

Noninvasive Mapping of Regional Response to Segmental Allergen Challenge Using Magnetic Resonance Imaging and [F-18]Fluorodeoxyglucose Positron Emission Tomography

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Magnetic resonance (MR) and positron emission tomography (PET) imaging techniques were coregistered to demonstrate regional ventilation and inflammation in the lung for in vivo, noninvasive evaluation of regional lung function associated with allergic inflammation. Four Brown Norway rats were imaged pre- and post segmental allergen challenge using respiratory-gated He-3 magnetic resonance imaging (MRI) to visualize ventilation, T₁-weighted proton MRI to depict inflammatory infiltrate, and [F-18]fluorodeoxyglucose-PET to detect regional glucose metabolism by inflammatory cells. Segmental allergen challenges were delivered and the pre- and postchallenge lung as well as the contralateral lung were compared. Coregistration of the imaging results demonstrated that regions of ventilation defects, inflammatory infiltrate, and increased glucose metabolism correlated well with the site of allergen challenge delivery and inflammatory cell recruitment, as confirmed by histology. This method demonstrates that fusion of functional and anatomic PET and MRI image data may be useful to elucidate the functional correlates of inflammatory processes in the lungs. Magn Reson Med 53:1243–1250, 2005. © 2005 Wiley-Liss, Inc.

Key words: MRI; PET; hyperpolarized gas; lung; inflammation

New methods for both functional and high-resolution anatomic imaging of small animals have recently emerged (1–3). Fusion of multiple imaging modalities provides the advantage of combining anatomic and physiologic measures for regional assessment and treatment of lung and

other diseases in humans and small animal models. Asthma research in particular can benefit from fusion of components measuring regional lung ventilation, inflammation, and metabolism to be able to understand the role of inflammatory processes in the development of airway obstruction.

The airway obstruction in human asthma appears to be heterogeneously distributed in the lungs, as demonstrated physiologically (4) and with imaging (5,6). Invasive bronchoscopy and biopsy (7) have been the primary methods to determine the regional nature of physiologic and molecular changes associated with asthma. However, sampling is necessarily limited in the number of biopsies that can be obtained and regions of the airways that are accessible. Allergen models in small animals have been developed for study of the inflammatory response and its effect on global measures of airway physiology (8). The complexity of lung diseases such as asthma makes noninvasive regional studies of lung physiology in animal models particularly desirable because detailed histology can be obtained for correlations with observed functional changes.

Imaging methods including magnetic resonance imaging (MRI) and positron emission tomography (PET) are under development for noninvasive assessment of lung function. In conventional proton MRI of the lung, the signal is much lower than that of surrounding tissues due to low tissue density in the lungs. As a result, fluid buildup from disease and inflammation results in high signal relative to background (9,10). The location and dose of allergen delivered have been shown to correlate with increased signal, associated with fluid accumulation (10), relative to background.

Under MRI, hyperpolarized He-3 acts as a contrast agent to visualize airways and distal airspaces in the lungs (11,12). He-3 MRI has been demonstrated in humans for application in respiratory lung disease (6,12–14) and in small animals using respiratory-gating and specialized ventilation systems (3,15,16).

[F-18]Fluorodeoxyglucose PET ([F-18]FDG-PET) has been used to image glucose metabolism associated with inflammatory diseases of the lungs (17,18). Metabolic processes result in trapping of the radioactive tracer F-18 within the cell and can be correlated to the glucose metabolism rate (19,20). Increased metabolic activity in the

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lungs has been correlated with increased levels of activated neutrophils, likely stemming from a respiratory burst (17).

We have developed methods for acquiring and fusing functional images of small animal airways using a multimodality approach consisting of the three methods described above including T_1 -weighted proton MRI, He-3 MRI, and [F-18]FDG-PET for the study of inflammation, ventilation, and glucose metabolism, respectively. This approach allows visualization of the relationship among processes associated with allergic inflammation in asthma. To our knowledge, this is the first fusion imaging of the key physical components of allergic inflammation in vivo, with histologic confirmation of the imaging results. The methods have been developed in rats and are capable of providing in vivo imaging for longitudinal studies in the same animal.

METHODS

Five BN rats were imaged; one received a methacholine challenge and the other four animals received segmental allergen challenges. Segmental allergen challenges were delivered to allow comparison between contralateral lungs postchallenge as well as pre- to postchallenge in the same lung. Following imaging, animals were sacrificed and histology was performed to confirm the location of insufflation, inflammation response, and cell makeup.

