

Allergen-induced Lung Inflammation in Actively Sensitized Mice Assessed with MR Imaging¹

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Purpose:

To demonstrate the feasibility of using proton magnetic resonance (MR) imaging to noninvasively detect extravascular and luminal fluid in a murine model of allergen-induced airway inflammation.

Materials and Methods:

The Basel Veterinary Authority approved this experiment. Actively sensitized female Balb/c mice received ovalbumin or saline and underwent MR imaging (a) once 24 hours after the fourth administration of ovalbumin or saline ($n = 25$) or (b) several times between and after ovalbumin or saline administrations ($n = 22$) to determine the volume of fluid signal induced by an allergen. Images were acquired in spontaneously breathing animals, without cardiac or respiratory gating. Signal detected with a gradient-echo sequence was compared with bronchoalveolar lavage (BAL) fluid parameters and with perivascular and peribronchial edema and mucus observed at histologic analysis.

Results:

Up to 24 hours after the fourth administration of ovalbumin, intense and continuous fluid signals (volume, 40–50 μL) were detected in proximal lung regions. At 72 hours after the fourth administration of ovalbumin, remaining signals ($21.1 \mu\text{L} \pm 3.8$) had a discontinuous texture. The number of eosinophils in the BAL fluid at 24 and 72 hours and their activation were higher in mice that received ovalbumin than in those that received saline. Histologic analysis revealed edema and secreted mucus in the early phase, whereas only mucus was encountered in the late phase.

Conclusion:

These findings suggest that the main component of the early response was plasma leakage (edema), while the main component of the late response was secreted mucus. With the technique validated, the basis for pharmacologic studies in this murine model of lung inflammation with use of MR imaging as a noninvasive readout was provided.

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Over the past decade, there has been a shift to use murine models to investigate the molecular basis of asthma and other respiratory disorders. Murine models offer the prospect of enabling one to determine the complex interaction between cytokines, chemokines, and growth-related peptides in disease pathogenesis. Receptors and intracellular signaling pathways that are subsequently activated can be studied because of the availability of monoclonal antibodies and techniques for targeted gene disruption and gene incorporation for individual mediators, receptors, and proteins (1,2). Evidently, these are essential tools in the drug discovery process.

Plasma leakage, edema formation, and mucus hypersecretion contributing to luminal airway narrowing and thus impairment of lung function are major characteristics of asthma in humans (3–5). The way one of these features of airway inflammation is emphasized basically enables one to determine the differences between several of the murine models described in the literature (6–10). Inflammation established by repeated administration of allergens to actively sensitized animals is commonly

assessed with terminal procedures, such as histologic examination or bronchoalveolar lavage (BAL) fluid analysis.

In this study, we sought to demonstrate the feasibility of using proton magnetic resonance (MR) imaging to noninvasively detect extravascular and luminal fluid in a murine model of allergen-induced airway inflammation. The fluid signal intensity detected with a gradient-echo sequence (11–13) was compared with (a) the protein concentration in the BAL fluid, (b) the number and activation of inflammatory cells recovered in the BAL fluid, and (c) the perivascular edema, peribronchial edema, and mucus observed at histologic analysis.

Materials and Methods

This work was performed within the frame of research activities of Novartis Pharma (Basel, Switzerland). The company had no financial interest in the results reported in this article. Experiments were performed with the approval of the Basel Veterinary Authority (license number 1989).

Animals

In this study, we used 47 Balb/c female mice (Janvier, Le Genest-St-Isle, France) that weighed between 20 and 25 g. The mice were housed in a temperature- and humidity-controlled environment, with free access to standard mouse chow and tap water.

Sensitization and Allergen Challenge

Ovalbumin (250 µg/mL) (Fluka, Buchs, Switzerland) was mixed in a blender with aluminum hydroxide (10 mg/mL) in saline for 30 minutes on ice and in-

jected intraperitoneally (0.2 mL per animal) on days 0 and 14. As shown in Figure 1, on days 18–21, the mice were lightly anesthetized with 2% isoflurane (Abbott, Cham, Switzerland) and 100 µg of ovalbumin in 25 µL of saline (29 actively sensitized ovalbumin-challenged mice [hereafter, ovalbumin-challenged mice]) or 25 µL of saline (18 actively sensitized saline-challenged mice [hereafter, saline-challenged mice]) administered intranasally via a micropipette (12.5 µL per nostril) by means of breathing aspiration. Mice were handled in a 45° dorsal decubitus position. Animals were allowed to recover immediately thereafter.

Experimental Design

Two examinations were performed, and the number of MR images acquired in each study differed (Fig 1). In the single-acquisition study, 15 ovalbumin-challenged mice and 10 saline-challenged mice were examined once 24 hours after the fourth challenge. We performed BAL fluid analysis (10 ovalbumin-challenged mice, five saline-challenged mice) or histologic analysis (five ovalbumin-challenged mice, five saline-challenged mice) immediately after MR imaging.

