Ventilation Defects Observed with Hyperpolarized ^3He Magnetic Resonance Imaging in a Mouse Model of Acute Lung Injury

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Regions of diminished ventilation are often evident during functional pulmonary imaging studies, including hyperpolarized gas magnetic resonance imaging (MRI), positron emission tomography, and computed tomography (CT). The objective of this study was to characterize the hypointense regions observed via ^3He MRI in a murine model of acute lung injury. LPS at doses ranging from 15–50 μg was intratracheally administered to C57BL/6 mice under anesthesia. Four hours after exposure to either LPS or saline vehicle, mice were imaged via hyperpolarized ^3He MRI. All images were evaluated to identify regions of hypointense signals. Lungs were then characterized by conventional histology, or used to obtain tissue samples from regions of normal and hypointense ^3He signals and analyzed for cytokine content. The characterization of ^3He MRI images identified three distinct types of hypointense patterns: persistent defects, atelectatic defects, and dorsal lucencies. Persistent defects were associated with the administration of LPS. The number of persistent defects depended on the dose of LPS, with a significant increase in mean number of defects in 30–50- μg LPS-dosed mice versus saline-treated control mice. Atelectatic defects predominated in LPS-dosed mice under conditions of low-volume ventilation, and could be reversed with deep inspiration. Dorsal lucencies were present in nearly all mice studied, regardless of the experimental conditions, including control animals that did not receive LPS. A comparison of ^3He MRI with histopathology did not identify tissue abnormalities in regions of low ^3He signal, with the exception of a single region of atelectasis in one mouse. Furthermore, no statistically significant differences were evident in concentrations of IL-1 β , IL-6, macrophage inflammatory protein (MIP)-1 α , MIP-2, chemokine (C-X-C motif) ligand 1 (KC), TNF α , and monocyte chemoattractant protein (MCP)-1 between hypointense and normally ventilated lung regions in LPS-dosed mice. Thus, this study defines the anatomic, functional, and biochemical characteristics of ventilation defects associated with the administration of LPS in a murine model of acute lung injury.

Keywords: ventilation defect; lipopolysaccharide; lung inflammation; magnetic resonance imaging; cytokines

CLINICAL RELEVANCE

The study defines the anatomic, functional, and biochemical characteristics of ventilation defects associated with exposure to bacterial LPS.

Physiologic and inflammatory responses to acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) were described in humans. However, the fundamental mechanisms that lead to ALI/ARDS have been difficult to elucidate because many key variables cannot be controlled precisely in critically ill patients. In this regard, animal models reproduce many aspects of the human condition, and do so in controlled experimental settings. When selecting an animal model, however, specific features of human ALI/ARDS mimicked by the model may vary across species and between different models (1).

Murine models are increasingly used to study the pathogenesis of ALI/ARDS. Understanding the applicability of murine models to humans requires a detailed functional characterization of changes in lung anatomy and physiology in the mouse. Traditionally, the study of lung function in the mouse includes either direct measures of airway resistance and lung compliance using invasive forced oscillation techniques (2, 3), or proxies for resistance and compliance, using noninvasive, unrestrained barometric plethysmography. These methods offer unique insights into the global aspects of lung function in murine models. However, lung function is often heterogeneous, especially in disease states, and this drives the need to develop functional imaging methods.

Recently, visualizing regional ventilation patterns in humans and animals became possible through hyperpolarized ^3He magnetic resonance imaging (HP ^3He MRI), which uses a gaseous contrast agent to visualize lung air spaces directly (4–6). This technique was further extended to evaluate several lung physiologic parameters, including fractional ventilation, oxygen depletion rates, ventilation–perfusion ratios, and apparent diffusion coefficients (7–9). However, even simple spin density imaging of HP ^3He demonstrated a heterogeneous distribution of ventilation in a variety of diseases in humans, including asthma, chronic obstructive pulmonary disease, and cystic fibrosis (10). Included in these ventilation patterns are regions of decreased signal intensity compared with the surrounding lung parenchyma, commonly referred to as “ventilation defects” (11–13). Although these hypointense regions were previously described in humans and animal models, little is known about their characteristics, distribution, and etiology.

