

Also important to the study of heterogeneous ventilation in allergic asthma is modeling the disease in animals. Mouse models are especially useful given the abundance of well-characterized strains with known genotypes and their frequent utility in the study of human diseases. By using mouse models of asthma, previous studies have shown areas of decreased ventilation with similar characteristics to ventilation defects noted in humans (19).

To date, ^3He MRI has been used only to image methacholine (MCh)-induced ventilation defects in small groups of animals (6–8). A critical next step is to validate the utility of ^3He MRI methodology, identify image signatures that differentiate treatment groups, and connect imaging to respiratory mechanics measurements. Furthermore, concurrent examination with imaging and traditional methods of respiratory mechanics allows direct visualization of parameters that using respiratory mechanics alone can only be inferred. Hence, we hypothesized that direct visualization of airways and airspaces by ^3He MRI before, during, and after MCh challenge, would enhance the interpretation and be consistent with traditional methods of respiratory mechanics and bronchoalveolar lavage inflammatory cell analysis in differentiating the effect of corticosteroid treatment in a disease model relevant to allergic asthma.

MATERIALS AND METHODS

Animals. All studies were conducted under protocols approved by the Duke University Institutional Animal Care and Use Committee and utilized 6- to 8-wk-old male Balb/C mice (Jackson Labs, Bar Harbor, ME) prepared in three treatment groups: 1) ovalbumin-sensitized and challenged (Ova/Ova, $n = 5$), 2) ovalbumin-sensitized and challenged while being treated with the anti-inflammatory corticosteroid dexamethasone (Ova/Ova+Dex, $n = 3$), and 3) ovalbumin-sensitized, but phosphate buffered saline (PBS)-challenged controls (Ova/PBS, $n = 4$). MR imaging was performed on mice from each treatment group. Separate additional animals were prepared at the same time and used for traditional lung functional and inflammatory characterization with forced oscillation respiratory mechanics testing ($n = 4$ animals/group) and cell analysis with bronchoalveolar lavage (BAL, $n = 4$ animals/group), respectively. The BAL animals were separate from the respiratory mechanics animals, although they were prepared identically and at the same time.

Ovalbumin sensitization. Ovalbumin (Ova) was used to sensitize and challenge mice to create the widely utilized model of allergic inflammation (22). Mice were sensitized and challenged with Ova (Ova/Ova group) or sensitized with Ova, but challenged with PBS (Ova/PBS

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group), as described in a prior study (19). To investigate the potential effects of corticosteroids on ablating the inflammatory response, a third group of mice (Ova/Ova+Dex) was prepared in the same manner as Ova/Ova animals, except an intraperitoneal injection of dexamethasone (Vetoquinol USA) at 1 mg/kg was administered 24 h prior to the first challenge and then 30 min prior to each Ova challenge.

Respiratory mechanics and bronchoalveolar lavage. Total lung resistance in response to MCh challenge was measured using the flexiVent system (flexiVent, SCIREQ, Montreal, Canada), which uses a 2-s sine-wave, 2.5 Hz forced oscillation technique to assess lung mechanics. Mice were anesthetized with 60 mg/kg Nembutal, tracheotomized with a cannula, and then connected in a supine body position to a small animal ventilator with a positive end-expiratory pressure (PEEP) of 3 cmH₂O and 0.2 ml tidal volume. The animals were then given a neuromuscular blockade (0.8 mg/kg Pancuronium Bromide, Sigma-Aldrich). Following baseline resistance measurements, MCh at a concentration of 80 µg/ml was administered via a 3-s bolus to the jugular vein to achieve a dose of 80 µg/kg, and mechanics indexes were measured over a 5-min period. MCh was delivered intravenously rather than by aerosol to maintain consistency with the ³He MRI studies, which currently accommodate only injection delivery. Two models of respiratory mechanics were used to assess lung mechanics: the linear first-order single-compartment model, which provides resistance of the total respiratory system, and the constant-phase model (2), which uses forced oscillation to differentiate between central airway Newtonian resistance (*R_n*), peripheral lung damping (*G*, tissue resistance), and elastance (*H*, tissue stiffness).

Bronchoalveolar lavage was performed after euthanasia and removal of the chest wall to allow complete deflation of the lungs. A cannula was positioned within the trachea, and the lungs were lavaged to 25 cmH₂O pressure with three consecutive saline fills. Lavage volumes are reported as the total return volume for three fills for each mouse.

Animal preparation for MRI. Mice used in the imaging studies were anesthetized with an 85 mg/kg intraperitoneal dose of pentobarbital sodium (Nembutal Sodium Solution, OVATION Pharmaceuticals, Deerfield, IL). Anesthesia was maintained with doses of 20 mg/kg pentobarbital sodium delivered approximately every 45–60 min or when the heart rate increased above 500 beats/min.

Mice were ventilated through a tracheostomy tube cut from a 2.5-cm section of an 18-gauge intravenous catheter (Hospira, Lake Forest, IL). The tube was inserted below the cricoid cartilage and advanced 5 mm. It was then fastened in place with a suture to ensure a gas-tight seal. A jugular catheter was used for MCh delivery using a 2-Fr. catheter (Sherwood Medical, Tullamore, Ireland). The animal was ventilated using a custom hyperpolarized gas- and MR-compatible ventilator (14) set at 100 breaths/min, with each cycle consisting of a 240-ms inspiration, 100-ms breath-hold for image acquisition, and 260-ms exhalation period. After the lines and probes were placed, the animal was set on a cradle that fit into a 64.8-MHz tuned birdcage coil optimized for the ³He signal (5.5 cm long and 3.5 cm in diameter) with the lungs positioned in the center of the coil. The entire assembly was then placed in the magnet, and the animal's rectal temperature was used to provide feedback to a system circulating warm air through the bore to keep the animal at 37°C.

