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Aerobic conditioning and allergic pulmonary inflammation in mice. II. Effects on lung vascular and parenchymal inflammation and remodeling

R. P. Vieira, V. F. de Andrade, A. C. S. Duarte, A. B. G. dos Santos, T. Mauad, M. A. Martins, M. Dolhnikoff and C. R. F. Carvalho

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Utility of magnetic resonance imaging and nuclear magnetic resonance-based metabolomics for quantification of inflammatory lung injury

N. J. Serkova, Z. Van Rheen, M. Tobias, J. E. Pitzer, J. E. Wilkinson and K. A. Stringer

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Lung inflammation and vascular remodeling after repeated allergen challenge detected noninvasively by MRI

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¹Discovery Technologies and ²Respiratory Diseases Research Department, Novartis Institutes for BioMedical Research, Basel, Switzerland; ³Sackler Institute of Pulmonary Pharmacology, King's College London, London, United Kingdom; and ⁴Faculty of Pharmacy, University Louis Pasteur-Strasbourg-I, Illkirch, France

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Tigani B, Cannet C, Karmouty-Quintana H, Blé F-X, Zurbrugg S, Schaublin E, Fozard JR, Beckmann N. Lung inflammation and vascular remodeling after repeated allergen challenge detected noninvasively by MRI. *Am J Physiol Lung Cell Mol Physiol* 292: L644–L653, 2007. First published November 3, 2006; doi:10.1152/ajplung.00122.2006.—Magnetic resonance imaging (MRI) has been used previously to follow noninvasively inflammatory processes in rat acute models of lung inflammation. Here the technique was applied to a model involving repeated intratracheal administration of ovalbumin (OA). Anatomical MRI was performed at different time points with respect to a single or multiple OA challenges in Brown Norway rats actively sensitized to the allergen. Vascular permeability was assessed using dynamic contrast-enhanced MRI (DCE-MRI). Bronchoalveolar lavage (BAL) fluid analysis and histology were performed to validate the MRI data. The time course of MRI signals after a single OA challenge reached a maximum at 48 h and decreased significantly at 96 h. After the second and subsequent challenges, the maximum signal occurred at 6 h with a time-dependent decline over the remainder of the time course. A reduction of the inflammatory response following repeated administration of OA was also detected by BAL fluid analysis. The decrease in vascular permeability assessed by DCE-MRI in repeatedly OA-challenged rats was consistent with the thickening of the vascular wall for vessels of diameter up to 300 μm revealed by histology. Angiogenesis of vessels smaller than 30 μm was also detected histologically. These results suggest that MRI can be used to detect the inflammatory response and vascular remodeling associated with chronic airway inflammation in rat models involving repeated administration of allergen. As the contrast agent used in the DCE-MRI experiments is approved for clinical use, there is potential to translate the approach to patients.

angiogenesis; asthma; chronic inflammation; magnetic resonance imaging; vessel remodeling

ASTHMA IS A COMPLEX CHRONIC inflammatory disease of the airways that involves the activation of many inflammatory cells, in particular mast cells, macrophages, and eosinophils (2, 3). Remodeling of the airway wall is a distinctive feature of asthma, characterized by hypertrophy and hyperplasia of airway smooth muscle, increase in mucous glands, thickening of the reticular basement membrane, and changes of airway blood vessels (13, 23). Although the presence of vascular remodeling in asthma and other airway diseases is well documented (20, 21, 24, 29, 32, 33, 36), the mechanisms and therapeutic implications of alterations in blood vessels of the airways are only beginning to be elucidated, and changes in the microvas-

culature still represent an important gap in the understanding of the pathophysiology of asthma and other chronic inflammatory airway diseases.

Airway inflammation and remodeling characterizing asthma occur not only in central airways but also in the distal lung and in the parenchyma. Along with the capacity to produce T helper type 2 (Th2) cytokines and chemokines (34), the distal airways have been found to be a major contributor to the airflow obstruction in asthmatic patients (9, 22, 57, 58, 60). Infiltration of inflammatory cells and release of Th2-type cytokines has also been detected in the lung parenchyma (27), and inflammation at distal sites is generally more severe than large airway inflammation (12, 14, 19, 27, 28, 34). Remodeling in the lung periphery has also been detected (11).

The model of repeated low-dose allergen challenge has been applied to rats to study the major features of chronic airways inflammation. Remodeling of distal airways has been reported for this model (38). In the present study, signals detected by magnetic resonance imaging (MRI) in the lungs of actively sensitized Brown Norway (BN) rats following repeated intratracheal (IT) instillation of allergen (ovalbumin; OA) have been quantified and compared with the inflammatory status of the lung reflected by the degree of inflammatory cell infiltration and activation in the alveolar space determined by bronchoalveolar lavage (BAL) fluid analysis and by the eosinophil infiltration assessed histologically. Because of the increasing interest in the understanding and characterization of vascular remodeling associated with chronic airway inflammation, we have also evaluated the possibility of assessing noninvasively vascular permeability in the parenchyma following repeated administration of OA by dynamic contrast-enhanced MRI (DCE-MRI). Histological analysis of the lung tissue was performed to define the vascular changes.

METHODS

Animals

Male BN rats (Iffa-Credo, L'Arbresle, France) weighing 250–300 g were used. They were housed in a temperature- and humidity-controlled environment, having free access to standard rat chow and tap water. All experiments were performed following the Swiss federal regulations for animal protection.

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Sensitization and Exposure

OA (20 $\mu\text{g/ml}$) was mixed (30 min on ice) with aluminium hydroxide (20 mg/ml) in saline and injected (0.5 ml/animal subcutaneously). Injection of OA, together with adjuvant, was repeated 14 and 21 days later.

