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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Abstract

Inflammatory effects in the rat lung have been investigated, non-invasively by magnetic resonance imaging (MRI), at early time points (3 and 6 h) after ovalbumin (OA) or endotoxin (lipopolysaccharide; 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, nor 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 upon both sites.

**Keywords:** edema; lipopolysaccharide (LPS); lung inflammation; magnetic resonance imaging; pleura
Introduction

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 autacoids, 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 neutrophils, 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 the 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 (lipopolysaccharide; 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 alpha (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 broncho-alveolar 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.

Methods and Materials

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 grams were used in the study. Animals were kept at an ambient temperature of 22 ± 2°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 16): OA (20 µg/ml) was mixed (30 min on ice) in a blender (Polytron, Kinematica Ltd.) with aluminum hydroxide (20 mg/ml) and injected subcutaneously (0.5 ml per animal). Injection of OA was repeated 14 and 21 days later. Sensitized animals were used in experiments between days 28 and 35.

Ovalbumin or Lipopolysaccharide Exposure
For challenge, animals were anaesthetized (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 intra-tracheally (i.t.) through a cannula positioned above the carina.
**MRI**

For MRI, rats were anaesthetized with 2% isoflurane in a mixture of O₂/N₂O, administered via a face mask. Animals were placed in supine position in a cradle made of plexiglas. Body temperature was maintained at 37±1°C using warm air, regulated by a rectal temperature probe (DM 852, Ellab, Copenhagen). 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, Karlsruhe, Germany). For detection of fluid signals, a T1-weighted, gradient-echo sequence with following parameters was used: repetition time, 5.6 ms; echo time, 2.7 ms; flip angle of the excitation pulse, approximately 15°; field-of-view, 6x6 cm²; matrix size, 256x128; and slice thickness, 1.5 mm. A single slice image was obtained by computing the 2DFT of the averaged signal from 45 individual image acquisitions and interpolating the data set to 256 x 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 anaesthetizing and positioning the animal, did not exceed 25 minutes.

**MR Image Analysis**

The volume of fluid signal was quantified using a semiautomatic segmentation procedure implemented in the IDL (Interactive Data Language Research Systems, Boulder, Colorado, USA) environment (version 5.1) on an SGI O2 (Silicon Graphics Inc., Mountain View, California, USA) system. Images were first weakly lowpass filtered with a Gaussian profile filter and then transformed into a set of four grey 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). Since the fluid comprised high signal intensities in the original images, it was represented by the highest grey 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 in 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 post-challenge 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.

Post Mortem Analyses
Rats were killed by an overdose of pentothal (250 mg/kg i.p.) 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 so as to include the main bronchi as well as the pulmonary alveoli. After processing 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 of 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 x10 magnification on PAS / Alcian Blue stained slides. Morphometric analyses were performed with the software “Image Access 4.0” (Imagic, Glattpbrugg, 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 x10 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 \( \mu m \) 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 x10 magnification on PAS/Alcian blue stained slides stained. The circumference of each bronchus was manually delineated and the number of goblet cells counted. Results were expressed as the number of goblet cells per mm length bronchus. Only bronchi whose diameters comprised between 900 and 2300 \( \mu m \) were measured. The total goblet cell numbers (sum of those in the left, right caudal and right median lobes) are presented.

**Bronchoalveolar Lavage (BAL) Fluid Analysis**

A detailed description of the BAL procedure and the analysis of the parameters of inflammation in the BAL fluid has 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, Switzerland). Determination of eosinophil peroxidase was based on the oxidation of o-phenylenediamine by eosinophil peroxidase in the presence of hydrogen peroxide. Myeloperoxidase activity was measured in a photometric assay based on the oxidation of O-dianiside dihydrochloride by myeloperoxidase in the presence of hydrogen peroxide. The level of protein in the bronchoalveolar lavage fluid supernatants was
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 Coastar 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* (one tail) was performed for MRI, BAL fluid and histological data referring to edema. The *Mann-Whitney 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 LPS treated rats (figure 1 a). Determination of pleural edema signal volume by MRI showed a significantly greater response in OA and LPS treated rats (figure 1 b). Histological assessment of the pleural cavity revealed a more pronounced pleurisy in the OA compared to the LPS group (figure 1 c, 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 MR images of the lung (figure 2 a). However, OA treatment resulted in higher levels of fluid volumes than LPS challenge at both time points (figure 2 b). Post mortem bronchoalveolar lavage fluid analysis, performed 6 h after challenge, revealed greater increases in protein concentration and markers of cellular activation (MPO and EPO) in OA-treated animals compared to the LPS-treated group, albeit greater inflammatory cell recruitment (macrophages, eosinophils, 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 to saline instillation. Furthermore, whereas OA and LPS led to comparable levels of perivascular edema, peribronchial edema was significantly higher in OA-challenged lungs.
(figure 2 c, 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 (figure 2 e).

Discussion

Perhaps the most striking finding in the present study was the appearance of a marked MRI signal arising from the pleura six hours after OA and a signal of lower magnitude following LPS challenge (figure 1 a, 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 (figure 1 c, 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 intra-pleural 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 shown also that intra-thoracic OA administration (to the pleural cavity) resulted in an increase of leucocytes and protein concentration in the pleural cavity as early as 30 minutes after challenge, which was still detected 4 hours later (13, 26).

Due to 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 i.t. 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.
Intra-tracheal 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 volume was observed in allergen-treated animals. These MRI observations correlated with the determination of peribronchial edema by histology 6 h after challenge (figure 2 a, b, c). MRI detects the overall presence of edema, but due to limited spatial resolution 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 to 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-treated 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 cysteoyl 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 cys-LTs are potent inducers of vascular permeability in the airways, leading to the formation of edema (3, 17). The release of these mast cell derived pro-inflammatory mediators by OA may account for the increased levels of protein concentration and cellular activation (MPO and EPO) observed in BAL fluid, of 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 to endotoxin challenge (figure 2 c), albeit similar levels of perivascular edema (figure 2 d).