In the multiple-acquisition study, 14 ovalbumin-challenged mice and eight saline-challenged mice were examined 24 hours after the second and third challenges and at sequential time points after the fourth challenge (Fig 1). We

Advances in Knowledge

- Proton MR imaging was performed in spontaneously breathing animals without the use of cardiac or respiratory gating to detect fluid signal intensity in the lungs of actively sensitized allergen-challenged mice, and the signal intensity was correlated to inflammatory features in a murine model of allergic asthma.
- Repeated MR measurements emphasized two phases in the course of multiple allergen challenge: The early phase primarily reflected plasma leakage, while the second phase was related to secreted mucus.
- This model can be used to noninvasively study the pharmacodynamic effects of drugs and to validate targets in mice or transgenic mice.

Implication for Patient Care

- After the validation described in this article and considering the ability to use MR imaging to obtain repetitive measurements in the same animal, this technique will be useful for in vivo profiling of anti-inflammatory drugs in mice and in transgenic mice with use of this model of allergen-induced lung inflammation.

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Abbreviations:

ANOVA = analysis of variance
BAL = bronchoalveolar lavage

Author contributions:

Guarantors of integrity of entire study, F.X.B., N.B.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; manuscript final version approval, all authors; literature research, F.X.B., C.C., H.K.Q., N.F., A.T., N.B.; experimental studies, F.X.B., C.C., S.Z., H.K.Q.; statistical analysis, R.B.; and manuscript editing, F.X.B., N.F., A.T., N.B.

See Materials and Methods for pertinent disclosures.

performed BAL fluid analysis (10 ovalbumin-challenged mice, four saline-challenged mice) or histologic analysis (four ovalbumin-challenged mice, four saline-challenged mice) immediately after the last MR image was acquired 72 hours after the fourth challenge.

MR Imaging

In vivo measurements were obtained with a spectrometer (Biospec 47/40; Bruker Medical Systems, Karlsruhe, Germany) operating at 4.7 T and equipped with an actively shielded gradient system capable of generating a 200 mT/m gradient. A 32-mm-diameter birdcage resonator was used for excitation and detection. Gradient-echo images were acquired with the following parameters: 5.6/3.5 (repetition time msec/echo time msec), 100-kHz bandwidth, approximately 10° excitation pulse flip angle, 256×128 matrix, 0.75-mm section thickness, and 3×3 -cm field of view for transverse images or 5×3 -cm field of view for coronal or sagittal images. A single-section image (74-second acquisition time) was obtained by computing the two-dimensional Fourier transformation of the averaged signal intensity from 60 (for transverse or coronal sections) or 30 (for sagittal sections) individual acquisitions and interpolating the data set to 256×256 pixels. The entire lung was covered with 16 consecutive transverse sections. In addition, one sagittal median section and four consecutive coronal sections parallel to the main bronchi were acquired. Typical MR sections obtained in different orientations are shown in Figure 2.

During measurements, mice were anesthetized with 1.5% isoflurane in a 1:2 mixture of O_2 and N_2O that was administered via a face cone. Animals were allowed to breathe spontaneously, and neither respiratory gating nor cardiac gating was applied. A mean body temperature of $37^\circ\text{C} \pm 1$ (standard deviation) was maintained with warm air flow.

Image Analysis

For each time point, the high-signal-intensity area was quantified on each of

the 16 images that covered the entire lung by using a semiautomatic segmentation procedure implemented in the Interactive Data Language environment (Research Systems, Boulder, Colo) on a Linux system. Images were first low-pass filtered with a Gaussian profile filter and then transformed into a set of four gray-level classes by using adaptive Lloyd-Max histogram quantitation (14). The highest class in the transformed images was extracted interactively with a region grower, the border of which was drawn manually to control the growing. For a given time point, the total volume of high-intensity signals was calculated by adding the areas obtained for each of the 16 images and multiplying the summed value by the section thickness. Since high-intensity signals, which were mainly caused by vessels, were present on baseline images for each mouse, the corresponding baseline volume was subtracted from the volumes evaluated at further time points to reflect only the effects of the challenge.

Postmortem Analyses

Animals were sacrificed by means of intraperitoneal injection of 0.2 mL of pen-

tobarbitone (250 mg/kg) immediately after MR images were acquired.

BAL fluid analysis.—The trachea was cannulated, and the lungs were lavaged by gently injecting 0.5 mL of a solution described previously (15) eight times via the trachea, without any chest massage. The BAL fluid, obtained by means of aspiration after the first two lavages, was centrifuged (Omnifuge 2.0; Heraeus Sepatech, Stuttgart, Germany) for 8 minutes at 4°C and at a rate of centrifugation of 400 g, and the supernatant was frozen at -80°C for later protein and eosinophil peroxidase activity assays (described in detail by Tigani et al [16]). Briefly, protein levels in the supernatant were measured with a photometric assay that was based on the reaction of proteins with an alkaline copper tartrate solution and a Folin reagent. Determination of eosinophil peroxidase activity was based on the oxidation of *o*-phenylenediamine by eosinophil peroxidase in the presence of hydrogen peroxide.