Animal Procedures

All animal procedures conformed with the Guide for the Care and Use of Laboratory Animals and were approved by the University of Wisconsin Animal Care and Use Committee. Male inbred BN/SsNHsd (Brown Norway) rats were purchased (Harlan, Indianapolis, IN, USA) and housed in an accredited animal facility. Rats weighed 298–355 g at the time of the experiments. Rats were anesthetized for nonterminal studies using pentobarbital (15 mg i.p., followed by 5 mg supplemental doses as needed; Abbott Laboratories, North Chicago, IL, USA) and for terminal studies using urethane (450 mg i.p.; Sigma, St. Louis, MO, USA). An orotracheal tube was placed atraumatically to facilitate segmental instillations and mechanical ventilation (21). For allergen challenge studies, rats were sensitized to ragweed pollen extract (Hollister-Stier, Spokane, WA, USA) with a single subcutaneous injection of 1 mg extract protein mixed with adjuvant (Imject Alum; Pierce Biotechnology, Rockford, IL, USA) (22). After prechallenge imaging, segmental allergen challenges were achieved by advancing a catheter into an airway branch (21) and gently instilling 0.1 mL of sterile India ink-labeled buffer containing 1 mg of pollen extract. Postchallenge imaging was conducted 20–24 h after allergen challenge. Following postchallenge imaging, animals were euthanized, lungs were fixed with formalin, and paraffin sections were stained (Giemsa) and examined. A gross estimate of the area of allergen instillation was marked by the residual India ink.

MR Imaging

MR imaging was performed on a standard 1.5-T system with broadband capability (Signa LX, GE Medical Sys-

tems, Milwaukee, WI, USA). A dual-coil system was developed to allow imaging of both proton and He-3 frequencies, 63.9 and 48.6 MHz, respectively, without repositioning the subject. The coil is the size of a standard head coil to allow structural support of the animal cradles. The sensitive homogeneous region is approximately $8 \times 8 \times 5$ cm, making it well suited to small animal studies within a whole-body scanner. The attainable resolution is ~ 0.5 mm³ with an SNR of ~ 10 using a 160-point readout matrix at this FOV.

T_1 -Weighted MRI

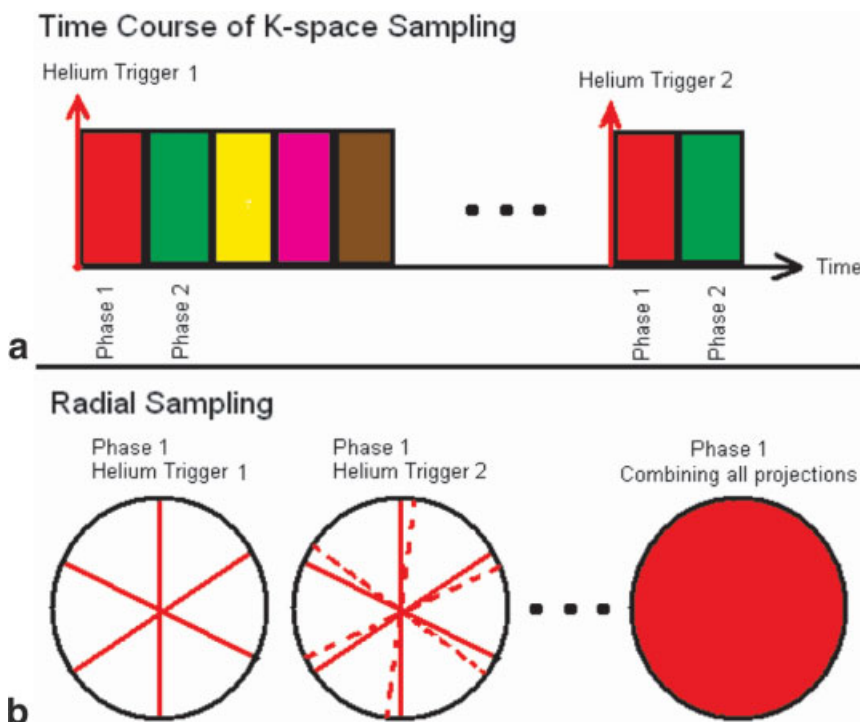
Inflammatory infiltrate in the lungs was imaged using a T_1 -weighted proton MR gradient echo Cartesian sequence designed to image hydrogen protons in water molecules. Imaging parameters included TR/TE = 100 ms/1.9 ms, flip angle of 21° , acquired and reconstructed resolution of $256 \times 256 \times 64$, 12 cm^2 FOV, and 0.5-mm slice thickness. This provided a nearly isotropic 0.5-mm³ voxel size. An ungated acquisition was performed while the animal was under ventilator assisted breathing. Since a tidal volume of 15% of total lung capacity was used, respiratory motion of the diaphragm and associated artifacts were small.