This study aimed to verify that hypointense regions could be observed by ^3He MRI in an LPS-induced model of ALI in mice, and to characterize these regions further by histology and cytokine analysis. The LPS murine model was chosen because its biochemical and histologic attributes are well-characterized

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in the pulmonary literature (1, 14). LPS-induced lung injury can also be induced and imaged in a single day while reproducibly showing physiologic evidence of airway inflammation (15). Mice underwent ^3He MR imaging 4 hours after the instillation of LPS, and regions of hypointense signal were quantified and characterized by type, size, and location within the lung. These investigations identified three types of hypointense patterns: persistent defects, atelectatic defects, and dorsal lucencies. Persistent defects were associated with the administration of LPS, whereas atelectatic defects predominated under conditions of low-volume ventilation and could be reversed with deep inspiration. In contrast, dorsal lucencies were present in nearly all mice, regardless of experimental conditions. To bolster imaging findings with histologic and biochemical data, experiments were performed to determine whether hypointense regions correlated with histologic findings, or exhibited atypical cytokine distributions when evaluated using bead-based multiplex immunoassays.

MATERIALS AND METHODS

Animal Preparation

All live animal experimentation was performed under a protocol approved by the Duke University Institutional Animal Care and Use Committee. C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME) at 8–10 weeks of age were separated into three experimental groups: the first group received LPS, the second group was instilled with saline, and the third group of naive mice received neither LPS nor saline. Specifically, the first group of mice received 50 μl of LPS in sterile saline (0.3–1.0 $\mu\text{g}/\mu\text{l}$ *Escherichia coli* 0111:B4; Sigma-Aldrich, St. Louis, MO) in the supine position via intratracheal instillation through a low dead volume 18-gauge tracheostomy tube inserted below the cricoid cartilage. A range of LPS doses from 15–50 μg was administered to assess the dosage dependence of the number, size, and locations of hypointense lung regions revealed by MRI. The second group received sterile saline in place of LPS, according to the same methods.

All animals were placed on a custom HP gas and MRI-compatible ventilator, which also maintained animal body temperatures at 37°C throughout imaging. Unless otherwise indicated, animals were ventilated in the supine position at either a normal physiologic tidal volume for mice (200 μl) or at a low tidal volume (150 μl), and at a rate of 100 breaths/minute. All mice were anesthetized with an 85 mg/kg intraperitoneal dose of pentobarbital (Nembutal Sodium Solution; Ovation Pharmaceuticals, Inc., Deerfield, IL), and anesthesia was maintained with doses of 20 mg/kg pentobarbital delivered approximately every 45–60 minutes, or when the heart rate increased above 500 beats/minute. A summary of data concerning the animals used in our treatment groups is provided in Table 1.

Magnetic Resonance Imaging

^3He was polarized to approximately 30%, using a prototype commercial polarizer (IGI.9600.He; MITI, Durham, NC). Immediately before imaging, ^3He was dispensed from the polarizer into a 250-ml Tedlar bag (Jensen Inert Products, Coral Springs, FL). HP ^3He MRI was performed using a 64.8 MHz tuned bird cage coil (5.5 cm long, 3.5 cm diameter) 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 Healthcare EXCITE 12 console (GE Healthcare, Waukesha, WI). Mice were imaged using a three-dimensional (3D) radial acquisition sequence with repetition time (TR) = 5 ms, echo time (TE) = 0.3 ms, bandwidth = 31.25 kHz, and field of view = $2 \times 2 \times 3.2 \text{ cm}^3$ at a resolution of $188 \times 188 \times 1,000 \mu\text{m}^3$. We required 11,512 radial views for a full sample of the image over 5.75 minutes at 20 views per breath during the 100-millisecond ventilation-hold incorporated into each breath.

