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Identification with MRI of the pleura as a major site of the acute inflammatory effects induced by ovalbumin and endotoxin challenge in the airways of the rat

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Quintana, Harry Karmouty, Catherine Cannet, Elisabeth Schaeublin, Stefan Zurbrugg, Rosemary Sugar, Lazzaro Mazzoni, Clive P. Page, John R. Fozard, and Nicolau Beckmann. Identification with MRI of the pleura as a major site of the acute inflammatory effects induced by ovalbumin and endotoxin challenge in the airways of the rat. *Am J Physiol Lung Cell Mol Physiol* 291: L651–L657, 2006. First published May 25, 2006; doi:10.1152/ajplung.00303.2005.—Inflammatory effects in the rat lung have been investigated, non-invasively by MRI, at early time points (3 and 6 h) after ovalbumin (OA) or endotoxin (LPS) challenges. Six hours after challenge with OA, a strong, even inflammatory signal was present around the periphery of the lung in a region corresponding to the pleura. Histological analysis confirmed the presence of marked edema associated with the pleural cavity of OA-treated animals. Lower levels of pleural edema were observed in MRI and histological evaluation of LPS-treated animals and no abnormality was observed in actively sensitized and naïve, saline-treated groups. Diffuse edematous signals were detected in the lung 3 and 6 h after challenge with OA or LPS; the signal volumes were larger at both time points following OA instillation. Bronchoalveolar lavage (BAL) fluid analysis performed 6 h after challenge revealed increased levels of protein and greater cellular activation in OA- than in LPS-treated animals. Furthermore, increased levels of peribronchial edema were found by histology 6 h after OA. BAL fluid and histological assessments demonstrated that the inflammatory signals were due to edema and not mucus as no significant changes in BAL mucin concentrations or differences in goblet cells were identified between OA or LPS challenge and their respective vehicle groups. Our data show that MRI is able to detect, non-invasively, inflammatory signals in both the lung and the pleura in spontaneously breathing animals, highlighting its potential to study the consequences of pulmonary insults on both sites.

edema; lipopolysaccharide; lung inflammation; magnetic resonance imaging

ASTHMA and chronic obstructive pulmonary disease (COPD) are chronic disorders of the lung that involve the interplay of many inflammatory and structural cells, resulting in the interaction of a wide variety of inflammatory mediators that include auto-coids, cytokines, chemokines, growth factors, and adhesion molecules. In asthma, these inflammatory mediators are thought to contribute to epithelial cell damage, bronchial hyperresponsiveness, plasma exudation, and edema of the airways mucosa (5). In COPD, chronic inflammation of the small airways and lung parenchyma, with the involvement of neu-

trophils, macrophages, and cytotoxic (CD8+) T lymphocytes, results in the destruction of the lung parenchyma by proteolytic enzymes (emphysema) and in mucus hypersecretion leading to severe airflow limitation (7). A common contributory mechanism in both asthma and COPD is the increase in permeability of the lung vasculature. The resulting effect is the leakage of fluid from the microvascular circulation into the surrounding tissue. Assessment of this fluid is important for diagnostic purposes and for planning and guiding treatment (8).

Actively sensitized Brown Norway (BN) rats are frequently used in asthma research (20). When exposed to allergen (typically ovalbumin; OA), sensitized animals develop airway hyperresponsiveness, eosinophilic inflammation, and an increase in activated T cells (CD25+) in the airways as well as an increase of fluid extravasation in the lungs.

An inflammatory response resembling in many aspects that observed in patients with COPD can be elicited experimentally in BN rats by the administration of endotoxin (LPS), a gram-negative bacterial wall constituent that is able to stimulate a variety of inflammatory cells (11), particularly macrophages and monocytes (21). LPS causes the release of several inflammatory mediators, including tumor necrosis factor- α (TNF- α) (28), and induces the accumulation of neutrophils in the pulmonary vasculature (23).

Distinct MRI signals can be detected non-invasively in the rat lung following OA or LPS challenge, reflecting the different pathophysiological effects that are induced by either substance. Challenge of actively sensitized BN rats with OA results in an even signal of high intensity, particularly at 24 h after instillation, correlating with increased protein concentration in bronchoalveolar lavage (BAL) fluid and with eosinophilic infiltration and activation (8, 35). LPS challenge of naïve BN rats results in a diffuse signal of lower intensity maximally observed 48 h after treatment and correlating with increased mucin concentration in BAL fluid analysis (35) and with an increase of goblet cells and flocculent mucosal material from histological analysis (10).

Up to now there has been no information on the changes induced by OA or LPS at shorter time intervals after allergen challenge. Thus in the present work we have used MRI to study the effects of OA and LPS acutely (3 and 6 h after challenge) in actively sensitized and naïve BN rats, respectively.

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MATERIALS AND METHODS

Experiments were carried out with the approval of the Veterinary Authority of the City of Basel (license no. 567).

Animals. Forty-eight male BN rats (Iffa Credo, L'Arbresle, France) weighing 250–300 g were used in the study. Animals were kept at an ambient temperature of $22 \pm 2^\circ\text{C}$ under a 12-h normal phase light-dark cycle and fed with NAFAG pellets (Nahr und Futtermittel AG, Gossau, Switzerland). Drinking water was freely available.

Sensitization. Twenty-four rats were sensitized to OA using the following standard protocol (see Ref. 16): OA (20 $\mu\text{g}/\text{ml}$) was mixed (30 min on ice) in a blender (Polytron, Kinematica) with aluminum hydroxide (20 mg/ml) and injected subcutaneously (0.5 ml/animal). Injection of OA was repeated 14 and 21 days later. Sensitized animals were used in experiments between days 28 and 35.