He-3 MRI

Hyperpolarized He-3 ventilation MRI was performed using a respiratory-gated 2D projection acquisition (PR) sequence with 10 images (phases) per respiratory cycle (Fig. 1). Hyperpolarized He-3 gas levels of 30–40% polarization were produced through spin exchange (23) using 1 g of optical pumped rubidium vapor held at 160°C and 8 atm. (IGI.9600, He, Amersham Health, Princeton, NJ, USA). A ventilation system was designed to maintain oxygenation of the animal by alternating He-3 breaths with air breaths while respiratory-gated MRI was performed. The ventilation system (Fig. 2) was composed of a computer running LabView software (National Instruments, Austin, TX, USA) for system control, a small animal ventilator with pneumatic valves for MR compatibility (CWE, Inc., Ardmore, PA, USA), and a PDX13–83-62P stepper motor (Parker, Rohnert Park, CA, USA) to drive a syringe system delivering breaths of He-3 to the lungs of the rat. Sync pulses from the computer controller triggered both the MRI scanner to begin data acquisition and the ventilator to deliver one helium breath for every three air breaths. A graph of the integrated signal of the lung volume over time (Fig. 3) shows the rapid change in signal due to respiration and RF saturation of He-3.

A PR acquisition sequence was chosen due to greater robustness against artifacts caused by changes in signal intensity during data acquisition than that provided by Cartesian acquisition techniques (16). Images were acquired using $\sim 4.5^\circ$ flip angle, ± 6.25 KHz bandwidth, TR/TE = 5.5 ms/1.8 ms, 8×8 cm FOV, and 32-mm slice thickness to fully cover the lung volume. Eighty readout points were acquired during each projection with synthesis to 128 points using homodyne reconstruction (24,25). Unique sets of projections from the same respiratory phase, acquired over multiple He-3 breaths, were combined to produce cine images with an effective time reso-

FIG. 1. (a) Respiratory-gated He-3 MRI data acquisition scheme. Each helium breath is divided in time into separate phases over which subsets of radial projections are acquired. (b) Subsets acquired during multiple breaths are recombined to produce a time-resolved image for each phase.



lution of 75 ms, depicting the ventilation cycle. Cine images were generated with a total of 204 combined projections per ventilation phase with only 12 projections acquired per phase of a given breath. Sampling density correction was performed by applying a Ram-Lak weighting filter to the projection data prior to regridding to Cartesian k -space (26). Each cine time frame was sinc-interpolated to a 256×256 matrix resulting in an in-plane reconstructed voxel size of $0.3 \times 0.3 \text{ mm}^2$.

Methacholine HCl ($30 \mu\text{mol}$; Sigma) was instilled into a lung segment in order to test the effects of an acute bronchoconstrictor stimulus on He-3 ventilation imaging.

image glucose metabolism. A transmission scan was initially performed for two of the animals to correct for inhomogeneities in tissue attenuation. Image acquisition was then initiated prior to i.v. injections of 75–110 MBq/kg of radioactive [F-18]FDG to observe tracer wash-in. Data were collected for 90 min to include tracer uptake and trapping. Image data were reconstructed using ordered subset estimation maximization, an iterative reconstruction algorithm widely used in PET imaging for reconstruction of images from data with low SNR (27). The images were generated at a $128 \times 128 \times 63$ matrix size, $0.5 \times 0.5 \times 1.2 \text{ mm}^3$ voxel size, and a resolution of $\sim 2 \text{ mm}$ FWHM.