MCh delivery for MRI. MCh was prepared fresh each day at a concentration of 80 µg/ml. MCh was introduced through a jugular intravenous catheter at a dose of 125 µg/kg, typically consisting of a 40-µl volume delivered over 3 s via a syringe pump, as previously described (19). MCh was pumped through ~2 m of extension tubing to the animal in the magnet bore. To ensure reliable MCh delivery to the animal, fresh MCh was primed through the extension tubing immediately prior to the 2D ³He MRI. Then, an initial bolus of 200 µl was used to clear MCh in the line and two to three subsequent test boluses were delivered to ensure that each caused a reproducible drop in heart rate. The animal was allowed to recover for 5 min after the last MCh test bolus and given a total lung capacity (TLC) breath to recruit alveoli. Such TLC breaths increased airway pressures from a

baseline of 5 to 10 cmH₂O to ~20 cmH₂O due to constant volume ventilation. After the MCh effect was reproducibly demonstrated and the animal was allowed to recover, a 125 µg/kg bolus of MCh was delivered and post-MCh 2D ³He MRI was initiated, as described next, to image the temporal dynamics of MCh response.

Image acquisition. ³He MRI was performed at 64.8 MHz in a 2.0-T horizontal 15-cm clear bore magnet (Oxford Instruments, Oxford, UK) with shielded gradients (18 Gauss/cm) controlled by a GE EXCITE 12 console. ³He (Spectra Gases, Alpha, NJ) was polarized to ~30% in batches of 1.2 liter using a prototype commercial polarizer (IGI.9600.He, MITI, Durham, NC). After hyperpolarization was stopped, the ³He in the optical cell decayed with a relaxation time *T*₁ = 30 h. ³He was dispensed into a Tedlar bag (Jensen Inert Products, Coral Springs, FL) in volumes of ~150 ml to acquire a single baseline 3D image or 250 ml to acquire both post-MCh 2D and 3D ³He MRI.

Once animals were localized in a supine body position in the magnet, 3D ³He MR images were acquired using a radial trajectory (21) with field of view of 2.0 × 2.0 × 3.2 cm and an encoding matrix of 128 × 128 × 32 to yield a resolution of 156 × 156 × 1,000 µm³. The 3D images used 11,512 radial views for full sampling and were acquired with TR = 5 ms, thus capturing 20 views per breath and taking 5.75 min to complete. During each breath, the flip angle was incremented using a variable flip angle scheme ending in a 90° pulse (23).

Two-dimensional ³He MRI used a radial acquisition with a field of view = 2.4 × 2.4 cm² and 128 × 128 matrix yielding a resolution of 188 × 188 µm². Images were acquired using TR/TE = 5/0.3 ms and BW = 31.25 kHz during the 100-ms ventilation inspiratory breath-hold periods. During each breath-hold, 20 k-space views were acquired, thus requiring 20 breaths (12 s) to fully sample the image with 400 radial views. The flip angle was incremented for each view to end with a 90° pulse. Such 2D images were acquired every 12 s for 10 total images consisting of one baseline image immediately followed by the MCh injection and nine post-MCh images. Immediately following the 2D image series, an additional 3D scan was acquired to visualize persistent ventilation defects induced by MCh challenge.

Imaging data analysis. All image visualization and analysis was performed using ImageJ software version 1.38x (NIH, Bethesda, MD, <http://rsb.info.nih.gov/ij/>). The high-sensitivity ³He coil used in this work did not permit proton imaging, which can be useful for automated segmentation of the thoracic cavity by virtue of its characteristic paucity of lung parenchymal signal. Therefore, ³He MR images were analyzed by manual tracing. In each ³He MR image slice, ventilation defects were identified, traced, and multiplied by slice thickness to establish a ventilation defect volume (VDV), as suggested by Mathew et al. (12). For the 2D images, all calculations used units of area rather than volume. To normalize these defect volumes, the thoracic cavity volume (TCV) was determined by tracing each ³He slice to identify the outer contours of the lung and again multiplied by slice thickness. In cases where ventilation defects were present, these contours were estimated using the operator's knowledge of normal ventilation patterns previously acquired in control mice (19). Ventilation defects were determined based on consensus of three authors (A.C.T., S.S.K., B.D.). Only slices with signal visibly increased above background (approximately twice background signal) were traced. Each thoracic cavity and ventilation defect was traced by a single operator (A.C.T.), who was not blinded to the treatment group.