An aliquot from the other six lavages was used for total cell counting in an automatic cytometer (Cobas Helios 5Diff; Hoffmann-La Roche, Axon Labo-

Figure 1

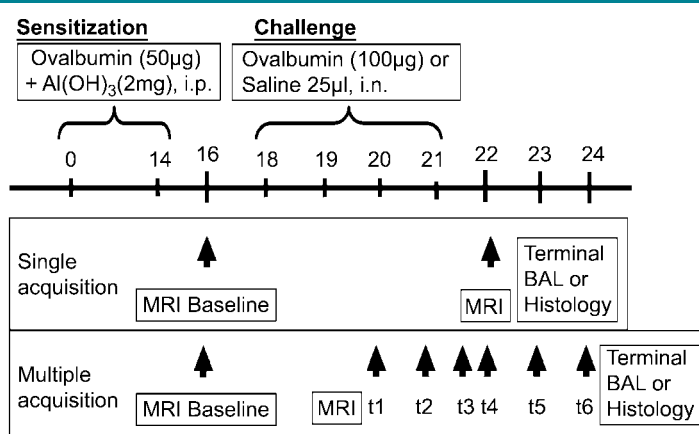


Figure 1: Diagram shows the experimental protocol. Sensitization lasted 14 days. Baseline MR images were obtained at day 16. Saline or ovalbumin challenges were performed from day 18 to day 21. In the single-acquisition study, MR imaging was performed 24 hours after the fourth challenge (on day 22). In the multiple-acquisition study, MR images were acquired 24 hours after the second challenge (*t1*), 24 hours after the third challenge (*t2*), and at various time points—namely, 6 hours (*t3*), 24 hours (*t4*), 48 hours (*t5*), and 72 hours (*t6*)—after the fourth challenge. In both experiments, mice were sacrificed immediately after the last MR image was obtained, and either BAL fluid analysis or histologic analysis was performed. *i.n.* = intranasal, *i.p.* = intraperitoneal.

ratory, Basel, Switzerland). From each aliquot, a sample containing approximately 50 000 cells was used for cyto-spin preparation. Cells were stained with Diff-Quik, and a differential count of 200 cells (eosinophils, macrophages, neutrophils, and lymphocytes) was obtained by observing cytospin slides under a classic light microscope (original magnification, $\times 100$; oil immersion) with standard morphologic criteria.

Histologic analysis.—The trachea was immediately ligated to avoid collapse, and the entire lung was removed and immersed in 10% neutral buffered formalin for 72 hours. The lungs were then cut longitudinally to include all lobes. After dehydration of tissues with

use of increasing graded series of ethylic alcohol and processing to paraffin wax, tissue slices were embedded in blocks. The 3- μ m-thick slices were cut in a manner that would enable us to observe the main bronchi of each lobe. Slices were stained with (a) hematoxylin-eosin to assess the general morphology, (b) Verhoeff-van Gieson dye to depict elastic fibers in arteries and to determine if perivascular edema was present, and (c) Alcian blue periodic acid-Schiff to depict excreted mucus and mucus-containing goblet cells.

Statistical Analyses

MR data were analyzed with statistical software (SYSTAT 12; Systat Software,

San Jose, Calif). Analyses were performed (a) on raw data, with use of the baseline signal intensity volumes as covariates, and (b) on the differential signal intensity volumes, obtained by subtracting the baseline signal intensity volume from the postchallenge signal intensity volume for each animal. Data from the single-acquisition study were evaluated with analysis of variance (ANOVA). For analysis of data from the multiple-acquisition study, we used an extension of ANOVA, called mixed-model analysis or ANOVA with random effects, to account for the longitudinal structure of the data. For multiple comparisons, ANOVA was followed by the Bonferroni correction.

BAL fluid parameters from ovalbumin- and saline-challenged mice were compared by using ANOVA followed by the Bonferroni correction to adjust for the two time points. Pearson correlation coefficients and corresponding confidence intervals were computed between the volumes of MR signal intensities and the corresponding BAL parameters for the same animals with statistical software (Unistat, version 5.6.02; Unistat, London, England).

Results

Figure 3a shows MR sections obtained through the chest in two mice 24 hours after the fourth intranasal administration of saline or ovalbumin (single-acquisition study). Fluid signal intensity, preferentially located in the proximal regions of the lung, was observed in ovalbumin-challenged mice. No extravascular fluid signal intensity was observed in saline-challenged mice. The mean volume of areas with fluid signal intensity detected with MR imaging is displayed as a baseline-subtracted volume in Figure 3b and shows a 13.9-fold increase in signal intensity in the ovalbumin-challenged animals compared with that in the saline-challenged animals.