For challenge with OA, animals were briefly anesthetized (4% Forene; Abbott, Cham, Switzerland) in an anesthetic chamber. OA (0.3 mg/kg dissolved in saline, 0.2 ml/animal) or vehicle (saline, 0.2 ml/animal) was administered IT, and the animals were allowed to recover.

Protocols

Actively sensitized rats were divided into the groups described below ($n = 7\text{--}12$ animals/group). All animals were imaged *in vivo* by MRI. Baseline MR images were acquired 48 h before the first challenge.

No exposure. Rats were examined by MRI and killed immediately after for histological analysis.

Single exposure. One week after sensitization, animals were given IT OA (0.3 mg/kg) or saline (0.2 ml IT). These animals were analyzed by MRI 24 h after challenge. They were killed immediately after the MRI session for BAL fluid analysis or histology.

Multiple exposures at 96-h interval. Rats were challenged four times with OA (0.3 mg/kg IT) or saline (0.2 ml IT), the first challenge taking place 1 wk after sensitization. The procedure adopted for a single exposure was repeated at 96, 192, and 288 h after the first challenge. For each animal, MR images were acquired 6, 24, 48, and 96 h after each OA challenge. Animals were killed after the 312-h acquisition (24 h after the 4th challenge) for BAL fluid analysis or histology.

MRI

Measurements were carried out with a Biospec 47/40 spectrometer (Bruker, Karlsruhe, Germany) operating at 4.7 T. A birdcage resonator of 7-cm diameter was used for excitation and detection. During MRI measurements, rats were anesthetized with 2–2.5% Forene in a mixture of $\text{O}_2/\text{N}_2\text{O}$ (1:2) administered via a face mask. The rats respired spontaneously during image acquisition, and neither respiratory nor cardiac triggering was applied. The body temperature of the animals was maintained at $37 \pm 1^\circ\text{C}$ by a flow of warm air regulated by a rectal temperature probe (DM 852; Ellab, Copenhagen, Denmark). Total examination time per animal, including positioning, ranged between 15 and 35 min, depending on the type of measurement.

Anatomical MRI. Anatomical MRI has been described in detail elsewhere (4). A gradient-echo sequence (16) with 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 was used throughout the study. A single slice image was obtained by computing the two-dimensional Fourier transform of the averaged signal from 60 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 75 s for a single slice. The entire lung was covered by 20 contiguous slices. The examination protocol for each animal consisted of acquiring a set of baseline images before the first challenge. Then, images were acquired at different time points after each OA challenge (see *Protocols* for details).

DCE-MRI. Vascular permeability was assessed by using a gradient-echo sequence and the following parameters: field-of-view $6 \times 10 \text{ cm}^2$; matrix size 64×128 ; slice thickness 6 mm; repetition time 2.21 ms; echo time 615 μs ; 28 averages. The coronal slab covered the whole lung, and acquisition time per image was 7.92 s. Twenty images were acquired sequentially. Immediately after acquiring the

tenth image, a bolus of Gd-DOTA (Dotarem diluted 1:16, 0.5 ml/kg; Guerbet, Aulnay-sous-Bois, France) was injected intravenously during ~ 1 s. The permeability surface area product was determined from the initial slope of the tracer uptake curves (52).

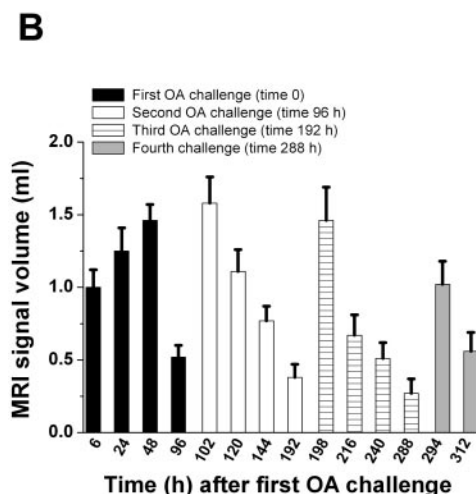
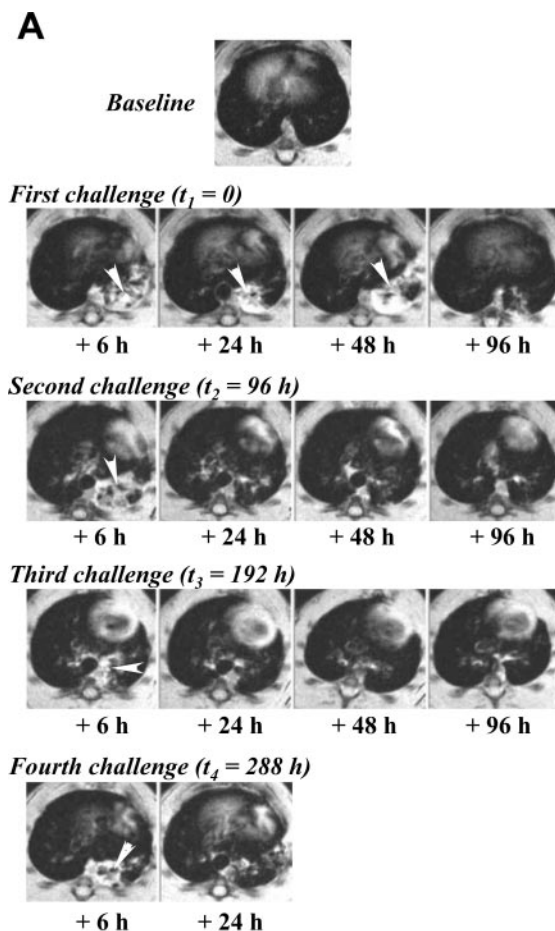


Fig. 1. A: axial sections through the thorax of an actively sensitized rat acquired at various time points following repeated intratracheal (IT) instillation of ovalbumin (OA) (0.3 mg/kg). The rat was challenged at time points 0, 96, 192, and 288 h. The animal respired freely during image acquisitions, and neither respiratory nor cardiac triggering was applied. The signal reflecting edema formation is indicated by the arrowheads. B: volume (means $\pm$ SE, $n = 4$) of fluid signals in the lungs determined from the magnetic resonance (MR) images. MRI, magnetic resonance imaging.