[F-18]FDG-PET Imaging

FDG microPET image acquisition (UW MicroPET P4, Concorde Microsystems, Knoxville, TN, USA) was used to

Image Registration

The image coregistration program was written and tested by the PET Imaging Center of UW Madison. A manual

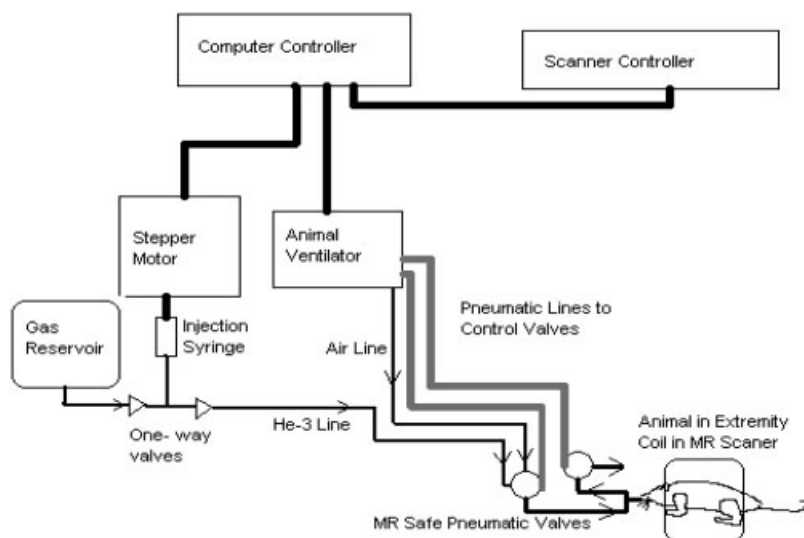


FIG. 2. Diagram of He-3 MRI ventilation system design.

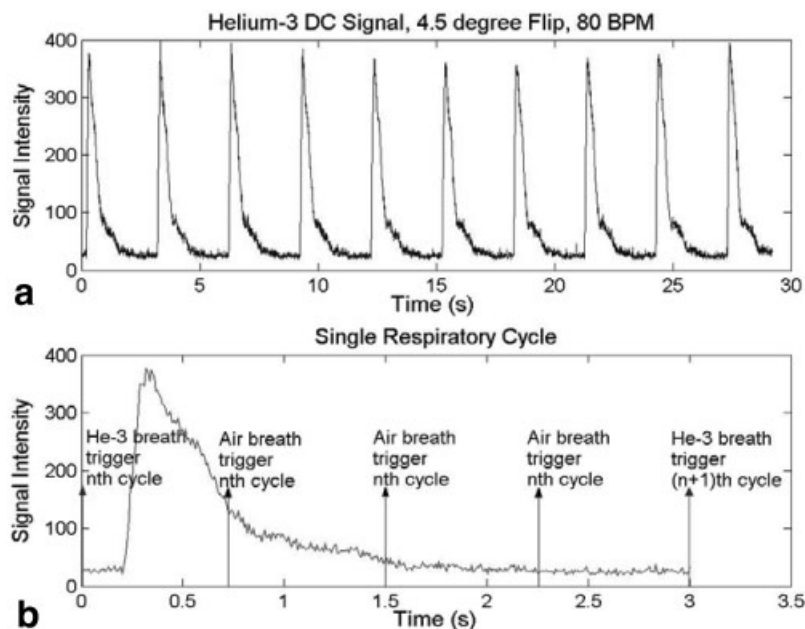


FIG. 3. He-3 integrated signal intensity (a) over multiple breaths and (b) over a single breath with the location of sync triggers to the ventilation system shown by arrows.

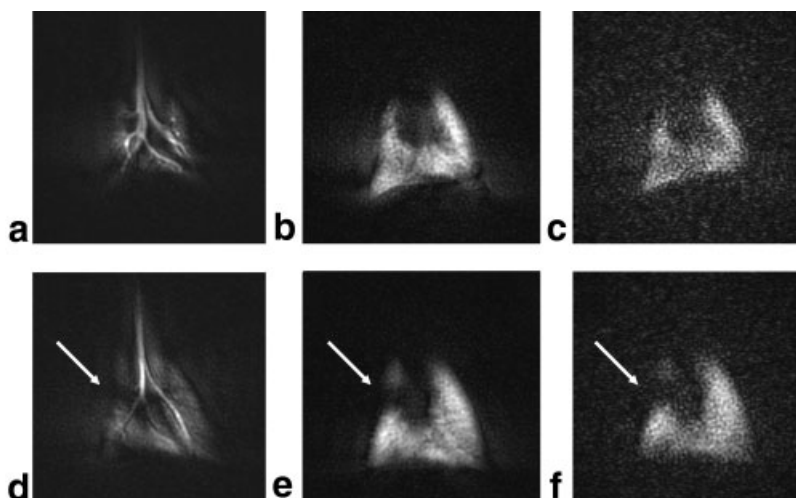
interactive method of volume alignment using simultaneous 2D and 3D visualization of the images was used for image registration. Input images were scaled first to obtain new volume images with cubic voxels of the same size. Next, the program displays four windows with 3D and 2D fused images and accepts operator commands to rotate or translate a selected object based on specific anatomic features common to both images. The ventilation phase of peak parenchymal He-3 enhancement was chosen for registration to the T_1 -weighted proton data. Because of the limited respiratory motion under tidal breathing, misregistration at the diaphragm was negligible (1–2 pixels or [1eq]1 mm) in comparison to the size of the defects measured in this study. A separate program reorients the original image volume to match the reference image and perform the final reslicing of the operand image based on the transformation parameters (three angles and three translations).