Ovalbumin or LPS exposure. For challenge, animals were anesthetized (4% isoflurane; Abbott, Cham, Switzerland) in an anesthetic chamber. Actively sensitized rats were exposed to OA (0.3 mg/kg; $n = 12$) or saline (0.2 ml; $n = 12$). Naïve rats received either LPS (1 mg/kg; $n = 12$) or saline (0.2 ml; $n = 12$). Substances were administered intratracheally through a cannula positioned above the carina.

MRI. For MRI, rats were anesthetized with 2% isoflurane in a mixture of $\text{O}_2/\text{N}_2\text{O}$ administered via a face mask. Animals were placed in supine position in a cradle made of plexiglas. Body temperature was maintained at $37 \pm 1^\circ\text{C}$ using warm air regulated by a rectal temperature probe (DM 852, Ellab, Copenhagen, Denmark). During examinations, animals breathed spontaneously and neither cardiac nor respiratory triggering was applied.

Measurements were performed using a Biospec 47/40 spectrometer (Bruker Medical Systems, Karlsruhe, Germany) operating at 4.7 T, equipped with an actively shielded gradient system capable of generating a gradient of 200 mT/m. The operational software of the scanner was Paravision (Bruker). For detection of fluid signals, a T1-weighted, gradient-echo sequence with the following parameters was used: repetition time, 5.6 ms; echo time, 2.7 ms; flip angle of the excitation pulse, $\sim 15^\circ$; field-of-view, $6 \times 6 \text{ cm}^2$; matrix size, 256×128 ; and slice thickness, 1.5 mm. A single slice image was obtained by computing the 2-dimensional Fourier Transformation of the averaged signal from 45 individual image acquisitions and interpolating the data set to 256×256 pixels. There was an interval of 530 ms between individual image acquisitions, resulting in a total acquisition time of 59 s for a single slice. The entire lung was covered by 18 consecutive transversal slices. The duration of a session, including anesthetizing and positioning the animal, did not exceed 25 min.

MRI analysis. The volume of fluid signal was quantified using a semiautomatic segmentation procedure implemented in the IDL (Interactive Data Language Research Systems, Boulder, CO) environment (version 5.1) on an SGI O2 (Silicon Graphics, Mountain View, CA) system. Images were first weakly low-pass filtered with a Gaussian profile filter and then transformed into a set of four gray level classes using adaptive Lloyd-Max histogram quantization. This method avoids operator bias due to the arbitrary choice of threshold levels on each image (8). Because the fluid comprised high signal intensities in the original images, it was represented by the highest gray level class in the transformed images. This class could be extracted interactively by use of a region grower. Because of the unknown extent of the fluid, no morphology parameters were incorporated into the region-growing process. Instead, a contour serving as a growing border was drawn to control region growing manually. The segmentation parameters were the same for all the analyzed images, chosen to segment regions corresponding to high intensity signals. Because the edematous signals and those from vessels were of comparable intensities, the volume corresponding to the vessels was assessed on baseline images and then subtracted from the volumes determined on postchallenge images. For the edematous responses of the pleura, signal volumes were determined at time points of 3 and 6 h

after challenge with OA, LPS, or saline; no baseline measurements were taken due to the lack of MRI-visible vessels at the pleural region.

Postmortem analyses. Rats were killed by an overdose of pentothal (250 mg/kg ip) administered 6 h after challenge and immediately following MRI signal acquisition.

Histological analysis. Lungs were inflated with 5 ml of 10% phosphate-buffered neutral formalin via a cannula inserted in the trachea. The lungs were then removed from the thorax and immersed in formalin between 24 and 72 h. The lung tissue was then sectioned transversally through the left lobe, the right apical, the right median, and the right caudal lobes to include the main bronchi as well as the pulmonary alveoli. After we processed to paraffin wax, sections were embedded in blocks. Slices of 3- μm thickness were cut from these blocks and then stained using Alcian blue-periodic acid-Schiff (PAS) stain for the determination of peribronchial edema and detection of goblet cells and acidic and neutral mucus. Staining using Verhoeff's reaction was performed for quantification of perivascular edema.

Determination of pleural edema. Two pictures of the left lobe and two pictures of the right caudal lobe of each animal were taken at the level of the hilus so that the visceral pleura (that closely covers the lung) and the parietal pleura (that is adherent to the thoracic wall and the diaphragm) were present. Pleural edema was defined as the presence of increased fluid in the pleural cavity (the space between the parietal and the visceral pleurae). For the quantification of pleural edema, the distances between the visceral and the parietal pleura at four different levels were determined.

Quantification of peribronchial edema. Three to four pictures of bronchi from each section of the left lobe (apical, median, and caudal) were captured at $\times 10$ magnification on PAS/Alcian blue-stained slides. Morphometric analyses were performed with the software Image Access 4.0 (Imagic, Glattbrugg, Switzerland) connected to a video camera Prog/Res/3008 (Jenoptic LOS, Eching, Germany). The areas of smooth muscle layer and peribronchial edema were manually circumscribed, and peribronchial edema was calculated as a percentage of smooth muscle layer area.

Quantification of perivascular edema. Five to eight pictures of arteries from each section of left, right caudal, and right median lobes were captured at $\times 10$ magnification on Verhoeff-stained slides. As before, morphometric analyses were performed with the software Image Access 4.0. The areas of external edema and external elastica lamina were manually circumscribed, and perivascular edema was calculated as a percentage of external elastica lamina area. Only vessels with internal diameters between 35 and 150 μm were measured. Approximately 25 vessels were assessed per animal. The total edema determined histologically (sum of the edema in the left, right caudal, and right median lobes) is shown as a percentage.