MR Image Analysis

The volume of the fluid signals was determined using a semiautomatic segmentation procedure described elsewhere (4).

BAL Fluid Analysis

A detailed description of the BAL procedure and the analysis of the parameters of inflammation have been provided (4, 51). Briefly, animals were killed with pentobarbital sodium (250 mg/kg ip), and the lungs were lavaged. For leukocyte numbers and cell differentiation, the automatic cell analyzing system was utilized (Cobas Helios 5Diff; Hoffmann-La Roche, Axon Lab). Determination of eosinophil peroxidase (EPO) was based on the oxidation of *o*-phenylenediamine by EPO 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 measured by a photometric assay, based on the reaction of protein with an alkaline copper tartrate solution and Folin reagent.

Histology

Immediately after MRI acquisitions, rats were killed by an overdose of pentobarbital (250 mg/kg ip). Lungs were fixed by slow in situ inflation (pressure of ~ 5 cmH₂O), with ~ 5 ml of 10% neutral phosphate-buffered formalin, pH 7.2, via the tracheal cannula. After removal from the thorax, lungs remained in formalin for a maximum of 72 h. Following fixation, lungs were trimmed, and three transverse sections were cut through the left lung (superior, median, and caudal parts) to include the main bronchi as well as the pulmonary alveoli.