The program is written in ANSI C language on a Sun Ultra 60 workstation under Solaris 2.8 operating system using 24-bit color for display in Open Windows. The XView library is used for the user interface and the XIL library for image display. Volume rendering is done based on Volpack library developed in 1994 by Philippe Lacroute and Marc Levoy at the Computer Systems Laboratory at Stanford University (28). The library was chosen for its fast and reliable performance and availability of the source code residing on a public domain (29).

Image Analysis

The He-3 defect volume was estimated based on the in-plane defect size and the lung thickness. The volume of T_1 -weighted proton MR defect was determined by summing affected regions selected manually in each 2D slice. Signal-to-noise measurements were made by dividing the

FIG. 4. Pre-methacholine challenge images showing (a) initial inspiration and filling of the major airways, (b) full inhalation and filling of the distal airspaces, and (c) residual signal in the lung following exhalation. Post-methacholine challenge images showing (d and e) the corresponding phases to the prechallenge case above. A ventilation defect is evident in the right lung following methacholine challenge (arrows).



mean signal in the region of interest by the SD within the background in air. Patlak analysis (20) and compartmental analysis are considered gold standard methods to measure glucose metabolism in [F-18]FDG-PET studies. Patlak analysis to determine the influx constant (K_i) was performed on the region of activation pre- and postchallenge using a custom software package written in IDL. Reconstructed image time frames for Patlak analysis were initially 5×2 min followed by 8×10 min time intervals. The arterial input function was measured by placing a region of interest over the left atrium of the heart (30).

RESULTS

He-3 Ventilation Imaging

To validate the described techniques, time-resolved ventilation images were acquired before and after a methacholine challenge to a segment of the right lung. The images show a defect present in the right lung in the region of the segmental challenge (Fig. 4, arrows). Systemic changes including generalized narrowing of the central airways (Fig. 4a versus d, and air trapping (Fig. 4c versus f are also visible after methacholine challenge. These results match those observed by Chen and Johnson for systemic i.v. delivery of methacholine (3). Typical results of the He-3 ventilation imaging for the allergen challenge experiments are shown in Fig. 5a and b for one of the animals. Images have been selected to present the lungs at end-inspiratory volume during tidal ventilation. Comparison of the pre- and postchallenge He-3 MR images shows a decrease or absence of signal in the left lung following allergen challenge indicative of a ventilation defect located in the mid-portion of the left lung. There are no visible changes in the right lung and no apparent postchallenge ventilation defects on that side.

Similar MRI results to those shown in Fig. 5 were obtained in the three additional animals although attenuation corrected PET data for Patlak analysis were not acquired for two of these animals. Quantitative results for the two animals undergoing both MRI and Patlak analysis of PET are summarized in Table 1.

T_1 -Weighted Proton Imaging

Increased T_1 -weighted proton signal is evident after allergen challenge in the mid and lower left lobe (Fig. 5d, arrow) compared with prechallenge images (Fig. 5c), indicating inflammatory infiltrate in this location due to edema formation and inflammatory cell influx. Furthermore, no changes are visible in the right contralateral lung in the postchallenge images.