Data Analysis

Data were analyzed using ImageJ software version 1.38x (<http://rsb.info.nih.gov/ij/>; National Institutes of Health, Bethesda, MD). Hypointense regions were characterized according to their area, type,

TABLE 1. NUMBERS OF MICE USED, ACCORDING TO TREATMENT GROUP AND VENTILATION PROTOCOL

Treatment Group	Normal Tidal Volume Protocol	Low Tidal Volume Protocol
LPS (15 μg)	4	
LPS (30 μg)	5	4
LPS (50 μg)	4	
Normal Saline	4	3
Naive	2	

Mice were exposed to LPS or normal saline and ventilated, using either a normal murine tidal volume (200 μl) or a low tidal volume (150 μl) to accentuate areas of atelectasis. The number of animals imaged in each treatment group according to ventilation protocol is shown.

number, and distribution in each lung lobe, and were visually identified as regions of lung showing decreased intensity relative to the surrounding lung parenchyma. Each hypointense region was quantified by its associated area, which was calculated by manually tracing its perimeter. If the hypointense region did not consume an entire lobe, the area was calculated from the individual slices in which it was largest. Hypointense areas that encompassed an entire lobe were estimated from a maximum intensity projection image of the 3D data, using the characteristic lung shape on the projection image. If more than one hypointense region was present in the same lobe of the same animal, their areas were added together.

Area was used rather than volume to avoid partial-volume complications, which were particularly challenging in mice. The hypointense area of a given lobe was normalized by dividing by the total lung area of that lobe, and a corresponding percentage was calculated. An example of this approach to estimating defect area is shown in Figure E1 of the online supplement. In the specific case of animals ventilated under normal tidal volumes, only persistent defects were analyzed for area.

The number of defects was compared between low and normal tidal volumes. The resistance of defects to a total lung capacity (TLC) maneuver served as a basis for their further classification as atelectatic or persistent. TLC breaths involved manually restricting the flow of ventilation from the exhalation valve for three breaths, to expand the lungs fully. This maneuver typically generated a peak inspiration pressure of approximately 25 cm H₂O. At normal tidal volume, the mean number of hypointense regions was compared in mice that received 15, 30, and 50 μg LPS versus mice dosed with saline. ANOVA with the Tukey *post hoc* test was performed using Prism version 4 software (GraphPad Software, Inc., La Jolla, CA). Statistical significance was defined as $P < 0.05$. Unless otherwise noted, data were expressed as mean $\pm$ SEM.

Histology

One naive mouse and two mice instilled with 50 μg LPS underwent ^3He MRI for histologic comparison. Immediately after MRI, the mice were killed by pentobarbital overdose (250 mg/kg, intraperitoneal). A cut was made across the upper abdomen, and the descending aorta and vena cava were sectioned. The endotracheal tube was connected to a solution of 10% phosphate-buffered formalin at a pressure of 25 cm H₂O. After 24 hours, the lungs were excised, processed for conventional histology, and stained with hematoxylin and eosin. Three-micron-thick sections were cut every 200 μm through the entire lung, along a plane similar to the MRI plane. The location of hypointense regions observed via ^3He MRI was identified on the histology slides, and tissues were examined using conventional light microscopy.

Lung Sampling for Cytokine Analysis

Regions of hypointense ^3He signal were identified from the MRI images, and tissues corresponding to these locations were dissected postmortem. Tissues were also collected from regions exhibiting a normal ^3He signal for comparison. All tissues were dissected immediately after animals were killed with pentobarbital. Each dissected sample contained approximately the same amount of lung tissue (1–5 mg). Dissected samples were lysed using the Bio-Plex Cell Lysis Kit (catalogue number 171-304011; Bio-Rad, Hercules CA) according

to the manufacturer's instructions. Lung lysates were diluted in lysis buffer containing 4 μ M PMSF, to achieve a final protein concentration of 50 μ g total protein/sample. Samples were mixed with Bio-Plex beads for cytokine analysis, using a Bio-Plex Suspension Array System powered by Luminex XMAP Technology (Bio-Rad). The cytokines measured included IL-1 β , IL-6, chemokine (C-X-C motif) ligand 1 (KC), monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1 α , MIP-2, and TNF α . Cytokine concentrations were normalized to the mass of the dissected lung tissue.