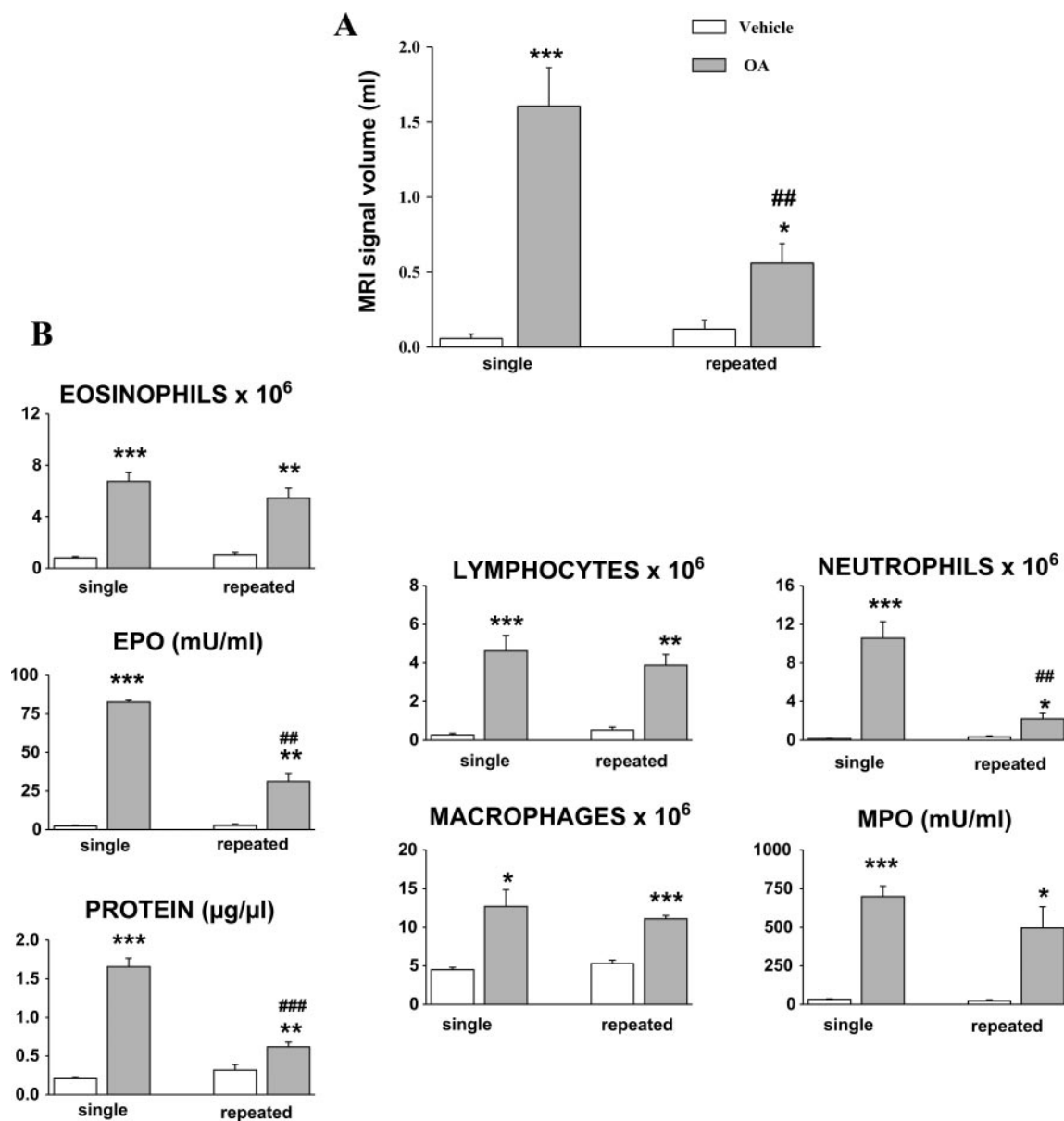


Fig. 2. A: volume of fluid signals detected by MRI in the lungs of actively sensitized Brown Norway (BN) rats 24 h after 1 or 4 IT instillations of vehicle or OA (0.3 mg/kg). B: inflammatory cell numbers, eosinophil peroxidase (EPO) and myeloperoxidase (MPO) activities, and protein concentrations detected in bronchoalveolar lavage fluid of the same animals. * $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$ indicates that the value is significantly different from the equivalent value in the vehicle-treated group. # $0.01 < P < 0.05$, ## $0.001 < P < 0.01$ indicates significant difference between singly and repeatedly OA-challenged rats. All values are expressed as means $\pm$ SE ($n = 6$ rats/group).

Sections were then dehydrated through increasing graded series of ethylic alcohol and embedded in one block of paraffin wax. Serial slices (3 μm) were cut and stained with 1) hematoxylin and eosin to assess the general morphology; 2) May-Grunwald Giemsa modified for paraffin sections to enable the quantification of eosinophils; 3) Verhoeff/Van Gieson's method for the demonstration of elastic fibers (measurement of vessel wall areas); and 4) immunohistochemical (IHC) staining with monoclonal antibody anti-smooth muscle actin (47) (counting of the number of blood vessels).

Eosinophils were counted around perivascular and peribronchial areas of each section (superior, median, and caudal) of the left lobe with a light microscope (Axioscop; Carl Zeiss, Feldbach, Germany) at $\times 400$ magnification using a 0.060-mm² calibrated grid. Approximately 20 grids were counted per section. For each animal, the mean numbers of peribronchial and perivascular eosinophils were calculated for the three sections.

Morphometric analyses were performed using the image analysis software Image Access 3.2 (IMAGIC, Glattbrugg, Switzerland). The stained slides were examined with a light microscope (DMR BE; Leica Microsystems, Glattbrugg, Switzerland) connected to a video digitizing camera (Prog/Res/3008; Jenoptic LOS, Eching, Germany). Fifteen pictures from the left lobe (5 of each part, i.e., superior, median, and caudal, respectively) were captured at 50-fold magnification on Verhoeff-stained slides. Approximately 60 arteries (diameters between 30 and 300 μm) were measured per lung. The areas of the external lamina elastica (EEL) and of the internal lamina elastica (IEL) were manually circumscribed. For each artery, the difference between the area of the EEL and IEL was calculated. A mean wall artery area was obtained for each animal.

Fifteen pictures from the left lobe (5 of each part, i.e., superior, median, and inferior, respectively) were captured at 100-fold magnification on IHC anti-smooth muscle actin-stained slides. Smooth muscle actin was demonstrated by IHC staining with monoclonal

antibody anti-human smooth muscle actin (M0851; Dako, Glostrup, Denmark) directed against smooth muscle cells, myofibroblasts, and myoepithelial cells. Briefly, microwave-assisted antigen retrieval was carried out for 20 min in 10 mM citrate buffer, pH 6.0. After being washed in TBS, endogenous peroxidase was quenched with 0.5% H₂O₂ in methanol for 20 min at room temperature. Nonspecific binding sites were blocked with 10% normal horse serum for 20 min at room temperature. The slices were further incubated overnight (room temperature) with primary antibody, anti-smooth muscle actin diluted 1/800 in 1% normal horse serum. Negative controls were incubated with mouse isotype control (Zymed, San Francisco, CA). After being washed in TBS, sections were incubated with biotinylated horse anti-mouse IgG, preabsorbed with rat serum (Vector, Burlingame, CA), followed by avidin-biotin horseradish peroxidase complex (Dako). Peroxidase activity was visualized by incubation in a solution of 3,3'-diaminobenzidine tetrahydrochloride (Mediate, Nunningen, Switzerland). Sections were counterstained with Mayer's hematoxylin and mounted with Pertex (Mediate). The internal diameter of vessels (veins and arteries) was manually delineated in each picture. Results were expressed as number of vessels sorted by internal diameter ranked in six categories: ≤ 10 μm , 11–20 μm , 21–30 μm , 31–40 μm , 41–50 μm , and ≥ 51 μm . All vessels were captured in the neighborhood of bronchi with diameters between 200 and 400 μm . For each animal, mean numbers of vessels were calculated for the 15 slices analyzed.

For assessment of perivascular edema, five to eight pictures of arteries from each part (i.e., superior, median, and inferior) of the left lobe were captured at $\times 10$ magnification on Verhoeff-stained slides. As before, morphometric analyses were performed with the software Image Access 3.2. 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 30 and 300 μm were mea-