Quantification of goblet cells. Three to seven pictures of bronchi from each section of left, right caudal, and right median lobes were captured at $\times 10$ magnification on PAS/Alcian blue-stained slides. The circumference of each bronchus was manually delineated, and the number of goblet cells was counted. Results were expressed as the number of goblet cells per millimeter length of bronchus. Only bronchi whose diameters comprised between 900 and 2300 μm were measured. The total goblet cell numbers (sum of those in the left, right caudal, and right median lobes) are presented.

BAL fluid analysis. A detailed description of the BAL procedure and the analysis of the parameters of inflammation in the BAL fluid have been provided earlier (10, 36). Briefly, after killing the animals with an overdose of pentothal, the lungs were lavaged. For leukocyte numbers and cell differentiation, an automatic cell-analyzing system was utilized (Cobas Helios 5Diff, Hoffmann-La Roche, Axon Laboratory). Determination of eosinophil peroxidase was based on the oxidation of *O*-phenylenediamine by eosinophil peroxidase in the presence of hydrogen peroxide. Myeloperoxidase (MPO) activity was measured in a photometric assay based on the oxidation of *O*-dianiside dihydrochloride by MPO in the presence of hydrogen peroxide. The level of protein in the BAL fluid supernatants was

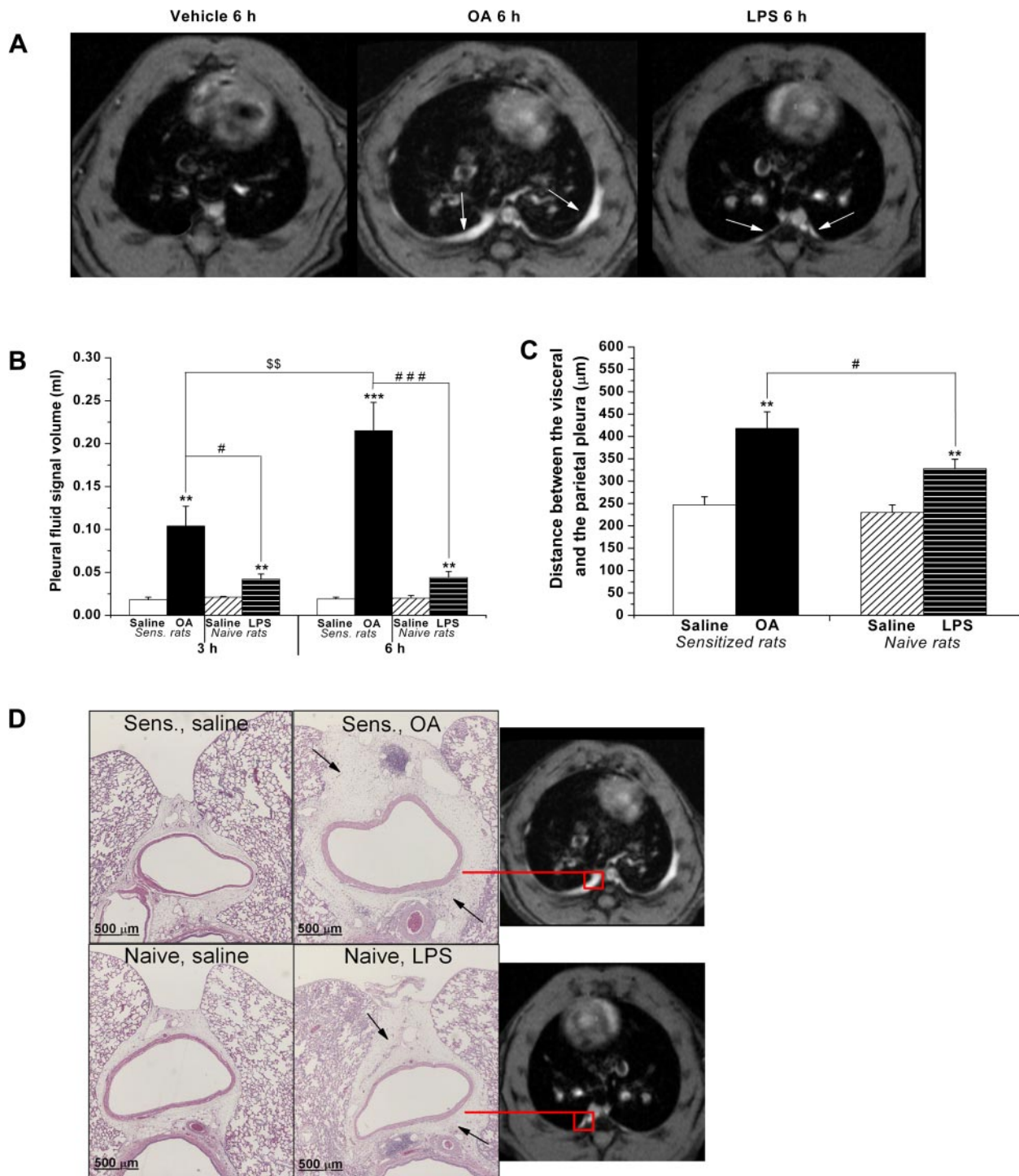
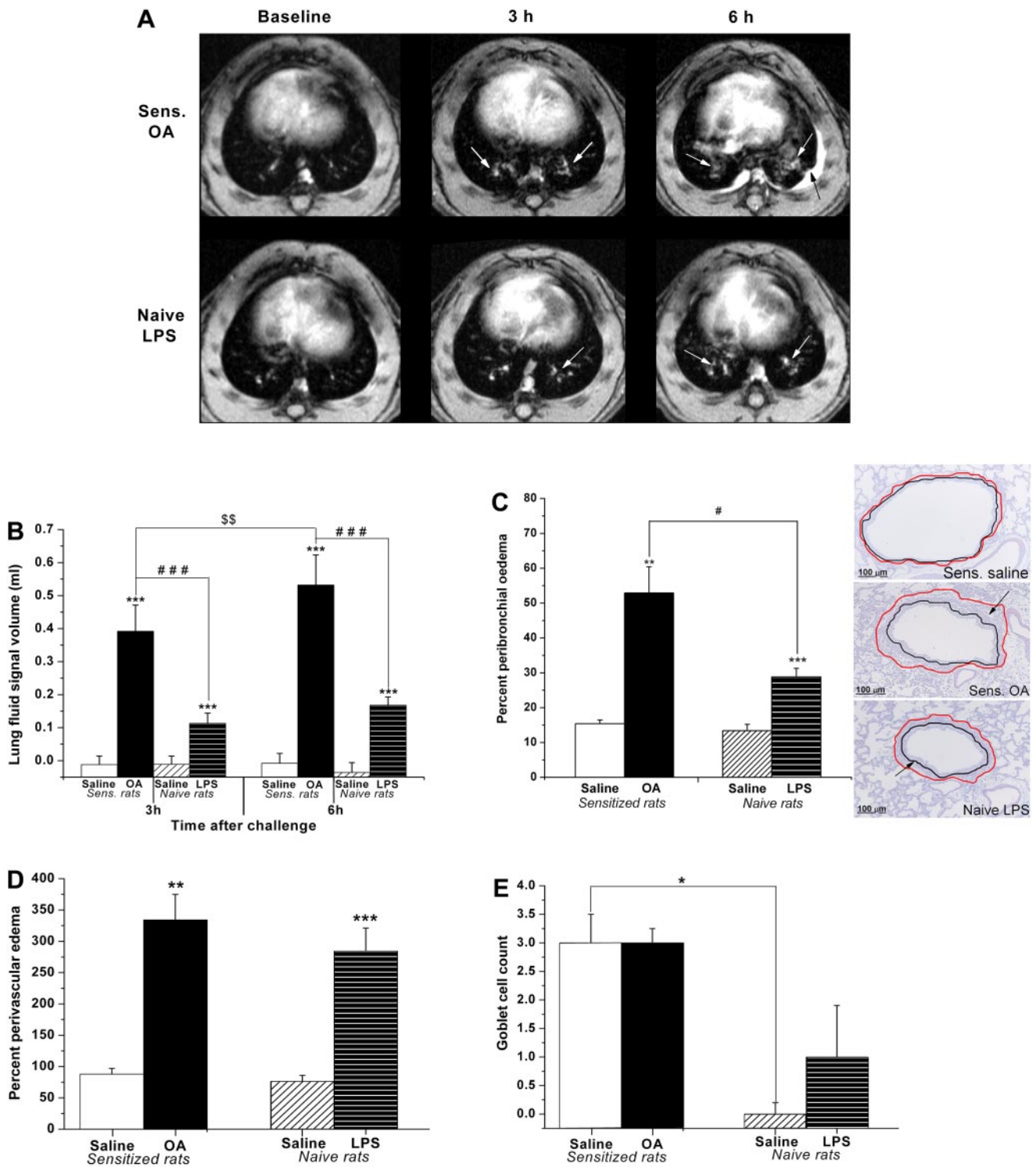