RESULTS

Three Distinct Types of Hypointense Regions

HP 3 He MRI allowed for the direct visualization of three distinct types of hypointense regions in mice. "Persistent defects" (Type 1) were evident primarily in mice ventilated under normal tidal volume. These defects tended to have a more ventral location, as opposed to "dorsal lucencies" (described below), and were of relatively small size ($1.4\% \pm 0.3\%$ of the total lung area). An example of this type of defect is shown in Figure 1. Persistent defects were so named because they were the only type of defect that persisted after TLC breath maneuvers. With the exception of one persistent defect noted in a saline-dosed mouse, all other persistent defects occurred in LPS-dosed mice.

Another type of hypointense region, evident primarily in mice ventilated under low tidal volume, was an "atelectatic defect" (Type 2). These defects were found in both LPS-dosed and saline-dosed mice. They averaged more than twice the size of the other types of defects, with a mean defect size of $4.7\% \pm 1.9\%$ of the total lung area. In two of four low tidal volume-

ventilated, LPS-dosed mice, these defects were large enough to encompass an entire lobe of the lung, specifically the left lung and the cranial lobe of the right lung. Atelectatic defects were so named because TLC breath maneuvers partly or completely resolved these ventilation defects. Examples of atelectatic defects found in LPS-dosed and saline-dosed mice are shown in Figures 2 and 3, respectively. Both of these examples illustrate the disappearance of ventilation defects after TLC breaths. Figure 2 is especially notable, because it simultaneously displays both persistent defects (which failed to resolve) and an atelectatic defect (which completely resolved after a TLC maneuver).

A third type of hypointense region was found in nearly all animals studied, regardless of treatment. This type, referred to as a "dorsal lucency" (Type 3), was so named because these regions were only evident in the most dorsal aspects of the lung. The term "lucency" was specifically used instead of "defect" because these regions were found in both naive and saline-treated control animals, implying they may normally be present. Examples of dorsal lucencies observed in the various treatment groups are depicted in Figure 1. The signal intensity of dorsal lucencies, which reflects the concentration of 3 He gas present and hence the degree of ventilation, was higher than in other types of defects, with signal intensities approximately half those seen in the a normal lung. Dorsal lucencies also tended to have a midline location. They were of intermediate size ($2.0\% \pm 0.3\%$ of total lung area), and were larger than persistent defects and smaller than atelectatic defects.

A detailed summary of the characteristics of all three types of hypointense regions identified is presented in Table 2.

LPS Dose and Ventilation Defect Relationships

To assess dose-response relationships, we plotted the number of ventilation defects per mouse as a function of LPS dose. The two types of defects induced by the administration of LPS, persistent and atelectatic, were included in this analysis. As shown in Figure 4, a significant trend ($P < 0.05$) was evident toward increasing numbers of defects with increasing dose of LPS. Although our sample size was small, a statistically significant difference in defect number was evident between animals

Figure 1. Ventilation defects in representative groups of mice. Each row contains images from a single mouse. Animals shown were imaged after treatment with LPS, saline, or no treatment (naive). *Left column:* Slices in which persistent (Type 1) defects (circled) are evident. In the 30- μ g LPS-dosed mouse, defects are evident in the medial lobe and left lung. A similar defect is evident in a normal saline-treated mouse. *Center column:* The next ventral slice in the image set for the same mice. In this slice, the defects are not apparent, demonstrating the three-dimensional nature of the defects. *Right column:* Dorsal lucencies (Type 3) (boxed) in the same mice.

Figure 2. Persistent and atelectatic defects observed in a single mouse instilled with a high dose of LPS (30 μ g) under different tidal volumes and after a total lung capacity (TLC) breath. Atelectatic defects (circles) are evident at low and normal tidal volumes, and decrease after TLC maneuver. Persistent defects (arrows) are apparent in the medial lobe. They persist through changes in tidal volume and the TLC maneuver.

that received high doses of LPS (30–50 μg) and saline-treated controls. No statistically significant difference in defect number was found with the low dose of LPS (15 μg). In mice dosed with 50 μg LPS, an average of 3.5 ± 0.6 persistent and atelectatic defects per mouse was evident. Only one persistent or atelectatic defect was noted among all normally ventilated saline-treated control mice, which resulted in an average of 0.25 ± 0.25 defects per mouse.