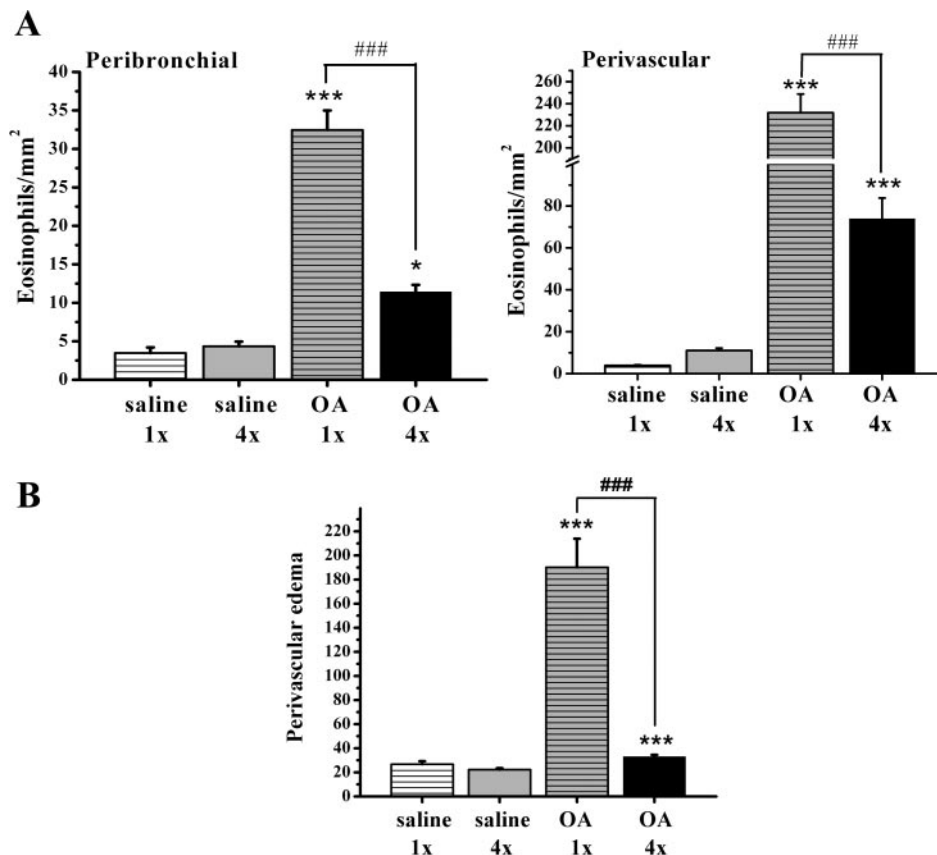


Fig. 3. A: peribronchial and perivascular eosinophil numbers (means $\pm$ SE, $n = 4$ rats/group) assessed histologically in actively sensitized animals 24 h after single or repeated challenge with allergen or saline. B: perivascular edema expressed as percent of vessel size determined by histology. The significance levels $*0.01 < P < 0.05$ and $***P < 0.001$ refer to statistical comparisons between 1) single OA (OA1x) and single saline (saline 1x) challenges and 2) reported OA (OA 4x) and reported saline (saline 4x) challenges. $###P < 0.001$.

sured. The total edema determined histologically is shown as a percentage.

Statistics

Mean values ($\pm$ SE) from n individual animals are presented. One-way ANOVA multiple comparison tests with the Bonferroni correction were carried out at each time point. Mann-Whitney non-parametric tests were applied to the histology data on angiogenesis. Student's t -test was also performed using vehicle-treated rats as the

control group. Significance was assumed at the 5% probability level. SigmaStat 3.1 (Systat Software, Point Richmond, CA) was used to perform ANOVA tests and t -tests, and SYSTAT 10.2 (Systat Software) was used to perform Mann-Whitney tests.

RESULTS

Figure 1A shows representative transverse sections through the thoracic region of actively sensitized BN rats before and at