MRI was sensitive enough to detect inflammation in the pleura and in the lung. Since 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. Based on previous observations (10), mucus could be considered as the primary contributor to the signals. But, the fact that (i) significant levels of edema were detected both histologically and in terms of protein concentration in the BAL fluid, (ii) no significant mucin was detected in the BAL, and (iii)
there was no significant increase of goblet cells or flocculent material in histological sections, indicates 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 to 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 which develops further over 24 h in the OA model (8), and which 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 comprises about 80% of the pulmonary volume, resulting in very short T2* relaxation times [of the order of 500 µs 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 i.t., leading to signals of mucus-like appearance that disappear within typically 30 minutes 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 upon 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.

Acknowledgements

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References


Table 1 - Mucin concentration and inflammatory cell infiltration and activation detected in BAL fluid of sensitized animals treated with saline (0.2 ml i.t.) or OA (0.3 mg/kg i.t.) and naïve animals treated with saline (0.2 ml i.t.) or LPS (1 mg/kg i.t.). Animals were killed 6 h after challenge with either substance. The significance levels *P < 0.05, **P < 0.01, and ***P < 0.001 refer to comparisons made between sensitized or naïve rats treated respectively with OA or LPS versus their respective vehicle (saline)-treated control groups (t-test with Bonferroni correction). The number of animals used in each group is shown in parentheses.

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<td>Protein concentration (µg/ml)</td>
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<td>EPO (mU/ml)</td>
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<td>20.1 ± 6.1</td>
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Figure 1

a) Image showing three different conditions: Vehicle 6 h, OA 6 h, and LPS 6 h.

b) Graph showing pleural fluid signal volume (ml) over time (3h and 6h).

- Saline Sens. rats
- Saline Naïve rats
- OA Sens. rats
- OA Naïve rats
- LPS Sens. rats
- LPS Naïve rats

c) Bar graph showing distance between visceral and parietal pleura (μm).
- Saline Sensitized rats
- Saline Naïve rats
- OA Sensitized rats
- OA Naïve rats
- LPS Sensitized rats
- LPS Naïve rats

d) Microscope images showing histological sections under different conditions.
Figure 2

(a) Representative images of lung fluid signal volume in sensitized (OA) and naive (LPS) rats at baseline, 3 h, and 6 h. Arrows indicate the increase in lung fluid signal volume.

(b) Graph showing the lung fluid signal volume in sensitized (OA) and naive (LPS) rats at 3 h and 6 h compared to saline controls. Significance levels are indicated by asterisks (*, **, ***).

(c) Histological images comparing airway hyperresponsiveness and goblet cell count in sensitized (OA) and naive (LPS) rats. Significance levels are indicated by asterisks (*, **).

(d) Graph showing the percent perivascular edema in sensitized (OA) and naive (LPS) rats compared to saline controls. Significance levels are indicated by asterisks (*, **, ***).

(e) Graph showing the goblet cell count in sensitized (OA) and naive (LPS) rats compared to saline controls.
Figure 1 – (a) Axial sections through the thorax of an actively sensitized rat treated with saline (top) or 0.3 mg/kg i.t. of OA (middle), and of a naïve rat treated with 1 mg/kg i.t. of LPS (bottom) acquired 6 hours after challenge. The white arrows show the appearance of pleurisy in OA and LPS treated animals. (b) Pleural fluid signal volumes determined from MR images acquired at 3 and 6 h after challenge in sensitized rats treated with saline (0.2ml i.t.) or OA (0.3mg/kg i.t.) and in naïve rats treated with saline or LPS (1 mg/kg i.t.). Values are means ± SEM. *** P<0.001 shows 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 differ 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 ± SEM) 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 hours 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 Students’ T-test (one tail) for figures (b) and (c).
**Figure 2 – (a)** Axial sections through the thorax of an actively sensitized rat treated with OA (0.3 mg/kg i.t.) and a naïve rat treated with LPS (1 mg/kg i.t.). The time points are relative to the intra-tracheal 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 MR images at baseline, 3 and 6 h after challenge in sensitized rats treated with saline or OA (0.3 mg/kg i.t.) and in naïve rats treated with saline or LPS (1 mg/kg i.t.). Values are means ± SEM. *** 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 differ from each other. ‡‡‡ P<0.001 refer to differences between OA and LPS at 3 and 6 h after challenge. **(c) Top:** Histological sections of a sensitized rat treated with saline (left) or OA (middle), and of a naïve rat treated with LPS (right) 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 arrow shows how the distance between both contours is increased following OA or LPS exposure. **Bottom:** Peribronchial edema assessed from the left, and right caudal lobes. Values (means ± SEM 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 [one tail]) than that in corresponding saline treated animals. # P<0.05 refer to differences in peribronchial edema between OA and LPS treatment. **(d)** Perivascular edema assessed from the left and right caudal lobes. Values (means ± SEM for 6 animals in each group) represent edema percent. The edema in OA and LPS and challenged rats was significantly greater (**0.001<P<0.01; *** P<0.001; Student’s t-test [one tail]) than that in corresponding saline treated animals. **(e)** Goblet cell number (means ± SEM, 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 i.t.) and in naïve rats treated with saline or LPS (1 mg/kg i.t.). * P<0.05 shows that the goblet cell number between sensitized and naïve animals treated with saline differ from each other (Mann-Whitney U test).