[F-18]FDG-PET Metabolic Imaging

Imaging performed prior to allergen challenge showed no significant FDG uptake in the lungs indicating little FDG metabolism in these organs (Fig. 5e); however, significant FDG uptake was detected in the same region of the lower left lung during postchallenge imaging (Fig. 5f, arrow). In the post-allergen challenge FDG images, the time activity curves showed FDG uptake continued to increase with time in the lower left lung postchallenge (Fig. 6). This

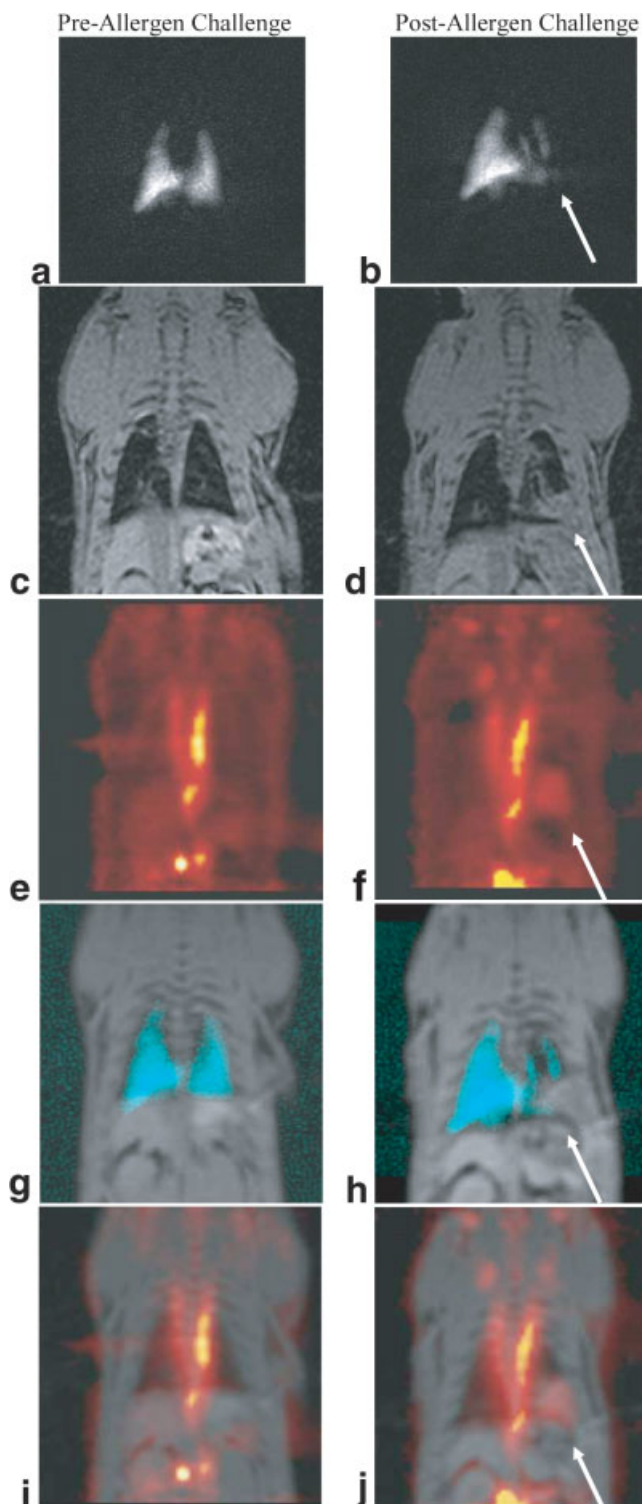


FIG. 5. Corresponding pre- and post-allergen challenge images. He-3 MR ventilation pre- (a) and post- (b) challenge showing a defect in the left lung, T_1 -weighted proton MR pre- (c) and post- (d) showing increased signal in the mid and lower left lung after challenge, [F-18]FDG-PET pre- (e) and post- (f) showing increased F-18 signal in the postchallenge left lung. Coredgistered He-3 ventilation and T_1 -weighted proton images pre- (g) and post- (h) showing correspondence of ventilation defect and increased proton signal in postchallenge. Coredgistered [F-18]FDG and T_1 -weighted proton image pre- (i) and post- (j) demonstrating increased glucose metabolism at the site of increased proton signal in the left lung postchallenge lung.

Table 1

Quantitative Measures of Ventilation, Inflammation, and Metabolism Pre- and Post-allergen Challenge

Animal	Ventilation (He-3 MRI), volume of defect (mm ³)	Inflammatory infiltrate (T_1 -weighted MRI)				Patlak influx constant [F-18]FDG-PET		
		Volume of defect (mm ³)	Mean SNR pre	Mean SNR post	Change	Pre (min ⁻¹)	Post (min ⁻¹)	Change
MR05	1400	620	24.2	103.6	79.4	0.00246	0.00902	0.00656
MR06	2300	1300	18.0	147.2	129.2	-0.00046	0.00461	0.00507

suggests active trapping of FDG inside the metabolically activated inflammatory cells, which is further confirmed by large allergen-induced changes in FDG influx constant measured by Patlak analysis (described below).