Persistent airway narrowing was quantitatively assessed by making maximum intensity projections of the pre- and post-MCh 3D ³He MR images. Transient airway narrowing was measured by reviewing the 10 2D post-MCh ³He MRI time course images to identify the frame in which maximal airway narrowing occurred (typically frame 2 or 3). In this image, the right bronchus diameter was measured immediately caudal to the origin of the accessory lobe bronchus, whereas the left bronchus diameter was assessed as it projects over the medial border of the left lung parenchyma. These points were chosen because they

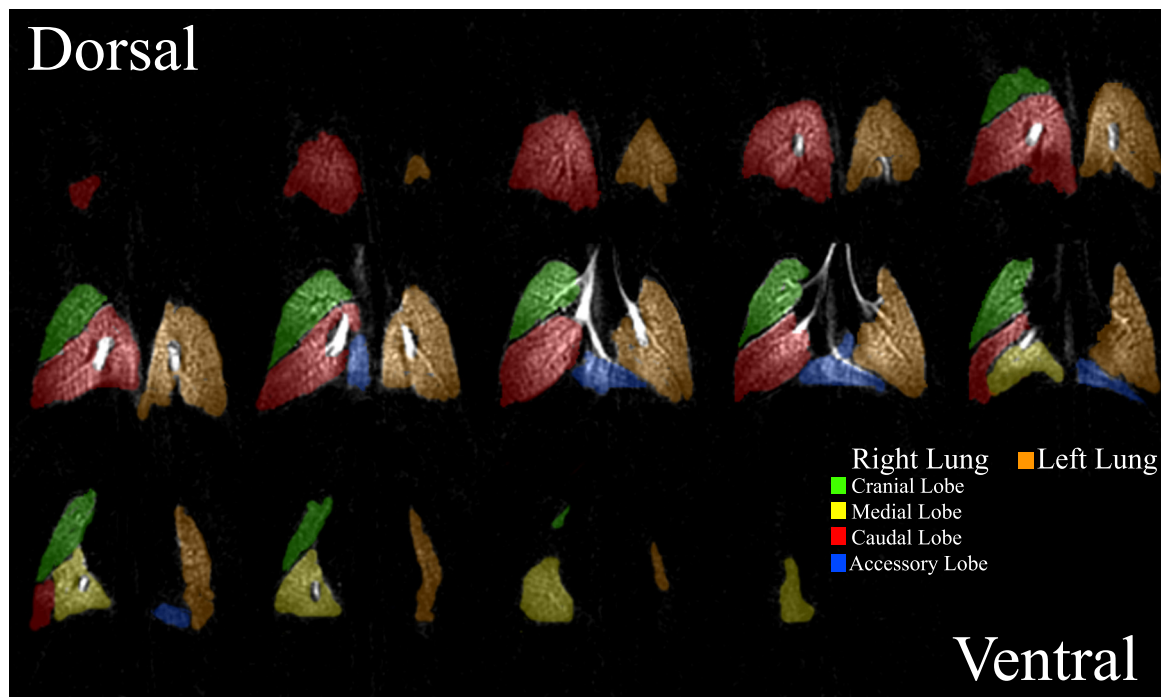


Fig. 1. In vivo map of the lung lobes acquired from an ovalbumin-sensitized/saline-challenged (Ova/PBS) Balb/C control mouse without methacholine (MCh) challenge. Images are sequential 1-mm slices through the mouse lung ordered *left to right* (in the dorsal to ventral direction). There are 4 lobes in the right lung: cranial, medial, caudal, and accessory. Left lung only contains 1 lobe.

are roughly equidistant from the carina, readily identifiable, and approximately equal in diameter. The degree of narrowing was quantified by the percentage change in bronchial diameter post-MCh relative to baseline and averaging the values for the right and left bronchi.

For each 3D ^3He MR image, VDV was divided by TCV to determine ventilation defect percentage (VDP) (12), which was used for statistical analysis. To analyze changes elicited by MCh challenge, the increase in VDP relative to baseline was calculated. To analyze the post-MCh 2D images, the second image (24 s) in the time series, which typically showed the greatest ventilation changes, was used to calculate changes in VDP.

Statistical comparisons of VDP at baseline and post-MCh challenge were made across treatment groups using the ANOVA Tukey-Kramer honestly significant difference method with JMP Pro statistical software Version 9 (SAS Institute, Cary, NC). Additionally, within each group, pre- and post-MCh pairwise comparisons were performed using one-tailed student *t*-tests. In cases where measurements showed nonsignificant differences between groups, post hoc estimates of the necessary group sizes were made using STPLAN software Version 4.5 (M.D. Anderson Cancer Center, Houston, TX) and assuming a

normal distribution with two samples of unequal variances, one-tailed test, and power = 0.80. Results were considered statistically significant for $P < 0.05$.

RESULTS

Baseline 3D ^3He MRI. As a starting point for image interpretation, Fig. 1 shows an in vivo image of a normal Balb/C control mouse prior to MCh challenge, demonstrating the normal lobar configuration of the mouse lung with four lobes of the right lung (cranial, medial, caudal, and accessory) and the single lobe of the left lung. In contrast, such baseline images exhibited striking ventilation defects in four of five Ova/Ova animals, where they encompassed an entire lung lobe (Fig. 2, A and B). The whole lobar defects in Ova/Ova animals were found in the left lung in three animals and the right medial lobe in one animal. Such whole lobar defects at baseline were not found in any Ova/Ova+Dex or Ova/PBS animals. No Ova/Ova+Dex animals exhibited any baseline ventilation defects (Fig. 2, C and D). However, three of four Ova/PBS