Evaluation of the defect areas showed differences among treatment groups. The largest defects were lobar atelectatic defects, found in LPS-dosed mice ventilated at low tidal volumes. Defects in the left lung and cranial lobe of the right lung encompassed an average of $13.6\% \pm 3.7\%$ and $5.3\% \pm 1.8\%$ of total lung area, respectively. No lobar defects were found in saline-treated mice. Also, no clear relationship was evident between LPS concentration and mean area of persistent defects.

The locations of ventilation defects in the 30- μg LPS-treated and saline-treated groups are shown in Figure 5. A similar distribution of ventilation defects was found for the 15- μg and 50- μg groups. In LPS-dosed mice at normal tidal volume, persistent defects showed a uniform distribution throughout all lobes of the lung, although some predilection was evident for the most ventral-superior portions and peripheral lung regions. Saline-treated mice ventilated at normal tidal volume had only a single persistent defect. In LPS-dosed mice ventilated at low tidal volumes, atelectatic defects were observed mainly in the superior portions of the lung and at increased number in the left lung compared with similar type defects in saline-treated mice, which displayed more defects in the accessory lobe and medial lobe. Dorsal lucencies primarily had a midline location in dorsal slices.

Histology

In one animal instilled with 50 μg LPS, HP ^3He MRI revealed multiple ventilation defects in the left and medial lobes. The upper left lobe was unventilated in all MRI images, yet when examined histologically, this region was indistinguishable from other parts of the lung. In all three mice examined using both ^3He MRI and histology, hypointense regions did not show corresponding abnormalities on histology. One exception occurred: an atelectatic defect, reduced by TLC maneuvers, correlated with an area of atelectasis in the medial lobe according to histologic examination (Figure 6). Throughout the lung, polymorphonuclear leukocytes were homogeneously distributed and were present within vessels, with evidence of diapedesis. Polymorphonuclear leukocytes also infiltrated the alveolar walls.

Proinflammatory Cytokine Concentrations in Areas of Ventilation Defect

We hypothesized that persistent ventilation defects may be attributable, at least in part, to increased concentrations of inflammatory mediators in those lung areas. To this end, persistent

Figure 3. Atelectatic defects are evident in a saline-treated mouse ventilated at a low tidal volume, before the TLC breath (Row 1). Atelectatic defects are circled. While continuing at low tidal volume ventilation but after a TLC breath (Row 2), each of the previously identified defects resolved. All images are from the same mouse.

defects in normally ventilated mice were dissected along with adjacent normally ventilated areas of lung. Samples of lung tissue from ventilation defect areas and “unaffected” areas were assayed for concentrations of proinflammatory cytokines, known to be upregulated in mice after exposure to LPS (16). The cytokines with the highest measured concentrations, normalized to lung tissue mass, were IL-1 β and MIP-2 (Figure 7), whereas the least abundant cytokines were IL-6 and TNF α . For each cytokine, we compared concentrations in LPS-dosed mice (with separate analyses for tissues from ventilation defect areas and adjacent “unaffected” areas) with those in saline-treated mice. LPS treatment resulted in significantly increased cytokine concentrations in both ventilation defect areas and “unaffected” areas of the lung, compared with saline treatment ($P < 0.001$). However, no significant differences were evident in cytokine concentrations between tissues associated with regions of hypointense signal versus normal ^3He signal. These results indicate that the ventilation defects we observed were likely not the result of an enhanced cytokine-mediated inflammatory process.