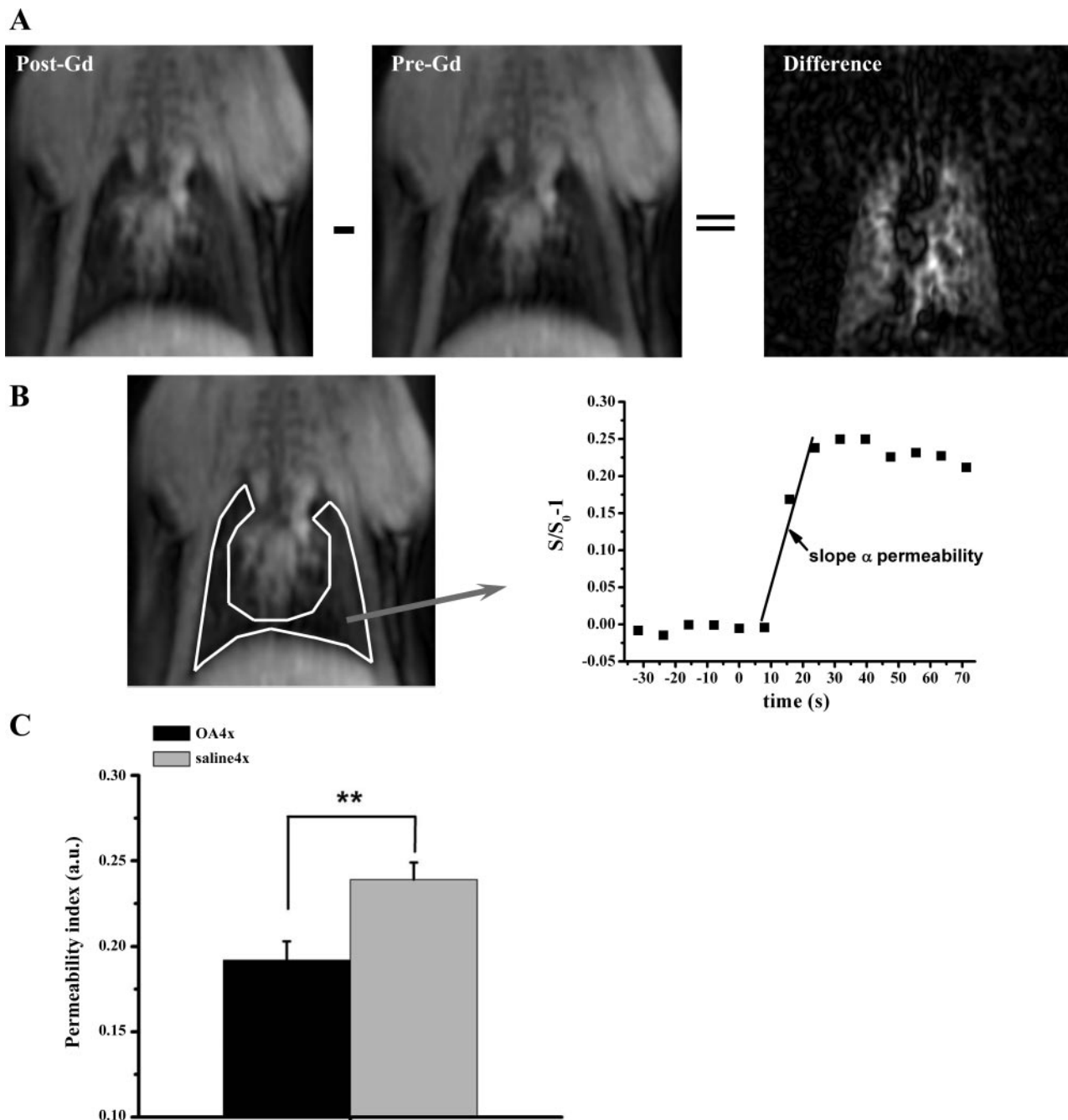


Fig. 4. A: coronal MR images acquired before and after intravenous administration of Gd-DOTA. The difference image shows that the contrast agent led to an increase of the signal in the lung parenchyma. B: mean image intensity was computed over a region-of-interest encompassing the entire lung (left) for the whole series of images comprising the dynamic contrast-enhanced MRI experiment. A relative permeability index was calculated from the steepness of the signal increase induced by Gd-DOTA (right). S_0 is the mean parenchymal signal intensity for the 10 images preceding administration of the contrast agent, and S is the mean signal intensity for a given image. C: Mean ($\pm$ SE, $n = 7$ rats/group) relative permeability was assessed in the lung. The level of significance $**0.001 < P < 0.01$ reflects statistical comparisons between multiple saline and OA challenges.

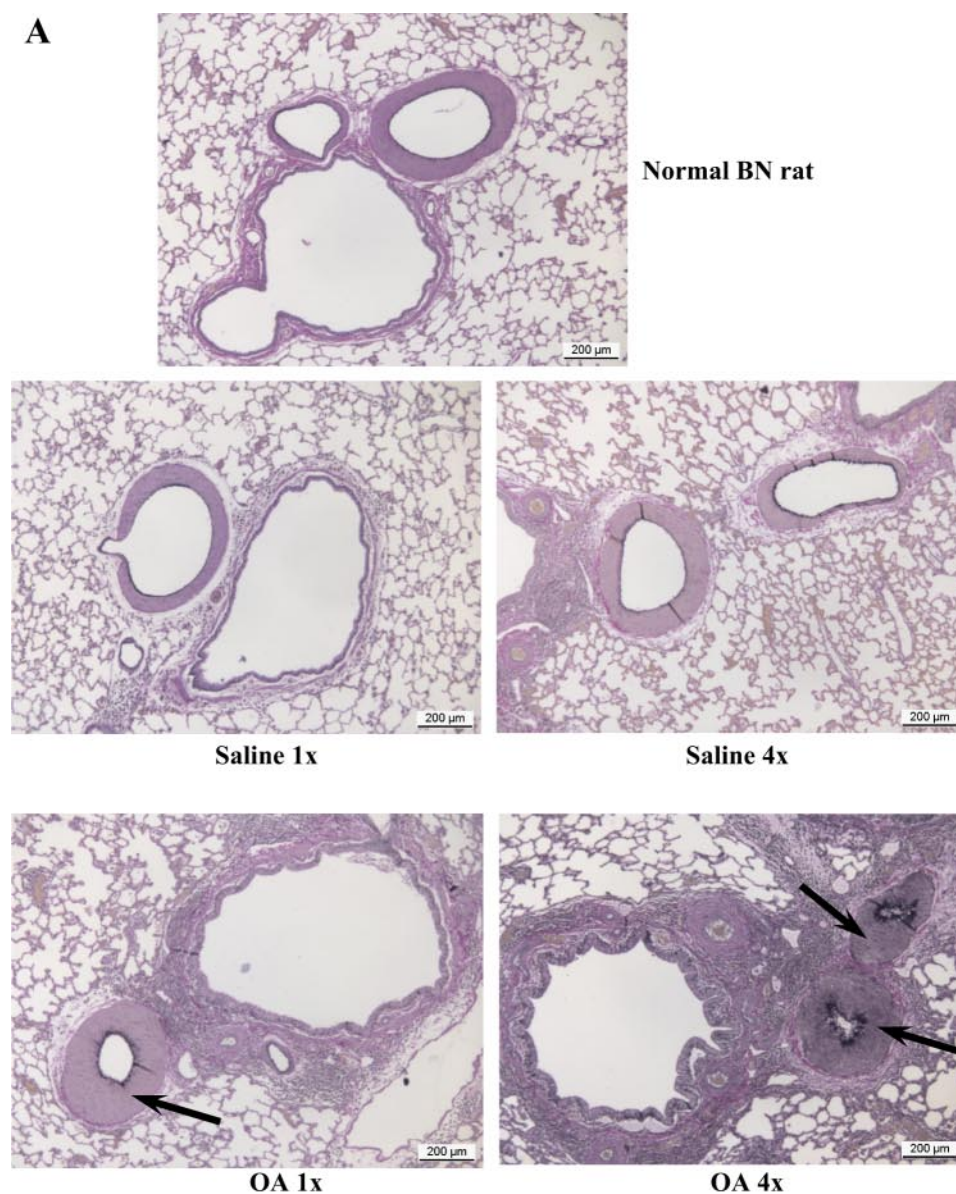
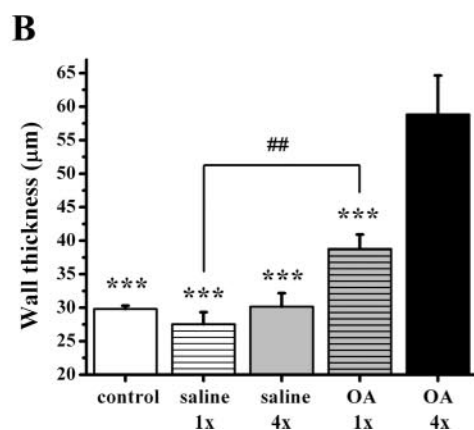