Fig. 1. A: axial sections through the thorax of an actively sensitized rat treated with saline (left) or 0.3 mg/kg intratracheally of ovalbumin (OA; middle) and of a naïve rat treated with 1 mg/kg intratracheally of LPS (right) acquired 6 h after challenge. The arrows show the appearance of pleurisy in OA- and LPS-treated animals. B: pleural fluid signal volumes determined from MRI acquired at 3 and 6 h after challenge in sensitized rats treated with saline (0.2 ml intratracheally) or OA (0.3 mg/kg intratracheally) and in naïve rats treated with saline or LPS (1 mg/kg intratracheally). Values are means $\pm$ SE. *** P < 0.001 and **0.001 < P < 0.01 show that the value differs significantly from the value in the respective vehicle-treated group. \$\$0.001 < P < 0.01 shows that the MRI signal volume 3 and 6 h after OA challenge in sensitized rats differs from each other. #0.001 < P < 0.01 and ### P < 0.001 refer to differences between OA and LPS at 3 and 6 h after challenge, respectively. C: pleural edema assessed as the distances (means $\pm$ SE) between the visceral and parietal pleura from the left and right caudal lobes. The distance between challenged rats was significantly greater (** P < 0.01; Student's t -test) than that in corresponding saline-treated animals. There was a significant difference between OA- and LPS-treated animals (# P < 0.05). D: histological sections of the left lobe of naïve rats or actively sensitized rats, 6 h after challenge. The arrows show the edematous response elicited by allergen and endotoxin in the pleural cavity. The number of animals per group was 6; samples were analyzed with the Student's t -test (1 tail) for B and C. Sens., sensitized.



measured by a photometric assay, based on the reaction of protein with an alkaline copper tartrate solution and Folin reagent. Mucus in BAL fluid was assessed using the sandwich enzyme-linked lectin assay as described earlier (10) by measuring the optical density of flat-bottomed Costar plates coated with Ulex europaeus agglutinin-1 (Sigma) at a wavelength of 492 nm using a SpectraMax 250 plate reader (Molecular Devices, Surrey, UK).