DISCUSSION

This study confirmed that LPS-induced ALI in mice is associated with the formation of ventilation defects that can be detected by HP ^3He MRI. Because the gaseous contrast agent in HP ^3He MRI directly visualizes airspaces, hypointense regions represent areas of the lungs that are not as well ventilated as surrounding regions. Thus persistent defects, which were found primarily in LPS-dosed mice, imply that prolonged periods of hypoventilation occurred in specific regions of the lung corresponding to the

TABLE 2. CHARACTERISTICS OF VENTILATION DEFECT TYPES

Type of Hypointense Region	Ventilation Pattern That Elicits	Resistant to TLC Breath	Size of Defect	Location	Slices	Signal Intensity	Found in Naive Mice?
Type 1: persistent defect	Normal and low tidal volume	Yes	$1.4\% \pm 0.3\%$	All lobes	Ventral	Background	No
Type 2: atelectatic defect	Low tidal volume	No	$4.7\% \pm 1.9\%$	All lobes; greatest in left lung and cranial lobe	All	Background	No
Type 3: dorsal lucency	Normal and low tidal volume	Yes	$2.0\% \pm 0.3\%$	Dorsal medial lobe and left lung; midline	Dorsal	Higher	Yes

Persistent defects are found predominantly with normal tidal volume (200 μL) ventilation throughout the lung. Atelectatic defects are larger and are seen primarily during low tidal volume (150 μL) ventilation. Dorsal lucencies are evident in the most dorsal slices of all animals regardless of treatment, generally at a midline location. Defect size was determined as the percentage of total lung area comprised of ventilation defect area, as described in MATERIALS AND METHODS. Values under “Size of Defect” are means $\pm$ SEM.

Figure 4. Mean number of ventilation defects per mouse in each treatment group. A statistically significant difference was evident in mean number of defects between saline-treated mice and mice that received high doses of LPS (30 µg and 50 µg) ($P < 0.05$). Error bars indicate the SEM. Difference in number of defects observed between saline treatment and the low LPS dose (15 µg) was not statistically significant.

ventilation defects. Ventilation defects were recently described in a murine model of LPS-induced acute lung injury (17).

What are the underlying mechanisms responsible for the development of persistent ventilation defects? Because none of the persistent defects involved an entire lobe of the lung, each lobe contained both normally ventilated and less well ventilated areas, confirming that regional ventilation is heterogeneous after the administration of LPS. None of the persistent defects appeared as large, wedge-shaped regions of hypoventilation, which would be characteristic of the obstruction or broncho-spasm of a proximal, major bronchus. Likewise, these defects are not likely a result of persistent large-caliber airway closure, because no such decreased airway diameter was evident in our image analysis or histology. Furthermore, because persistent defects remained after animals received total lung capacity

breaths, these defects were also unlikely to be the result of persistent atelectatic collapse of the airspaces. The resistance of persistent defects to TLC breaths raises the possibility that the decreased airway diameter of smaller bronchioles, alveolar interstitial edema, or pulmonary edema may be responsible for regionally reduced ventilation in these animals.

Unlike persistent defects, atelectatic defects were evident in saline-treated mice under low tidal volume ventilation. However, in LPS-dosed mice at low tidal volume ventilation, the atelectatic defects were accentuated in both size and number. Some degree of atelectasis is likely attributable to low-volume ventilation, which makes a smaller volume of gas available to fill alveoli. The accentuation of atelectasis in LPS-dosed animals

Figure 5. Map shows locations of ventilation defects observed in a mouse instilled with 30 µg LPS. Persistent defects are represented by large stars, and dorsal lucencies are represented by solid circles. The two upper rows show the locations of defects under normal tidal volume for LPS-dosed mice (upper row) and saline-treated mice (middle row). A large number of persistent defects are evident in LPS-dosed mice. By contrast, only one persistent defect is apparent in a saline-treated mouse. The lowest row shows dorsal lucencies, which tended to be in midline locations. All treatment groups are shown together, because the administration of LPS or saline did not affect the number of dorsal lucencies. One animal was imaged in the prone position and also showed dorsal lucencies, indicating that dorsal lucencies were not restricted to dependent regions of the lung.