The course of the MR signal intensity volume (baseline volume subtracted from volumes evaluated at further time points) for the multiple-acquisition study is shown in Figure 4a. An overall comparison of data obtained in ovalbu-

Figure 2

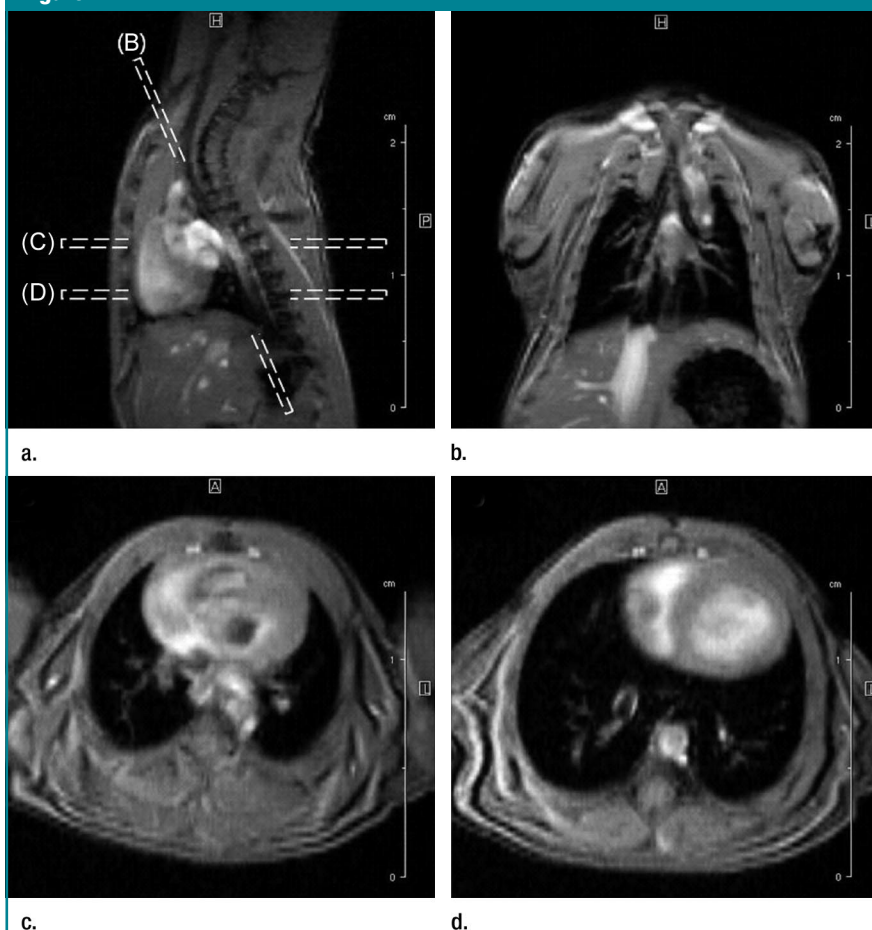
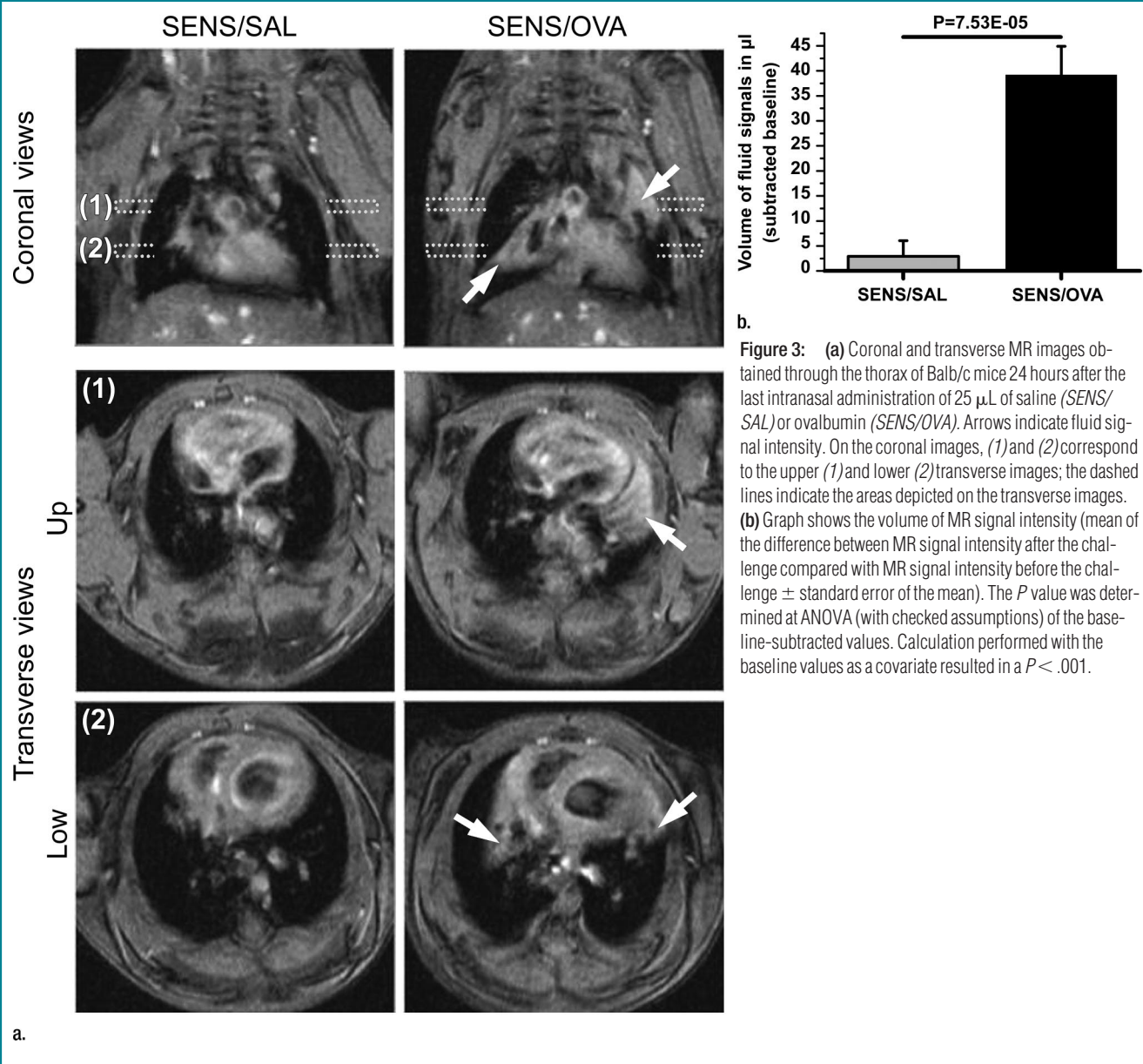