Statistics. Student's *t*-test (1 tail) was performed for MRI, BAL fluid, and histological data referring to edema. The Mann-Whitney's *U*-test was chosen for analysis of histological data relating to goblet cell numbers.

RESULTS

Six hours after challenge with OA, MRI images revealed a prominent edematous signal at the edges of the lung corresponding to the pleura. A similar although less intense signal was seen in LPS-treated rats (Fig. 1A). Determination of pleural edema signal volume by MRI showed a significantly greater response in OA- than LPS-treated rats (Fig. 1B). Histological assessment of the pleural cavity revealed a more pronounced pleurisy in the OA compared with the LPS group (Fig. 1, C and D).

At the early time points of 3 and 6 h after instillation of OA or LPS, irregular fluid signals of similar appearance were apparent in MRI of the lung (Fig. 2A). However, OA treatment resulted in higher levels of fluid volumes than LPS challenge at both time points (Fig. 2B). Postmortem BAL fluid analysis, performed 6 h after challenge, revealed greater increases in protein concentration and markers of cellular activation (MPO) and eosinophil peroxidase (EPO) in OA-treated animals compared with the LPS-treated group, although greater inflammatory cell recruitment (macrophages, eosinophils, and neutrophils) was observed in the LPS-treated rats (Table 1). No increased mucin levels were detected using the lectin enzyme assay in BAL fluid samples from either group (Table 1). Increased levels of peribronchial and perivascular edema were determined by histology at 6 h after OA or LPS compared with saline instillation. Furthermore, whereas OA and LPS led to comparable levels of perivascular edema, peribronchial edema was significantly higher in OA-challenged lungs (Fig. 2, C and D). No significant differences in goblet cell numbers were observed between OA or LPS and the corresponding saline-challenged animals; however, actively sensitized rats presented higher levels of goblet cells than naïve animals (Fig. 2E).

DISCUSSION

Perhaps the most striking finding in the present study was the appearance of a marked MRI signal arising from the pleura 6 h after OA and a signal of lower magnitude following LPS challenge (Fig. 1, A and B). The MRI signal correlated with histological analysis revealing severe edema in the pleural cavity of OA-treated animals. LPS rats scored slight edema, and no abnormality was observed in saline- (vehicle-) treated, sensitized, or naïve rats (Fig. 1, C and D). The pleura is a target site for toxic effects induced by a variety of particles (15). A high density of mast cells is distributed throughout the lung pleura in rodents (37). Models of antigen-induced pleurisy based on intrapleural injection of OA in sensitized rats have been described (4, 33, 27). Furthermore, it has been shown *in vitro* that pleural mast cells are important contributors to histamine and leukotriene release (38). *In vivo* studies performed by Nagase et al. (30) in BN rats showed that 5-hydroxytryptamine (5-HT) and leukotriene (LTD₄), both known to be released by mast cells (14), are involved in the early response to OA in sensitized animals. Experiments in actively sensitized rats have also shown that intrathoracic OA administration (to the pleural cavity) resulted in an increase of leukocytes and protein concentration in the pleural cavity as early as 30 min after challenge, which was still detected 4 h later (13, 26).

Because of its innate close proximity to the lung, the pleura is prone to react to inflammatory events occurring in the lung parenchyma (2). Changes in pleural permeability leading to exudative pleural effusions with high protein content have been observed in local or systemic diseases (24). Exposure of pleural mesothelial monolayers to agents such as thrombin, LPS, or bacteria can induce changes in pleural permeability to proteins (1, 22). Thus it is plausible that local parenchymal inflammation induced by intratracheal OA or LPS challenge may lead to a disruption in pleural permeability, resulting in an increase of fluid in the pleural space. Moreover, migration of neutrophils, mononuclear phagocytes, and lymphocytes from the vascular compartment to the pleural space occurs during inflammation (12, 29, 34). Therefore, the migration of activated inflammatory cells to the pleural space following OA challenge may contribute further to allergen-induced pleurisy.

Intratracheal instillation of OA or LPS resulted in the appearance of similar irregular MRI fluid signals at 3 and 6 h after challenge with either substance. A larger fluid signal

Fig. 2. A: axial sections through the thorax of an actively sensitized rat treated with OA (0.3 mg/kg intratracheally) and a naïve rat treated with LPS (1 mg/kg intratracheally). The time points are relative to the intratracheal challenges. The white arrows show the appearance of diffuse fluid signals in the lungs. Inflammation of the pleura was apparent 6 h after OA challenge (black arrows). B: lung fluid signal volumes determined from MRI at baseline, 3 and 6 h after challenge in sensitized rats treated with saline or OA (0.3 mg/kg intratracheally) and in naïve rats treated with saline or LPS (1 mg/kg intratracheally). Values are means $\pm$ SE. ****P* < 0.001 shows that the value differs significantly from the value in the respective vehicle-treated group. \$*P* < 0.05 shows that the MRI signal volume 3 and 6 h after OA challenge in sensitized rats differs from each other. ###*P* < 0.001 refers to differences between OA and LPS at 3 and 6 h after challenge. C: histological sections (right) of a sensitized rat treated with saline (top) or OA (middle) and of a naïve rat treated with LPS (bottom) 6 h after challenge. The black contour outlines the smooth muscle layer of the bronchus; the red contour outlines the extent of peribronchial edema; and the black arrows show how the distance between both contours is increased after OA or LPS exposure. Left: peribronchial edema assessed from the left and right caudal lobes. Values (means $\pm$ SE for 6 animals in each group) represent edema percent. The edema in OA- and LPS-challenged rats was significantly greater [***0.001 < *P* < 0.01 and ****P* < 0.001; Student's *t*-test (1 tail)] than that in corresponding saline-treated animals. #*P* < 0.05 refers to differences in peribronchial edema between OA and LPS treatment. D: perivascular edema assessed from the left and right caudal lobes. Values (means $\pm$ SE for 6 animals in each group) represent edema percent. The edema in OA- and LPS-challenged rats was significantly greater [***0.001 < *P* < 0.01; ****P* < 0.001; Student's *t*-test (1 tail)] than that in corresponding saline-treated animals. E: goblet cell number (means $\pm$ SE, *n* = 6 rats per group) in individual bronchi determined from the left lobe, right median, and right caudal taken 6 h after challenge in sensitized rats challenged with saline or OA (0.3 mg/kg intratracheally) and in naïve rats treated with saline or LPS (1 mg/kg intratracheally). **P* < 0.05 shows that the goblet cell number between sensitized and naïve animals treated with saline differs from each other (Mann-Whitney's *U*-test).