Fig. 5. *A*: histological analysis of vessel wall thickness. Animals were challenged once or 4 times every 96 h with either saline or OA. Lungs were removed immediately after MRI acquisition at 24 h after the last challenge. Tissue sections were stained with the Verhoeff/Van Gieson's method. Magnification is $\times 50$. Enlargement of the vessel wall is indicated by the arrow. *B*: wall thickness (means $\pm$ SE, $n = 7-8$ rats/group) of pulmonary vessels of BN rats assessed histologically. Control refers to nonactively sensitized (naïve) rats, whereas the other bars concern actively sensitized animals. Vessels analyzed had diameters between 30 and 300 μm . The significance levels *** $P < 0.001$ correspond to ANOVA comparisons with respect to repeatedly OA-challenged animals. ## $0.001 < P < 0.01$ represents the level of significance of a t -test comparison between the indicated groups of rats.



various time points following repeated IT instillation of OA (0.3 mg/kg) every 96 h. The corresponding mean volumes of fluid signals assessed from the MR images are presented in Fig. 1B. After the first challenge, signals were clearly evident at 6 h, progressed to a maximum at 48 h, and were significantly reduced at 96 h. After the second and subsequent OA challenges, development of the MRI signals followed a different course. The maximum volume of signals appeared at 6 h with a time-dependent decline over the rest of the time course (Fig. 1B).

Challenge of actively sensitized BN rats with OA led to an inflammatory response in the airways as assessed by changes in the BAL fluid leukocyte numbers, EPO and MPO activities, and protein concentrations measured 24 h postchallenge. Figure 2A shows that the volume of the signal detected by MRI at 24 h after the fourth OA challenge was reduced by 65% compared with the signal measured after a single challenge. Comparison of the BAL fluid markers revealed that eosinophil, macrophage, and lymphocyte numbers were similar in rats exposed either once or four times to OA (Fig. 2B). However, neutrophil numbers (−80%), EPO (−62%) and MPO (−30%) activities, and protein concentrations (−63%) were all significantly reduced in rats exposed four times at 96-h intervals ($P < 0.05$) (Fig. 2B). By contrast, in actively sensitized animals challenged with vehicle, no significant signal was detected by MRI 24 h following either a single or repeated challenge every 96 h (Fig. 2A). Furthermore, the levels of inflammatory markers in the BAL fluid were the same in rats challenged once or four times with saline (Fig. 2B).

Histology revealed peribronchial and perivascular inflammation following single OA challenge, determined by assessing eosinophil infiltration (Fig. 3A). Repeated OA instillation led to reduced eosinophil numbers compared with those elicited by a single challenge. This was reflected also in reduced perivascular edema determined histologically in repeatedly challenged lungs compared with single OA administration (3B).

For assessing the permeability of vessels, the contrast agent Gd-DOTA was administered intravenously as a bolus during the acquisition of a series of images. Administration of the contrast agent led to an increase of the parenchymal signal (Fig. 4A). The vessel permeability, estimated from the initial slope of the tracer uptake curves (Fig. 4B) (52), was significantly decreased in the lungs of rats challenged four times with OA compared with those challenged four times with saline (Fig. 4C). There were no differences in permeability between repeatedly vehicle-challenged rats and both singly OA- and vehicle-challenged animals.

Histological analysis performed on arteries having diameters between 30 and 300 μm revealed that repeatedly challenged, OA-treated animals exhibited a marked increase in vascular wall thickness compared with rats that received single or repeated vehicle administration as well as compared with singly OA-treated rats (Fig. 5). In addition, the mean thickness of the vascular wall after a single OA challenge was significantly different from that in singly vehicle-challenged animals.

An increased number of vessels smaller than 30 μm was observed in the lung tissue taken from singly and repeatedly OA-challenged rats compared with those assessed in saline-challenged and in nonchallenged, naïve control animals (Fig.