Figure 2: (a) Sagittal median, (b) coronal, and (c, d) transverse gradient-echo MR images obtained through the thorax in a naïve (nonsensitized nonchallenged) mouse. In a, (B), (C), and (D) correspond to the areas depicted in b, c, and d, respectively.

Figure 3



min-challenged mice with data obtained in saline-challenged mice was performed with mixed-model analysis and showed a significant difference ($P < .001$ with the baseline as a covariate, $P < .001$ when the baseline was subtracted) between the experimental conditions. For each day, significant differences between ovalbumin-challenged mice and saline-challenged mice were apparent (Fig 4a). In ovalbumin-challenged mice, MR signal intensity was

strong and continuous at early time points and became less intense and discontinuous at later time points after the fourth ovalbumin challenge (Fig 4b).

BAL fluid parameters were assessed immediately after the MR acquisitions performed 24 and 72 hours after the fourth challenge, and they are summarized in the Table. At both time points, the total cell count was significantly higher in BAL fluid samples obtained in ovalbumin-challenged mice than in sam-

ples obtained in saline-challenged mice. In the saline-challenged mice, macrophages constituted the largest cell type proportion, whereas in the ovalbumin-challenged mice, eosinophils were the predominant cell type at both time points. Neutrophil counts were significantly increased 24 hours after the fourth ovalbumin challenge but returned to baseline levels 72 hours after this challenge. Lymphocyte counts were also elevated 24 hours after the fourth

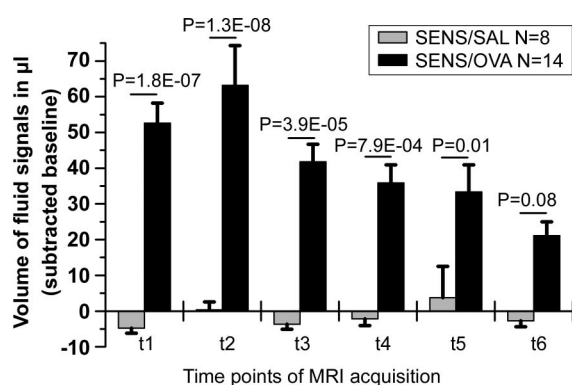
ovalbumin challenge and were further increased at the subsequent time point (72 hours after the fourth ovalbumin challenge). Eosinophil peroxidase activity and protein levels were also significantly increased at 24 and 72 hours after the fourth ovalbumin challenge (Table). The volume of fluid signal intensity in the lungs

detected with MR imaging significantly correlated with the total protein level and the number of eosinophils quantified in the BAL fluid samples, as well as with the activation of the eosinophils assessed by means of eosinophil peroxidase activity (Table).

Histologic analysis of Verhoeff-van

Gieson-stained lung tissue slices obtained from ovalbumin-challenged animals revealed edema in the perivascular region, with marked peribronchial and perivascular polynuclear cell infiltration, 24 hours after the last challenge (Fig 5); this finding was confirmed with hematoxylin-eosin staining (not shown).