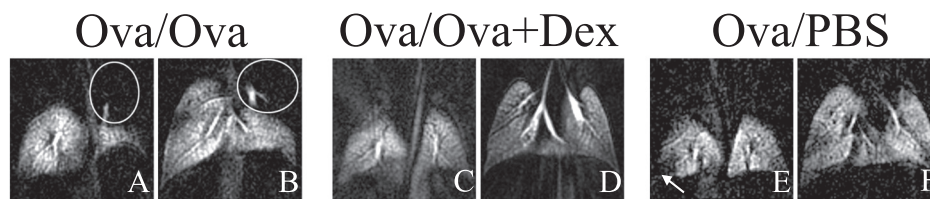


Fig. 2. Baseline Defects. Ovalbumin sensitized/ovalbumin challenged (Ova/Ova) animals displayed marked whole lobar defects in 4 of 5 animals even before MCh was introduced. Whole lobar defects were not seen in the Ova/Ova challenged treated with the corticosteroid dexamethasone (Ova/Ova+Dex) or Ova/PBS animals. A and B: coronal slices 2-mm apart in an Ova/Ova mouse at baseline. Circles in both panels indicate areas where the left lung should be evident, but is not visualized because it is not ventilated. C and D: corresponding slices in an Ova/Ova+Dex mouse exhibiting no baseline defects and full ventilation to both the right and left lungs. E and F: corresponding slices in an Ova/PBS mouse with a small posterior caudal lobe defect at baseline (arrow).

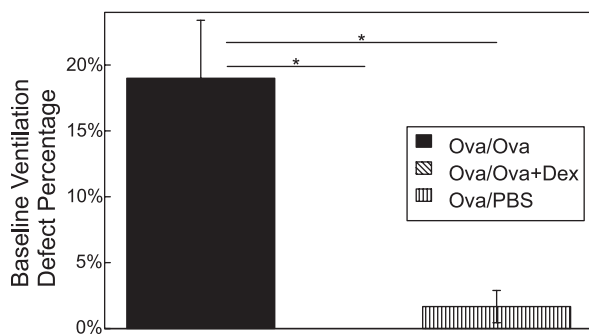


Fig. 3. Baseline ventilation defect percentage (VDP) in Ova/Ova (black), Ova/Ova+Dex (diagonal stripe), and Ova/PBS mice (vertical stripes). No baseline ventilation defects were identified in Ova/Ova+Dex animals. Baseline VDP was significantly different (*) between Ova/Ova and Ova/Ova+Dex groups ($P = 0.009$), as well as between Ova/Ova and Ova/PBS groups ($P = 0.01$). Error bars indicate standard error of the mean.

animals did show small defects at baseline (Fig. 2, E and F). (Note that several of the 3D image panels show a vertical streak artifact, which is caused by ^3He signal in the trachea that extends outside of the imaging field of view and thus “wraps” back into the image.) Quantitatively, the ventilation defect percentage in Ova/Ova animals was $19 \pm 4\%$ of baseline volume (Fig. 3), which was significantly different from both the Ova/Ova+Dex (0% , $P = 0.009$) and Ova/PBS ($2 \pm 1\%$, $P = 0.01$) groups.

Post-MCh 2D ^3He MRI. Two-dimensional images acquired prior to and immediately following MCh challenge are presented in Fig. 4. The Ova/Ova animals exhibited markedly decreased ventilation during the two images (0–24 s) immediately following MCh administration in four of five animals. These animals also exhibited significant narrowing of the mainstem bronchi ($35 \pm 11\%$, $P = 0.03$). All of the Ova/Ova animals regained ventilation patterns on the 2D scans similar to their pre-MCh image by the 10th image of the temporal series (2 min post-MCh).

For the Ova/Ova+Dex group, all three animals showed narrowing of the visible major airways after MCh challenge ($52 \pm 12\%$, $P = 0.05$). One of the Ova/Ova+Dex animals did

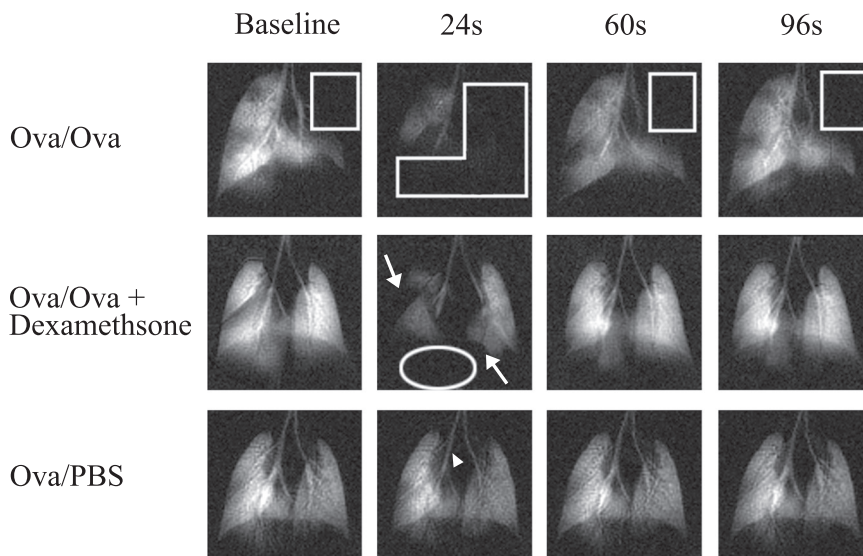
show a 36% decrease in ventilation in the two images (12–24 s) following MCh challenge. Another animal developed a sizeable defect in the right cranial lobe after MCh challenge, which did not completely recover by the 10th image of the temporal series. A third animal did not demonstrate perceptible ventilation defects on 2D images.