Table 1. BAL fluid parameters

	Sensitized Rats		Naïve Rats	
	Saline (6)	OA (6)	Saline (6)	LPS (6)
Total cells $\times 10^6$	6.6 $\pm$ 0.6	25.4 $\pm$ 4.4 $\dagger$	7.9 $\pm$ 0.8	80.5 $\pm$ 3.9 $\ddagger$
Protein concentration, μ g/ml	0.2 $\pm$ 0.02	1.3 $\pm$ 0.2 $\dagger$	0.2 $\pm$ 0.04	0.6 $\pm$ 0.05 $\ddagger$
Eosinophils $\times 10^6$	0.4 $\pm$ 0.05	2.8 $\pm$ 0.6*	0.6 $\pm$ 0.1	4.3 $\pm$ 0.4 $\ddagger$
EPO, mU/ml	2.7 $\pm$ 0.3	53.2 $\pm$ 1.5 $\ddagger$	2.8 $\pm$ 1.1	16.8 $\pm$ 3.1 $\dagger$
Macrophages $\times 10^6$	3.5 $\pm$ 0.3	9.4 $\pm$ 1.6*	3.8 $\pm$ 0.2	10.6 $\pm$ 0.8 $\ddagger$
MPO, mU/ml	46.6 $\pm$ 7.8	10,16.3 $\pm$ 129.9 $\dagger$	54.2 $\pm$ 20.2	282.7 $\pm$ 34.6 $\dagger$
Neutrophils $\times 10^6$	2.36 $\pm$ 11.52	11.52 $\pm$ 2.2 $\dagger$	2.9 $\pm$ 0.9	65.6 $\pm$ 2.4 $\ddagger$
Mucin concentration, mU/ml	16.3 $\pm$ 4.3	20.1 $\pm$ 6.1	9.9 $\pm$ 2.2	12.3 $\pm$ 2.9

Values are means $\pm$ SE. Mucin concentration and inflammatory cell infiltration and activation detected in bronchoalveolar lavage fluid of sensitized animals treated with saline (0.2 ml it) or ovalbumin (OA) (0.3 mg/kg it) and naïve animals treated with saline (0.2 ml it) or LPS (1 mg/kg it). Animals were killed 6 h after challenge with either substance. * P < 0.05, $\dagger P$ < 0.01, and $\ddagger P$ < 0.001 refer to comparisons made between sensitized or naïve rats treated, respectively, with OA or LPS vs. their respective vehicle (saline)-treated control groups (t -test with Bonferroni correction). The number of animals used in each group is shown in parentheses.

volume was observed in allergen-treated animals. These MRI observations correlated with the determination of peribronchial edema by histology 6 h after challenge (Fig. 2, A–C). MRI detects the overall presence of edema, but due to limited spatial resolution, it is unable to discriminate between the perivascular and peribronchial components (8, 9). Early after challenge, the levels of perivascular edema were similar in the OA and LPS groups, but peribronchial edema was accentuated following allergen. This explains the increased MRI fluid signal volume in OA- compared with LPS-treated animals. However, at later time points, e.g. 24 or 48 h after challenge, the major contribution to the MRI signals may originate from perivascular edema (36).

BAL fluid analysis showed an increase of inflammatory parameters in both OA- and LPS-treated animals 6 h after challenge. LPS caused a greater influx of inflammatory cells than OA treatment, whereas the latter manifested increased levels of markers of inflammatory cell activation (EPO and MPO) and protein concentration. OA challenge in sensitized BN rats leads primarily to the release of inflammatory mediators derived from mast cell degranulation (18, 19, 16), of which 5-HT (31) and cystenyl leukotrienes (LTC₄, LTD₄, LTE₄) (14, 25) play an important role in the early time points after OA challenge (31). 5-HT is a potent inducer of microvascular leakage (6), and cystenyl leukotrienes are potent inducers of vascular permeability in the airways, leading to the formation of edema (3, 17). The release of these mast cell-derived proinflammatory mediators by OA may account for the increased levels of protein concentration and cellular activation (MPO and EPO) observed in BAL fluid in allergen- and not endotoxin-treated animals (Table 1). Furthermore, the increased levels of protein concentration and cellular activation (MPO and EPO) in OA-challenged rats may explain the increased peribronchial edema observed in allergen-treated animals compared with endotoxin challenge (Fig. 2C), although they had similar levels of perivascular edema (Fig. 2D).