Figure 6. Correlation of lung histology with MRI. A single mouse was imaged immediately after a TLC breath, and 4 hours after instillation of 50 µg LPS. (a) The atelectatic defect (circle) was partly reversed by a TLC breath, in contrast to the persistent defect (arrow). (b) Hematoxylin and eosin staining of fixed lung tissue from the same animal. The lobes include cranial (Cr), medial (M), caudal (Cl), and left (L). Atelectasis is apparent in the medial lobe (arrow), which may correspond to the defect visible using MRI. However, the other defects shown by MRI are not associated with distinguishable features on histologic examination. (c) The maximum intensity projection shows that no part of the upper left lobe is ventilated. (d) Histologic detail of the upper left lobe (boxed in panel b), unventilated by MRI. The histology is similar to that in other areas of the lung.

Figure 7. Analysis of inflammatory markers in dissected regions of 30- μg LPS-dosed versus saline-treated lungs. Cytokine analysis of samples obtained via image-guided sampling of LPS-induced ventilation defect areas (*LPS-VD*), uninvolved areas of lung from the same mouse (*LPS-Non VD*), and saline-treated controls showed a statistically significant increase in cytokine concentrations in lung tissue from LPS-dosed versus saline-treated mice ($P < 0.05$). However, no statistical difference was evident for any of the cytokines examined between ventilation defect areas of the lung and adjacent uninvolved lung tissue.

supports the hypothesis that the decreased ventilation of respiratory units may be attributable to localized inflammation, induced by exposure to LPS. Indeed, atelectasis was shown to occur histologically, as was the case in other studies of LPS-dosed animals (18). However, the results of our cytokine analyses suggest that increased inflammation was not present in ventilation defect areas of the lung. Consequently, regional differences in the inflammatory process may not fully explain the worsening of ventilation in LPS-dosed animals. Further studies will be necessary to determine if a localized reduction in surfactant concentrations or other mechanisms contribute to the increased size and number of atelectatic defects in mice exposed to a high dose of LPS. These studies will use the 30- μg LPS dose, given the nonsignificant differences we observed between the 30- μg and 50- μg LPS groups.

TLC breaths and larger tidal volumes may cause barotrauma in both LPS-dosed and saline-treated mice. Although especially

prominent in LPS-dosed mice, saline-treated mice also demonstrated smaller diameters of right and left mainstem bronchi before TLC breaths than afterward (Figures 2 and 3). Because the increase in airway diameter after the TLC maneuver was especially dramatic in LPS-dosed mice, LPS-induced, airway-centric inflammation may further injure airways and predispose animals to worsened barotrauma. In this regard, a previous study using animals showed that LPS-induced acute lung injury can be exacerbated by barotrauma (19).

The analysis of cytokines typically released after exposure to LPS did not indicate that increased inflammation occurred in ventilation defect areas of the lung relative to normally ventilated areas. Because the levels of proinflammatory cytokines were comparably increased in areas of ventilation defects and in surrounding normally ventilated lung parenchyma, we can surmise that LPS and the subsequent inflammatory response were distributed homogeneously throughout the lung.

However, because a margin of several millimeters was obtained around each defect to ensure it was properly sampled, any localized increase in cytokine concentrations within ventilation defects may have been diluted by the lung tissue in the margin.

Future research may apply several human imaging modalities to murine models of ALI. For example, computed tomography (CT) has contributed considerable insights into clinical ALI, and small-animal CT is becoming more widely available (20). CT studies may enable the addition of lung tissue density measurements to the ventilation information provided by MRI. The ^3He used in our studies is in short supply (21). In this regard, obtaining ventilation information from Xe-enhanced CT may be possible (22). Indeed, the quality of hyperpolarized ^{129}Xe MRI is improving dramatically, and can image the transfer of gas into the pulmonary blood (23, 24), which could provide additional insights in models of ALI.

In conclusion, this study describes the identification and characterization of three types of hypointense regions observed via ^3He MRI in mice after the administration of LPS. Histologic examination showed no unique tissue characteristics of the defects, except in the single case of an atelectatic defect visible using both MRI and histology. Similarly, cytokine analysis showed comparable inflammation in ventilation defect regions and adjacent unaffected regions of lung tissue. Our study demonstrates that HP ^3He MRI may be a valuable tool in the further characterization of regional ventilation patterns in murine models of ALI, and may help elucidate the etiology of ventilation defects that are pervasive in pulmonary diseases in humans.

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