For the Ova/PBS group, MCh challenge caused narrowing of all visible airways ($44 \pm 8\%$, $P = 0.01$). Only one of four animals developed perceptible ventilation defects on 2D imaging, visible for six frames (72 s) after MCh challenge.

Pairwise comparisons of 2D ^3He MRI within groups pre- and post-MCh demonstrated a statistically significant increase in VDP only in Ova/Ova animals ($+30 \pm 8\%$, $P = 0.008$) compared with nonsignificant changes of $+6 \pm 3\%$ in Ova/PBS animals ($P = 0.08$) and $+21 \pm 9\%$ in Ova/Ova+Dex animals ($P = 0.07$). Comparisons of post-MCh VDP increase among groups using the Tukey Kramer method showed no statistical significance due to variability within the relatively small groups in this study. To obtain significant differences in VDP increase between Ova/Ova and Ova/PBS would require an estimated 5 animals per group, but differentiating Ova/Ova from Ova/Ova+Dex would require 35. Similarly, distinguishing post-MCh VDP increase in Ova/Ova+Dex from Ova/PBS would require nine animals in each group.

Post-MCh 3D ^3He MRI. Comparison of post-MCh 3D ^3He MR images with 3D images acquired at baseline permitted identification of smaller, more persistent defects induced by MCh than those identified on 2D imaging (Fig. 5). For the Ova/Ova animals, large whole lobar defects were visible in every animal post-MCh (5 of 5), although only one of these had not been present at baseline. In one Ova/Ova animal, the whole right medial lobar defect present at baseline became fully ventilated after MCh challenge. However, the clearing of this defect was accompanied by development of a new whole left lung lobar defect. The whole lobar post-MCh defects were observed in the left lung for four of five Ova/Ova animals, whereas two of five Ova/Ova animals exhibited whole lobar defects in the right medial lobe. Quantitatively, pairwise comparisons within groups showed that MCh challenge significantly increased 3D VDP in all groups: Ova/Ova ($P = 0.001$),

Fig. 4. Representative ventilation defects observed on a 2D ^3He magnetic resonance imaging (MRI) temporal series by treatment group following a 125 $\mu\text{g}/\text{kg}$ bolus of MCh. Each row shows a baseline coronal image taken prior to MCh challenge, followed by representative panels from the 9 subsequent images taken at 12-s intervals following MCh. In an Ova/Ova mouse with a whole left lobar defect at baseline (boxes), the defect remained after MCh injection, along with nearly complete cessation of ventilation at 24 s post-MCh. In an Ova/Ova+Dex mouse, large ventilation defects developed, including a temporary whole right medial lobar defect (circle), as well as cranial and left lobe defects (arrows). In an Ova/PBS mouse, few defects were seen on 2D imaging following MCh exposure, although some bronchoconstriction was noted (arrowhead).



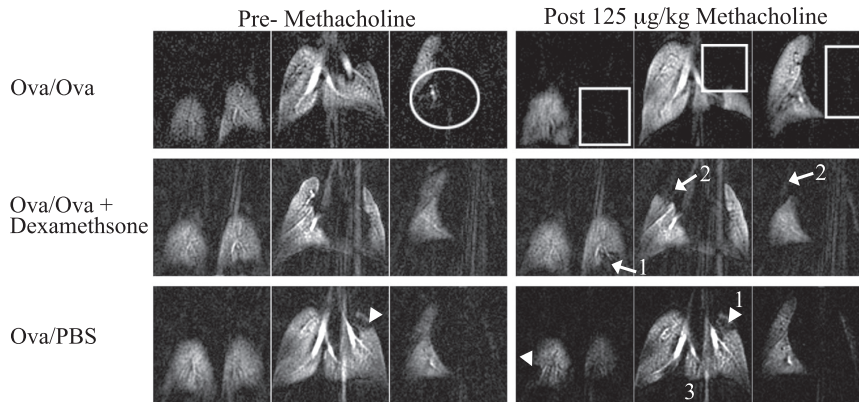


Fig. 5. Representative ventilation defects for the treatment groups observed at baseline and after MCh challenge using 3D ^3He MRI. Each set of 3 images are posterior, middle, and anterior slices taken from the ~ 12 – 15 1-mm coronal slices covering each mouse lung. In the Ova/Ova mouse, the circle marks the absence of ventilation to the medial lobe at baseline. However, after a 125- $\mu\text{g}/\text{kg}$ bolus of MCh, this lobe becomes ventilated, whereas the previously ventilated left lung develops a whole lobar defect (boxes). In the Ova/Ova+Dex mouse, defects are noted in the posterior left lung (1) and the apical cranial lobe (2). In the Ova/PBS mouse, there is a defect in the apical left lung (1) that is present in both the pre- and post-MCh images. In this animal, MCh also induced defects in the posterior caudal lobe (2) and the inferior caudal lobe (3).