Figure 4

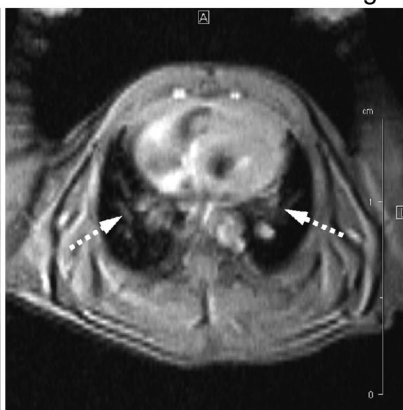
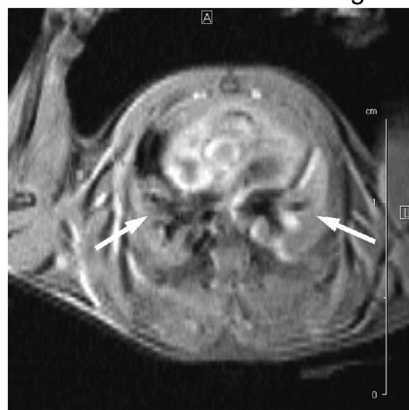


a.

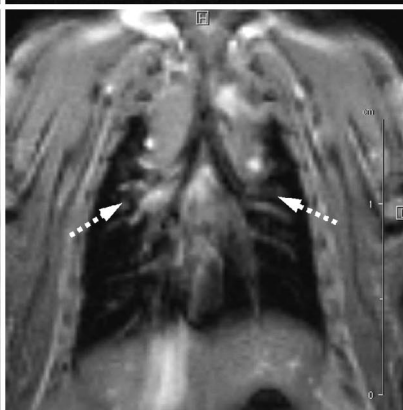
t3 : 6h after 4th OVA challenge

t6 : 72h after 4th OVA challenge

Transverse views



Coronal views



b.

Figure 4: (a) Graph shows course of volume of MR signal intensity (mean of the difference between MR signal intensity after the challenge compared with MR signal intensity before the challenge $\pm$ standard error of the mean) for mice in the ovalbumin-challenged group ($n = 14$, *SENS/OVA*) and mice in the saline-challenged group ($n = 8$, *SENS/SAL*) at different time points 24 hours after the second challenge (t_1), 24 hours after the third challenge (t_2), and 6 (t_3), 24 (t_4), 48 (t_5), and 72 (t_6) hours after the fourth challenge. Indicated P values determined at ANOVA with random effects (with checked assumptions) adjusted for multiple testing, with use of the Bonferroni correction for baseline-subtracted values. When the test was also performed on original values with baseline value considered as a covariate, the following P values were obtained: at t_1 , t_2 , 3, and t_4 , $P < .001$; at t_5 , $P = .01$; and at t_6 , $P = .06$. (b) Coronal and transverse MR sections obtained through the thorax in Balb/c mice are representative of fluid signal intensity area texture and volumes detected 6 and 72 hours after the fourth intranasal ovalbumin challenge ($100 \mu\text{g}$ in $25 \mu\text{L}$ of saline for 4 consecutive days). Solid arrows indicate intense and continuous fluid signals, whereas dashed arrows indicate less intense and discontinuous fluid signals.

Edema receded 72 hours after the fourth ovalbumin challenge; however, severe polynuclear cell infiltration was observed (Fig 5). At both time points, the cellular inflammation was predominantly located in the hilar region in the vicinity of the first-generation airways. Neither perivascular edema nor infiltration of polynuclear cells was observed in lung slices obtained from saline-challenged mice (Fig 5). Alcian blue periodic acid-Schiff staining revealed clear metaplasia of goblet cells and luminal mucus hypersecretion in all lung tissue slices obtained from ovalbumin-challenged mice 24 and 72 hours after the fourth challenge. Neither Alcian blue periodic acid-Schiff-positive cells nor luminal mucus secretion was observed in lung tissue slices obtained from saline-challenged mice.

Discussion

In this study, we found that multiple allergen challenges in mice induce the secretion of fluids in the lungs that are related to airway plasma leakage and mucus production and that can be monitored noninvasively with MR imaging. The qualitative finding that fluid signals were often located in anterior apical areas, which was confirmed with histologic analysis of the hilar region, is consistent with the fact that inflammatory processes occur in the main airways. However, since our goal was to validate MR imaging by comparing it with a global measure such as BAL fluid analysis, the overall volume of MR signal intensity in the lungs in response to ovalbumin administration was assessed. A quantitative location of data would not help the comparison with the established BAL fluid analysis, as there was a certain heterogeneity of responses. Quantification of the signal intensity was feasible and reproducible, despite the presence of large vessels and other anatomic structures, such as the heart, that occupied a large portion of the thoracic cavity.