MRI was sensitive enough to detect inflammation in the pleura and in the lung. Because the pleura is devoid of tissue-air interfaces, it is not surprising that even minor levels of edema gave rise to continuous, high intensity MRI signals in that area. However, irregular fluid signals of edematous origin were observed in the lung. On the basis of previous observations (10), mucus could be considered as the primary contrib-

utor to the signals. But, the facts that 1) significant levels of edema were detected both histologically and in terms of protein concentration in the BAL fluid, 2) no significant mucin was detected in the BAL, and 3) there was no significant increase of goblet cells or flocculent material in histological sections, indicate that the signals observed 3 and 6 h after challenge were most likely due to edema and not increased mucus production. Nevertheless, an increased goblet cell number was observed in sensitized animals compared with naïve animals. Such differences between sensitized and naïve animals have been described previously (32). Thus it is likely that early after challenge, OA and LPS activate a pathway that translates into the induction of a weak edematous response that develops further over 24 h in the OA model (8) and that precedes mucus release induced by LPS (10) observed at later time points.

The mucus-like appearance of the MRI lung edematous signals at 3 and 6 h following OA or LPS could possibly be explained by local magnetic field inhomogeneities produced by differences in magnetic susceptibilities between the lung tissue and air that comprise $\sim$ 80% of the pulmonary volume, resulting in very short T2* relaxation times [of the order of 500 μ s at 4.7 T (8)]. The present results suggest that for low levels of edema such as those present at these early points after challenge, the lung characteristics influence the T2* relaxation mechanism of the water. In other words, the presence of low fluid volumes in the lung can result in a signal appearance that does not allow a discrimination between edema and mucus. This is also the case when low volumes of saline are administered intratracheally, leading to signals of mucus-like appearance that disappear typically within 30 min following instillation. At later time points, e.g., 24 h following OA, the presence of marked edema ensures a relaxation mechanism dominated by free water that leads to a strong and uniform signal (8), thereby supplanting the influences of the lung characteristics on the signal behavior.

In summary, our results point to the opportunity of non-invasively studying with MRI the influence of pulmonary insults on inflammatory events in the pleura. Furthermore, histological analyses suggested that the early inflammatory responses following OA and LPS and detected by MRI in the lungs were primarily due to peribronchial edema.



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REFERENCES

1. **Antony VB.** Cytokines in pleural disease. In: *Cytokines in Pulmonary Infectious Disease: Pathogenesis and Therapeutic Strategies*. Edited by Nelson S and Martin T. New York: Dekker, 1998, p. 123–136.
2. **Antony VB.** Immunological mechanisms in pleural disease. *Eur Respir J* 3: 539–544, 2003.
3. **Arakawa H, Lötval J, Kawikova I, Löfdahl CG, and Skoogh BE.** Leukotriene D₄- and prostaglandin F₂-induced airflow obstruction and airway plasma exudation in guinea-pig: role of thromboxane and its receptor. *Br J Pharmacol* 110: 127–132, 1993.
4. **Bandeira-Melo C, Bonavita AG, Diaz BL, E Silva PM, Carvalho VF, Jose PJ, Flower RJ, Perretti M, and Martins MA.** A novel effect for annexin 1-derived peptide Ac2–26: reduction of allergic inflammation in the rat. *J Pharmacol Exp Ther* 313: 1416–1422, 2005.
5. **Barnes PJ.** Pathophysiology of asthma. *Br J Clin Pharmacol* 42: 3–10, 1996.
6. **Barnes PJ, Chung KF, and Page CP.** Inflammatory mediators of asthma: an update. *Pharmacol Rev* 50: 515–596, 1998.
7. **Barnes PJ.** New treatments for COPD. *Nat Rev Drug Discov* 1: 437–446, 2002.
8. **Beckmann N, Tigani B, Ekatodramis D, Borer R, Mazzoni L, and Fozard JR.** Pulmonary edema induced by allergen challenge in the rat: noninvasive assessment by magnetic resonance imaging. *Magn Reson Med* 45: 88–95, 2001a.
9. **Beckmann N, Tigani B, Mazzoni L, and Fozard JR.** MRI of lung parenchyma in rats and mice using a gradient-echo sequence. *NMR Biomed* 14: 297–306, 2001b.
10. **Beckmann N, Tigani B, Sugar R, Jackson AD, Jones G, Mazzoni L, and Fozard JR.** Noninvasive detection of endotoxin-induced mucus hypersecretion in rat lung by MRI. *Am J Physiol Lung Cell Mol Physiol* 283: L22–L30, 2002.
11. **Bradley SG.** Cellular and molecular mechanisms of action of bacterial endotoxins. *Annu Rev Microbiol* 33: 67–94, 1979.
12. **Broadbush VC, Herbert CA, Vitangcol RV, Hoeffel JM, Bernstein MS, and Boylan AM.** Interleukin-8 is a major neutrophil chemotactic factor in pleural liquid of patients with empyema. *Am Rev Respir Dis* 146: 825–830, 1992.
13. **Diaz BL, Serra MF, Alves AC, Cordeiro SB, Martins MA, and E Silva PM.** Local exposure to salbutamol or Bt2 cyclic AMP inhibits pleural exudation and leukocyte influx caused by antigen in rats. *Eur J Pharmacol* 296: 173–80, 1996.
14. **Foster A, Letts G, Charleson S, Fitzsimmons B, Blacklock B, and Rokach J.** The in vivo production of peptide leukotrienes after pulmonary anaphylaxis in the rat. *J Immunol* 141: 3544–3550, 1988.
15. **Gelzleichter TR, Bermudez E, Mangum JB, Wong BA, Moss OR, and Everitt JL.** Pulmonary and pleural responses in Fischer 344 rats following short-term inhalation of a synthetic vitreous fiber. II. Pathobiologic responses. *Fundam Appl Toxicol* 30: 39–46, 1996.
16. **Hannon JP, Tigani B, Williams I, Mazzoni L, and Fozard JR.** Mechanism of airway hyperresponsiveness to adenosine induced by allergen challenge in actively sensitized Brown Norway rats. *Br J Pharmacol* 132: 1509–1523, 2001.
17. **Henderson WR.** The role of leukotrienes in inflammation. *Ann Intern Med* 121: 684–697, 1994.
18. **Holgate ST, Benyon RC, Howarth PH, Agius R, Hardy C, Robinson C, Durham SR, Kay AB, and Church MK.** Relationship between