Ova/Ova+Dex ($P = 0.04$), Ova/PBS ($P = 0.04$). As shown in Fig. 6, for the Ova/Ova group, VDP increased by $14 \pm 2\%$, which was significantly different from the 4.1% increase seen in Ova/PBS animals ($P = 0.01$). Although the increase in VDP of $7 \pm 2\%$ in Ova/Ova+Dex mice was greater than Ova/PBS mice and less than Ova/Ova mice, these differences were not statistically significant. To obtain significant differences in post-MCh 3D VDP increase between Ova/Ova+Dex and Ova/Ova would have required an estimated 6 animals per group, whereas distinguishing Ova/Ova+Dex from Ova/PBS would have required 12 per group.

All treatment groups demonstrated modest residual airway narrowing on post-MCh 3D ^3He MRI compared with baseline images. However, these differences were only statistically significant for Ova/PBS mice ($11 \pm 3\%$, $P = 0.03$). Airway narrowing for Ova/Ova mice was $20 \pm 12\%$ ($P = 0.09$) and for Ova/Ova+Dex was $15 \pm 9\%$ ($P = 0.12$).

Inflammatory cell response in BAL and respiratory mechanics using forced oscillation technique. Analysis of BAL for total cells and inflammatory cell differentials in each treatment group showed that the Ova/Ova group elicited a significant increase in BAL cellularity compared with the Ova/PBS group ($P = 0.001$), and this increase reflected primarily a macrophage and an inflammatory cell-mediated BAL response. Dexamethasone treatment partially reversed the Ova-induced cellularity with respect to total cells and cell differentials for polymorphonuclear neutrophils (PMNs) and eosinophils, with significant reductions compared with the BAL cellularity of the

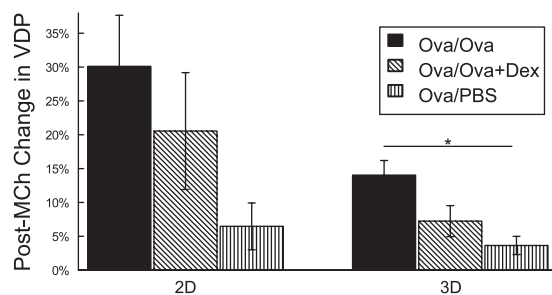


Fig. 6. Absolute increase in VDP by group induced by MCh-challenge and measured using both 2D time-resolved imaging and 3D MRI. Both the 2D and 3D MRI show a trend of greater MCh response in Ova/Ova relative to the other groups, although statistical significance is only reached for the post-MCh 3D analysis between the Ova/Ova and Ova/PBS animals. Error bars indicate standard error of the mean.

Ova/Ova group ($P = 0.008$). As anticipated, the Ova/PBS animals showed the fewest BAL inflammatory cells (Fig. 7).

Mean changes in total airway resistance in response to intravenous MCh showed that Ova/Ova animals exhibited the greatest increase in total lung resistance after intravenous MCh challenge and, as expected, Ova/PBS animals the smallest (Fig. 8). The Ova/Ova+Dex group demonstrated a beneficial effect due to dexamethasone, with total resistance during both the 0- to 2-min and 2- to 5-min measurement periods being decreased by $\sim 50\%$ compared with the Ova/Ova group.

To more closely analyze the relationship between ^3He MRI and traditional airway mechanics measurements, the mechanics measurements were separated into total resistance, Newtonian resistance, elastance (G) and tissue damping (H). Moreover, these indexes were averaged over the periods of 0–2 min and 2–5 min, roughly corresponding to the time window during which dynamic 2D ^3He MR images and post-MCh 3D ^3He MR images were acquired (Fig. 8). In the 0- to 2-min interval, the total resistance was significantly increased in the Ova/Ova relative to Ova/Ova+Dex ($P = 0.02$) and Ova/PBS groups ($P = 0.02$). The total resistance continued to be elevated in similar proportions during the 2–5-min interval. The Newtonian resistance made only a relatively modest contribution to total lung resistance ($\sim 20\%$) in all groups in the 0- to 2-min period and an even smaller contribution during the 2- to 5-min

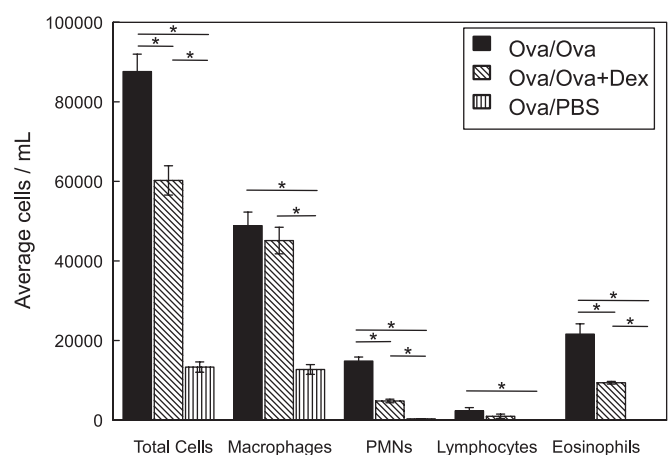


Fig. 7. Bronchoalveolar lavage data by treatment group. Differences between groups ($n = 4$ mice/group) for respective total cells, and cell differentials were significant (*) at $P < 0.05$. Error bars indicate standard error of the mean.