Image Fusion

The fusion images of anatomic T_1 -weighted proton MR with He-3 MR ventilation and [F-18]FDG-PET metabolism are also shown in Fig. 5. The registered ventilation/ T_1 -weighted images show correspondence of increased proton signal in regions of decreased He-3 signal (blue) in the middle and lower left lung after allergen challenge (Fig. 5h, arrow). One area of ventilation defect is devoid of proton signal in the apical left lung (Fig. 5h). No abnormalities are visible in the prechallenge case (Fig. 5g). The fused data for the coregistered glucose metabolism and T_1 -weighted images (Figs. 5i and j) show a match of enhanced glucose metabolism to the site of inflammatory infiltrate in the postchallenge image (Fig. 5j); again no such irregularities exist in the prechallenge images (Fig. 5i).

Quantification of Antigen-Induced Changes

Measures of the allergen-associated changes in He-3 MR ventilation images, T_1 -weighted proton MR images, and [F-18]FDG-PET images are summarized for two rats in Table 1. Changes in T_1 -weighted MR signal and the Patlak influx constants (K_i) for the [F-18]FDG-PET images of lungs were significantly increased postchallenge relative to prechallenge (Table 1), indicating increased inflammatory infiltrate and glucose uptake in the basal left lung

following allergen challenge. In both rats, the allergen-induced changes at the site of challenge are several times that of the prechallenge measures. Furthermore, allergen-induced changes are of similar magnitude, as expected for the equivalent allergen dose delivered to each animal.

Validation of Imaging Results

The site of insufflation was found to be the base of the left lung as determined by the India ink included in the allergen challenge (Fig. 7a). This site coincides with the location of the ventilation defect, region of inflammatory infiltrate, and region of increased metabolism detected with MRI and PET. The location of cellular infiltrate was confirmed to match the extent of the inflammatory infiltrate by histology. Giemsa-stained lung sections taken from the basal region of the left lung show large concentrations of neutrophil, eosinophil, and macrophage inflammatory cells (Fig. 7b). The right lung section shows no such inflammation (Fig. 7c). Furthermore, the apical regions outside the affected region of the left lung show no abnormal inflammatory cell infiltrates in the airway walls or alveoli (Fig. 7d). The measured volumes of the ventilation defect and affected region of inflammatory infiltrate corroborate

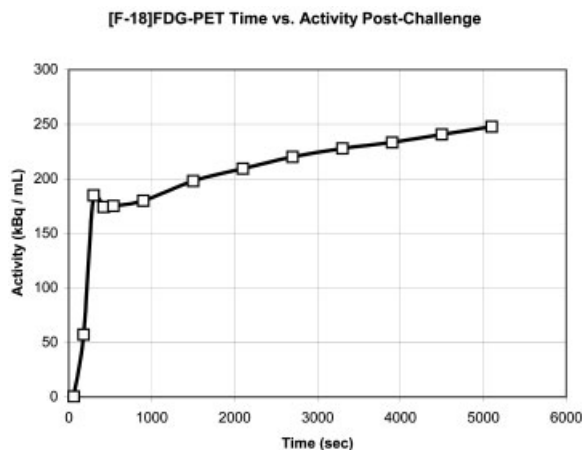


FIG. 6. [F-18]FDG-PET activity over time in the region of increased metabolic activity in Fig. 5f postchallenge.