A significant increase in fluid volume was observed after ovalbumin administration as early as 24 hours after the second challenge. The maximum net

Inflammatory Parameters in BAL Fluid Recovered from Lungs of Sensitized Mice Challenged with Saline or Ovalbumin 24 or 72 Hours after the Fourth Challenge

Parameter	24 hours after the Fourth Challenge		P Value	72 hours after the Fourth Challenge		Correlation with MR Signal Intensity (n = 29)		
	Saline-Challenged Mice (n = 5)	Ovalbumin-Challenged Mice (n = 10)		Saline-Challenged Mice (n = 4)	Ovalbumin-Challenged Mice (n = 10)	P Value	r Value	95% Confidence Interval
Total cell count (10 ⁶ cells per milliliter of BAL fluid)	0.187 ± 0.014	1.328 ± 0.108	<.001	0.178 ± 0.015	1.53 ± 0.192	<.001	0.50	.006 0.16, 0.73
Macrophages (10 ⁶ cells per milliliter of BAL fluid)	0.176 ± 0.014	0.268 ± 0.025	.06	0.168 ± 0.015	0.361 ± 0.044	.038	0.10	.6 −0.27, 0.45
Eosinophils (10 ⁶ cells per milliliter of BAL fluid)	0.003 ± 0.001	0.989 ± 0.126	<.001	0.004 ± 0.001	1.078 ± 0.132	<.001	0.54	.002 −0.22, 0.76
Lymphocytes (10 ⁴ cells per milliliter of BAL fluid)	0.3 ± 0.1	4.3 ± 0.4	<.001	0.3 ± 0.1	9.0 ± 2.0	.046	0.21	.28 −0.17, 0.54
Neutrophils (10 ⁴ cells per milliliter of BAL fluid)	0.5 ± 0.1	2.8 ± 0.4	.004	0.4 ± 0.1	0.1 ± 0.1	.014	0.01	.96 −0.35, 0.38
Total protein level (mg/mL of BAL fluid)	0.214 ± 0.015	0.568 ± 0.043*	<.001	0.211 ± 0.018	0.409 ± 0.035	.01	0.73	<.001 0.50, 0.87
Eosinophil peroxidase activity (U/mL of BAL fluid)	0.151 ± 0.083	2.709 ± 0.321	<.001	0.114 ± 0.06	2.562 ± 0.412	.006	0.54	.002 0.22, 0.76

Note.—Unless otherwise indicated, data are means ± standard errors of the mean. The P values 24 and 72 hours after the fourth challenge refer to an assumption of difference between the ovalbumin-challenged mice and the saline-challenged mice at the same time point with ANOVA with Bonferroni correction to adjust for the two time points. The Pearson correlation coefficient (r value) was used to assess the correlation with MR signal intensity between each BAL parameter and the volume of fluid signals detected with MR imaging, with probability (P value) for the two-sided alternative hypothesis $r \neq 0$.

volume (measured 24 hours after the third challenge) did not exceed 15% of the total lung volume in the mouse, whereas the maximum net volume exceeded approximately 40% of the total lung volume in actively sensitized Brown Norway rats that underwent one ovalbumin challenge (15–17). The relatively low volumes of fluid signal intensity detected in our study were in agreement with observations that showed that, independent of the allergen dose, little plasma exudation occurs in mouse airways, especially in late phases (18,19). One possible reason for this result could be that eosinophils are prominent effector cells in murine models of asthma (20), whereas mast cells are the major type of cell involved in the early inflammatory response in ovalbumin-challenged rats (and asthmatic humans) (21,22). When activated, mast cells are able to release mediators, such as histamine, that are responsible for plasma leakage from bronchial vasculature (23).

Evaluation of BAL fluids revealed an increase in the total number of cells in the airway space of ovalbumin-challenged mice compared with that in sa-

line-challenged mice. Eosinophils were the main cell type present in the BAL fluid 24 and 72 hours after the last allergen challenge. The proportions of other cell types were comparable to those in earlier reports (10,12). Consistent with the BAL fluid results, peribronchial and perivascular airway eosinophilia, considered to be a hallmark of human asthma (20), was seen on histologic samples obtained in ovalbumin-challenged mice. This observation was also made in other murine models of allergen-driven lung inflammation (8,24,25). In addition to high eosinophil cell counts in BAL fluid, luminal eosinophil activation, which was represented by eosinophil peroxidase levels, was markedly increased in ovalbumin-challenged mice and found to be correlated with volumes of fluid signal intensity detected with MR imaging. Although activation of eosinophils within the pulmonary tissue, shown by their degranulation into the airway lumen of allergic mice (26), remains controversial (27–29), it has been established that, once activated, products from their granules are associated with both plasma leakage and mucus hypersecretion (30,31).

Typically, up to 24 hours after the fourth ovalbumin challenge, MR signals were intense and continuous; however, at 72 hours after the fourth ovalbumin challenge, they were discontinuous and less intense. Previous results obtained in rats that underwent an allergen challenge and those that were subjected to an endotoxin pulmonary challenge (15, 16) showed that intense and continuous MR signals reflected predominantly edema-like responses, while discontinuous signals were related to secreted mucus. On the basis of those observations, we hypothesized that in the present murine model, the first phase of the allergic response detected with MR imaging was dominated by plasma leakage, while at a later phase, the main component of the signal was caused by secreted mucus. The following observations enabled us to substantiate this hypothesis: (a) the higher total protein level in the BAL fluid (ie, a relevant indicator of the luminal part of plasma leakage) in ovalbumin-challenged mice 24 hours after the fourth challenge, (b) the peribronchial and perivascular edema detected with histologic analysis at this time point, and (c) goblet cell metaplasia and mucus hypersecretion seen in histologic samples obtained in ovalbumin-challenged animals at early and late time points. However, 72 hours after the fourth ovalbumin challenge, no edematous tissue was seen at histologic analysis; in fact, only goblet cell hyperplasia and mucus-obstructed airways were seen. It has been shown that allergen-induced inflammatory cell persistence causes prolonged goblet cell metaplasia and mucus secretion (31). This finding is consistent with our observation of residual mucuslike MR fluid signal intensity 72 hours after the fourth intranasal administration of ovalbumin and with the histochemical observation of numerous Alcian blue periodic acid–Schiff–positive epithelial cells and mucus in airways at that time point. Because mucus contained in goblet cells is poorly hydrated, it is reasonable to assume that the long-lasting MR signal intensity after multiple ovalbumin challenges originated from excreted mucus, which is a major contributor to airway obstruction.