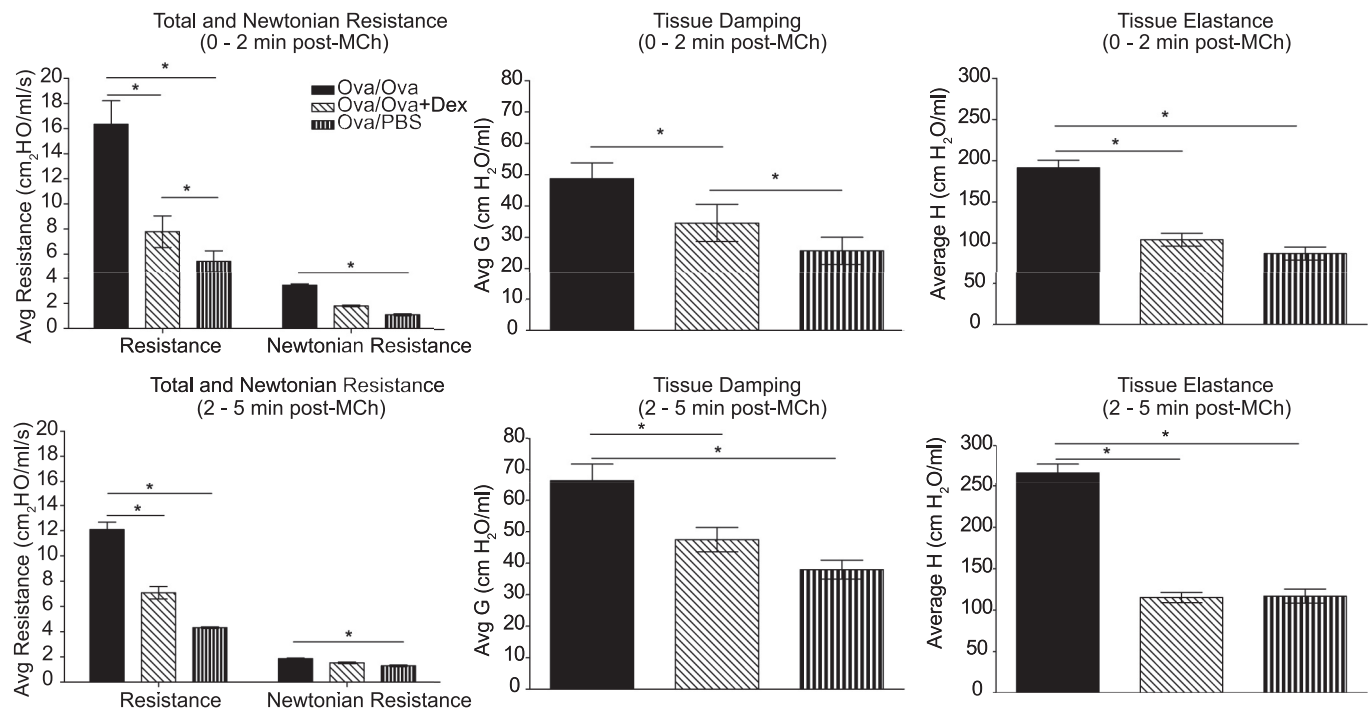


Fig. 8. Respiratory mechanics indexes averaged over the time intervals closely corresponding to dynamic 2D ^3He MRI (0–2 min, *top*) and 3D ^3He MRI (2–5 min, *bottom*). See METHODS for descriptions of total and Newtonian resistance and tissue damping (G) and tissue elastance (H). *Compared differences significant at $P < 0.05$ ($n = 4$ mice/group). Error bars indicate standard error of the mean.

period. The peripheral tissue damping was significantly elevated in the Ova/Ova mice ($P < 0.0001$), and this was somewhat attenuated by dexamethasone treatment ($P = 0.003$). During the 2- to 5-min measurement period, effects on tissue damping were sustained and differences persisted between Ova/Ova, Ova/Ova+Dex, and Ova/PBS groups. Similarly, elastance was significantly greater in the Ova/Ova group relative to the others ($P < 0.0001$ for both groups) at both the 0- to 2-min and 2- to 5-min intervals. The dexamethasone treatment appeared more beneficial for ameliorating the effect of MCh on tissue elastance than tissue damping.

DISCUSSION

The imaging results shown in this work are consistent with expectations regarding the varying levels of inflammation for the three groups. BAL analysis demonstrated significantly more inflammatory cells and individual cell types in Ova/Ova animals than Ova/PBS and showed that these cells were diminished in the dexamethasone-treated group. Consistent with these findings, ^3He MRI showed that the Ova/Ova group exhibited larger ventilation defects both at baseline and after MCh challenge than the other groups. ^3He MRI showed that the most responsive animals had large regions of absent ventilation rather than multiple, smaller ventilation defects.

Perhaps the most striking finding was the presence of large ventilation defects in the lungs of the Ova/Ova mice prior to MCh challenge. In fact, such baseline defects were also described by Risse et al. (15), who described the treatment effect of steroids on Ova/Ova treated rats using ^3He MRI. Although our sample size was small, it is somewhat striking to see the prevalence of these baseline defects in the left lung. This suggests that the regional inflammatory response was so in-

tense as to have led to airway edema and potential for airway closure, which would limit and/or severely curtail lobar ventilation. Such airway closure and atelectasis in allergically inflamed airways of mouse models has previously been noted by Lundblad et al. (11) using micro-CT and other investigators who have noted edema in these mice using conventional proton MRI (3). Future ^3He MRI studies would benefit from an accompanying ^1H MRI scan to test for edema, as well as the ability to scan animals longitudinally over a period of days to observe the resolution of defects. Notably, the baseline defects we observed in this study are a feature that cannot be identified by conventional and more invasive methods such as forced oscillometry, which typically measures only relative changes in baseline resistance of the airways in total in response to MCh challenge. Moreover, in our study these baseline defects were the strongest imaging features that clearly distinguished all three groups. Hence, identification of these focal baseline defects may be a promising marker for testing the efficacy of different therapeutic compounds.