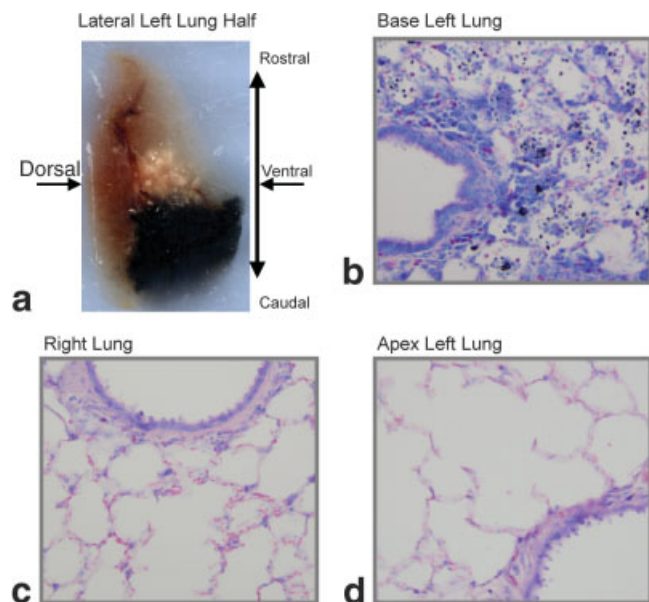


FIG. 7. (a) Paraffin block of the left lung showing site of allergen insufflation in the basal left lung marked by black India ink. Magnified Giemsa-stained tissue sections showing (b) left lung base with eosinophilic inflammatory infiltrate and (c) right lung and (d) left lung apex with no infiltrate present.

the qualitative observations in the fusion images (Fig. 5h) and matched well with the dorsal/ventral and rostral/caudal dimensions of the site of the challenge (Fig. 7a). Similar results were found for the other three animals.

DISCUSSION

This work demonstrates the use of *in vivo*, noninvasive imaging methods to detect changes in lung disease and evaluate the roles played by different physiologic processes through acquiring and fusing functional and anatomic image data. The model demonstrated here compares the location and extent of ventilation defects, inflammatory infiltrate, and changes in glucose metabolism to the location and extent of allergen insufflation. This feature makes the technique attractive for evaluation and validation of the role of the various components of lung diseases and their visibility using imaging techniques. Specifically, changes were observed and validated using pre- and post-challenge imaging for methacholine and segmental allergen challenges in BN rats. Local and systemic ventilation changes were visible following methacholine challenge, indicating local airway closure at the site of challenge and central airway narrowing most likely due to delivery of methacholine through the circulatory system.

In all four segmental allergen challenge experiments, ventilation defects, inflammatory infiltrate, and enhanced glucose metabolism were detected using imaging and confirmed at the location of insufflation through tissue samples and histology. No abnormalities were detected in images of the prechallenge lungs or the contralateral post-challenge lungs. The estimated volume of lost ventilation was found to be larger than the volume of inflammatory infiltrate (Table 1). This may result from ventilation defects due to smooth muscle contraction beyond the region of inflammatory infiltrate or reflect a limit on the lower threshold of detectability for T_1 -weighted infiltrate. However, the boundaries of the inflammatory infiltrate match the region of FDG uptake (Fig. 5j) suggesting that the mismatch in regional ventilation and inflammation is real. In T_1 -weighted MRI and [F-18]FDG-PET Patlak studies, the allergen-induced increase in mean SNR and Patlak influx constant was found to be well over 100% of the prechallenge values in both rats. This suggests the high detection sensitivity of these techniques for measuring allergen-induced changes.

The techniques developed in this work are principally designed to validate the ability to noninvasively detect lung ventilation and inflammation regionally using two emerging techniques for functional lung imaging: MRI and PET. Further studies will be directed at quantifying the dose-dependent response of the signal and volume changes measured in the images to better understand how a known, spatially localized perturbation is manifest in the images. The applications of these techniques include a wide range of studies in animal models including probing the underlying structural and functional relationships in asthma models as well as the ventilatory and inflammatory processes associated with rejection in lung transplant models and other inflammatory diseases of the lung. Furthermore, these methods are not isolated to animal studies and indeed PET (31) and MRI (6) are areas of active re-

search for functional lung imaging in human asthma. Specifically, studies have shown that regional inflammation and ventilation have the potential to provide a better understanding of the local processes, regional patterns, and temporal dynamics of airway closure and inflammation in this disease. Therefore, the concept of applying the registered PET and MRI techniques that are demonstrated in the present work can readily be extended to humans, albeit with modifications to increase acquisition speed and/or incorporate respiratory compensation over time-averaged acquisitions.

In conclusion, these studies demonstrate the feasibility of using multiple imaging techniques in coregistration to locate and quantify pulmonary inflammatory processes and their functional consequences. Studies in animals will allow systematic evaluations of potential mechanistic links between lung structure and function, with histologic correlates of the imaging. Existing methods for [F-18]FDG-PET imaging in humans, along with evolving experience using He-3 MRI in humans, could make these techniques readily adaptable to human use.

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