Figure 5

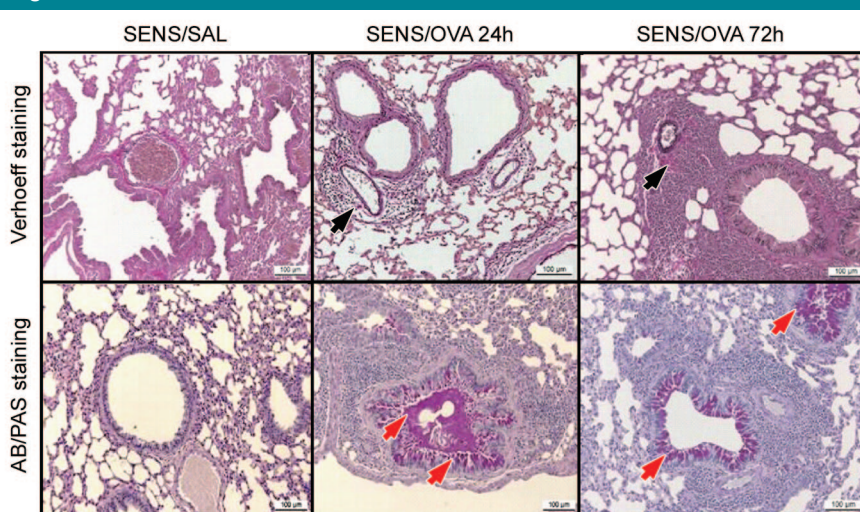


Figure 5: Histologic slices from the left lung of actively sensitized mice that were challenged with saline (left column, *SENS/SAL*) or ovalbumin (*SENS/OVA*) and sacrificed 24 (center column) or 72 (right column) hours after the last challenge show perivascular edema and polynuclear infiltration (black arrows) after Verhoeff–van Gieson staining (top row) and mucus plugs and goblet cell metaplasia (red arrows) after Alcian blue periodic acid–Schiff staining (bottom row).

Other researchers have shown the usefulness of proton MR imaging in the detection of infection-related changes in the lungs of spontaneously breathing mice (32,33). However, in those studies, image acquisition was triggered by electrocardiography. We used an approach that was similar to one previously used in rats (15,16,34), and, to our knowledge, we are the first to describe the feasibility of detecting inflammation-related changes in the lungs of spontaneously breathing mice by applying proton MR imaging without cardiac or respiratory gating. The gradient-echo sequence was used to obtain sharp images in the mouse chest without any triggering, as potential artifacts caused by movement were suppressed by averaging the echo signals from a number of individual image acquisitions (35). Since the animals were breathing spontaneously, no intubation or mechanical ventilation was necessary. Thus, this procedure easily enables repetitive measurement to be performed and circumvents lung damage that might be caused by mechanical ventilation (36).

Models of inhaled and segmental allergen challenge with subsequent fiberoptic BAL and biopsy have been developed to examine allergen-induced leukocyte infiltration in humans (37) and have been used to study disease mechanisms (38) and test the effects of treatments (39) in patients. Imaging can potentially add value to such studies by providing spatial information. To our knowledge, the only article to address imaging after an allergen challenge in human subjects reported on the use of scintigraphy (40). Considering the limited spatial resolution achieved with scintigraphy, MR imaging might be an alternative in proof-of-concept studies related to this model. The work presented in our article could help guide investigations in which MR imaging is considered as an additional imaging modality.

In conclusion, although the lung is challenging to examine with MR imaging, our results show that when this technique is applied without respiratory or cardiac gating in spontaneously breathing mice, it can be used to dis-

criminate leaking or mucus-secreting lung tissue from healthy lung tissue. The course of signal intensity changes in the lung after an allergen challenge points to two components—namely, plasma leakage and secreted mucus—which are relevant in characterizing the inflammatory state of the lung. With the ability to perform repetitive measurements in the same animal, this technique will be useful for in vivo profiling of antiinflammatory drugs in mice and in transgenic mice in which this model of allergen-induced lung inflammation is adopted.

Practical application: Our model and methods used to assess lung fluid may be useful in evaluating new drug therapies and may be applicable to other murine lung disease models characterized by inflammation and fibrosis.

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