^3He MRI after MCh-challenge showed the largest response in the Ova/Ova group, consistent with this group also exhibiting the highest level of airway inflammation. In the Ova/Ova group, MCh challenge led to near complete cessation of ventilation for a short period (12–24 s), even as the major airways remained visible. This combination of large ventilation defects and visible major airways suggests that in this allergic model, it is the small airways that exert a dominant role in MCh-induced airflow restriction. Here, we consider small airways to have a diameter < 1 pixel (188 μm for 2D images), which corresponds to third- or fourth-order airways, similar to definitions from previous micro-CT studies (17).

The Ova/Ova group also experienced the greatest increase in VDP on 3D ^3He MRI after MCh challenge. This suggests that the elevated inflammatory status of this group also predisposed it to increased responsiveness to MCh stimulation, with effects lasting at least several minutes following MCh challenge. Furthermore, the observation of such persistent ventilation defects, even after the visible bronchi had relaxed back to nearly baseline caliber, again suggests that inflammation/airways hyperreactivity affected primarily the smaller airways, such as the bronchioles.

The findings from ^3He MRI that MCh challenge affected primarily the peripheral lung are consistent with those derived from lung mechanics. We found with intravenous MCh challenge a significant enhancement in indexes of lung function identified with the peripheral lung, such as tissue damping (G) and elastance (H), and substantially less robust changes in the central airway parameter, Newtonian resistance. Such acute increases in G and H have been implicated by Irvin and Bates (9) as representing three potential mechanisms: derecruitment of lung units as airways close, temporal shifts of tissue movement, and inhomogeneity of airflow penetration/distribution. Indeed, our 2D ^3He MR images reveal narrowing of the visible major airways immediately after MCh challenge, primarily in the Ova/PBS and Ova/Ova+Dex groups, consistent with a slight increase in Newtonian resistance. However, the most pronounced effects observed during post-MCh 2D MRI were those of decreased or near complete loss of ventilation to distal units, which was particularly prominent in the Ova/Ova group. These imaging results are supported by the mechanics measurements and are consistent with the interpretation of only mild changes to the central conducting airways and a more predominant involvement of smaller airways within the lung periphery. Moreover, the creation of heterogeneously distributed ventilation defects in the Ova/Ova model, but not in the other groups, is consistent with observed enhancement of the indexes G and H in Ova/Ova. The observation on post-MCh 3D ^3He MRI that the visible central airways were no longer significantly narrowed is consistent with Newtonian resistance decreasing to nearly negligible levels during the 2- to 5-min period. Similarly, the retention of persistent ventilation heterogeneity in the Ova/Ova group relative to the other groups is consistent with the continued elevation of G and H values in this group during mechanics measurements.

In conclusion, this study demonstrates the impressive effects of inflammation on regional ventilation in various treatment groups. ^3He MR imaging results were 1) supported by inflammatory cell analysis in BAL and consistent with respiratory mechanics indexes derived in vivo specifically for mouse models and 2) predictive of airflow obstruction during MCh challenge. The imaging and respiratory mechanics measurements were able to determine statistically significant differences among groups in baseline ventilation defect volume and peripheral lung parameters, respectively, showing treatment effects that were diminished in Ova/Ova+Dex and Ova/PBS animals. Specifically, ^3He MRI supports the inferences from respiratory mechanics that the primary changes induced by MCh challenge in this model occur in the small airways. Finally, although agreement between ^3He MRI and respiratory mechanics after MCh was encouraging, the strongest differentiator of the three groups on ^3He MRI was baseline imaging prior to MCh challenge. Hence, ^3He MRI, even without the

need for MCh challenge, demonstrates predictive value as a functional biomarker at baseline in an allergic asthma model representative of inflammatory airway disease that can occur in humans.

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DISCLOSURES

Dr. Driehuys holds several patents related to hyperpolarized gas MRI. These patents have been licensed to GE Healthcare for commercial development. Although no such development is underway currently, Dr. Driehuys could potentially benefit financially from positive results relating to Hyperpolarized gases. Current royalties are below the \$10,000 mark.

AUTHOR CONTRIBUTIONS

Author contributions: A.C.T., D.M.S., W.M.F., and B.D. conception and design of research; A.C.T. and E.N.P. performed experiments; A.C.T., S.S.K., J.N., E.N.P., W.M.F., and B.D. analyzed data; A.C.T., S.S.K., J.N., E.N.P., D.M.S., W.M.F., and B.D. interpreted results of experiments; A.C.T., S.S.K., J.N., E.N.P., and B.D. prepared figures; A.C.T. drafted manuscript; A.C.T., S.S.K., J.N., E.N.P., D.M.S., W.M.F., and B.D. edited and revised manuscript; D.M.S., W.M.F., and B.D. approved final version of manuscript.

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