mediator release from human lung mast cells in vitro and in vivo. *Int Arch Allergy Appl Immunol* 77: 47–56, 1985.

19. **Kaliner M.** Asthma and mast cell activation. *J Allergy Clin Immunol* 83: 510–520, 1989.
20. **Karol MH.** Animal models of occupational asthma. *Eur Respir J* 7: 555–568, 1994.
21. **Kips JC, Tavernier J, and Pauwels RA.** Tumor necrosis factor causes bronchial hyperresponsiveness in rats. *Am Rev Respir Dis* 145: 332–336, 1992.
22. **Kroegel C and Antony VB.** Immunobiology of pleural inflammation: potential implications for pathogenesis, diagnosis, and therapy. *Eur Respir J* 10: 2411–2418, 1997.
23. **Lefort J, Singer M, Leduc D, Renesto P, Nahori MA, Huerre M, Creminon C, Chignard M, and Vargaftig B.** Systemic administration of endotoxin induces bronchopulmonary hyperactivity dissociated from TNF- α formation and neutrophil sequestration into the murine lungs. *J Immunol* 161: 474–480, 1998.
24. **Light RW and Rodriguez RM.** Management of parapneumonic effusions. *Clin Chest Med* 19: 373–382, 1998.
25. **Martin JG, Xu LJ, Toh MY, Olivenstein R, and Powell WS.** Leukotrienes in bile during the early and the late airway responses after allergen challenge of sensitized rats. *Am Rev Respir Dis* 147: 104–110, 1993.
26. **Martins MA, Lima MC, Bozza PT, Faria Neto HC, Silva PM, Sunkel CE, and Cordeiro RS.** Interference of the PAF receptor antagonist, PCA 4248, with the rat pleurisy evoked by inflammatory mediators or allergen. *Eur J Pharmacol* 237: 17–22, 1993.
27. **Medeiros MV, Macedo-Soares MF, De Luca IM, Hyslop S, De Nucci G, and Antunes E.** Contribution of C-fibers to leucocyte recruitment in bronchoalveolar lavage fluid and pleural cavity in the rat. *Eur J Pharmacol* 421: 133–140, 2001.
28. **Michie HR, Manogue KR, and Springgs DR.** Detection of circulating tumour necrosis factor after endotoxin administration. *N Engl J Med* 318: 1481–1486, 1988.
29. **Miller EJ and Idell S.** Interleukin-8: an important neutrophil chemotaxin in some cases of exudative pleural effusions. *Exp Lung Res* 19: 589–601, 1993.
30. **Nagase T, Lei M, Robatto FM, Eidelman DH, and Ludwig MS.** Tissue viscance during induced constriction in rabbit lungs: morphological-physiological correlations. *J Appl Physiol* 73: 1900–1907, 1992.
31. **Nagase T, Dallaire MJ, and Ludwig MS.** Airway and tissue behavior during early response in sensitized rats: role of 5-HT and LTD₄. *J Appl Physiol* 80: 583–90, 1996.
32. **Robinson PJ, Hegele RG, and Schellenberg RR.** Allergic sensitization increases airway reactivity in guinea pigs with respiratory syncytial virus bronchiolitis. *J Allergy Clin Immunol* 100: 492–498, 1997.
33. **Sampaio AL, Rae GA, and Henriques MM.** Role of endothelins on lymphocyte accumulation in allergic pleurisy. *J Leukoc Biol* 67: 189–195, 2000.
34. **Strieter RM, Standiford TJ, Huffnagle GB, Colletti LM, Lukacs NW, and Kunkel SL.** “The good, the bad, and the ugly”: the role of chemokines in models of human disease. *J Immunol* 156: 3583–3586, 1996.
35. **Tigani B, Schaeublin E, Sugar R, Jackson AD, Fozard JR, and Beckmann N.** Pulmonary inflammation monitored noninvasively by MRI in freely breathing rats. *Biochem Biophys Res Commun* 292: 216–221, 2002.
36. **Tigani B, Cannet C, Zurbrugg S, Schaeublin E, Mazzoni L, Fozard JR, and Beckmann N.** Resolution of the oedema associated with allergic pulmonary inflammation in rats assessed noninvasively by magnetic resonance imaging. *Br J Pharmacol* 140: 239–246, 2003.
37. **Wilkes LK, McMenamin C, and Holt PG.** Postnatal maturation of mast cell subpopulations in the rat respiratory tract. *Immunology* 75: 535–541, 1992.
38. **Wong WS, Spaethe SM, Henry DP, and Fleisch JH.** Contributory role of lung pleura to release of anaphylactic mediators from guinea pig lung in response to ovalbumin or A23187. *Biochem Pharmacol* 44: 1609–1615, 1992.