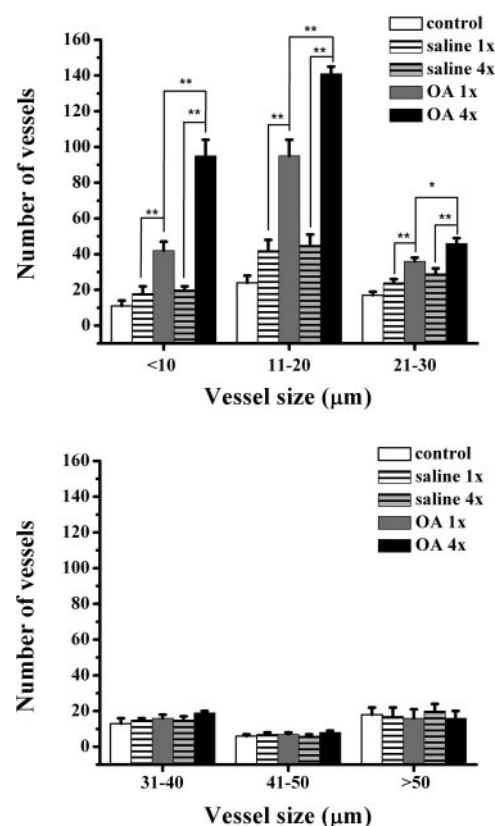


Fig. 6. Angiogenesis was quantified by histology in the lungs of BN rats under different treatment regimens. Control refers to nonactively sensitized (naïve) rats, whereas the other bars concern actively sensitized animals. All values are expressed as means $\pm$ SE ($n = 7$ rats/group). The levels of significance $*0.01 < P < 0.05$ and $**0.001 < P < 0.01$ correspond to statistical comparisons between the corresponding groups. For vessels smaller than 30 μm , there was a statistically significant difference between naïve controls and saline-challenged, actively sensitized rats.

6). Multiple OA challenge led to an increase in numbers of vessels smaller than 30 μm compared with a single instillation of OA. Compared with nonchallenged rats, saline administration led to a small increase in the number of vessels with sizes ranging between 11 μm and 30 μm . No change in the number of vessels was observed for diameters larger than 30 μm . These results suggest that a single or repeated administration of OA leads to angiogenesis of vessels smaller than 30 μm .

DISCUSSION

Actively sensitized BN rats exposed to allergen develop airway hyperresponsiveness and eosinophilic inflammation together with an increase in activated T cells (CD25+) in the airways (15, 18, 40), features that reflect key aspects of asthmatic inflammation. In this model, significant vascular leakage is found in the airway lumen, but not in the airway wall (35), thus accounting for the formation of edema that can be detected noninvasively using MRI (4, 50, 51). In the present experiments in which we have investigated with MRI the effects of repeated allergen administration, we have chosen a submaximal dose of OA (0.3 mg/kg). The choice of an interval of 96 h between two subsequent exposures was based on previous results showing that the inflammation detected as

fluid signals by MRI was markedly reduced at these time points after a single IT OA challenge of 0.3 mg/kg (4).

The time course of the development of MRI signals presented in Fig. 1B clearly shows a reduction in the overall lung inflammation after repeated allergen exposures compared with a single OA challenge. Parameters of inflammation in the BAL fluid revealed that EPO activity (a marker of eosinophil activation) and protein concentration (a marker of plasma extravasation into the alveolar spaces) were significantly decreased after four successive challenges of actively sensitized BN rats to OA. Furthermore, eosinophil, lymphocyte, and macrophage numbers in the BAL fluid over successive exposures remained unchanged with respect to the values after a single challenge. Histology demonstrated that peribronchial and perivascular eosinophil numbers following repetitive OA challenges were reduced compared with a single instillation. Our observations are consistent with other studies performed in guinea pigs (45), rats (15, 31), and mice (26) showing that repeated allergen challenges did not induce enhancement in numbers of cells in BAL compared with a single exposure. Reduction in eosinophilia (10) and lack of signs of activation of inflammatory cells in peripheral blood despite increased airway reactivity (42) were also observed in asthmatics following low, repeated doses of allergen. Adaptation or development of tolerance could be responsible for these effects (10, 26). Recent studies in mice (26) raised the possibility that immature myeloid dendritic cells generated under high IL-10 conditions, as in repeated allergen challenge (46), might induce T cell tolerance, thus playing a role in the resolution of airway hyperresponsiveness and airway allergic inflammation after repeated allergen challenge. Dendritic cells have been shown to play a role in the priming and activation of T lymphocytes (30, 56), thus having an important contribution in asthma pathogenesis. In vitro and in vivo studies demonstrated that IL-10 attenuates the differentiation and maturation of myeloid dendritic cells (17, 48).

By analogy to murine studies (49, 55), using histology we detected 1) angiogenesis and 2) remodeling of pulmonary vessels following single and repeated allergen challenge in the BN rat. Angiogenesis comprised vessels smaller than 30 μm . Vascular remodeling of pulmonary vessels having diameters between 30 and 300 μm involved a substantial increase of the vessel wall thickness. This result explains the decreased permeability assessed in vivo by MRI in repeatedly allergen-challenged rats compared with permeability indices obtained from animals exposed once to OA, since the leakage of the contrast agent from the intravascular to the extracellular space will be hindered at sites of increased vessel wall thickness (see next paragraph). Our histology and MRI observations are consistent with recent observations by Tormanen et al. (55) showing that central features of remodeling that take place in allergen-exposed airways are present also in pulmonary vessels. The mechanisms behind pulmonary vascular remodeling and its significance to the pathophysiology of allergic airway inflammation still need to be clarified. Increased levels of growth factors or of other mitogenic mediators present in inflamed regions (39) were suggested as possible effectors of hyperproliferative responses in OA-challenged airways and of the corresponding pulmonary vessel remodeling (55). Based on observations of eosinophilic inflammation in large pulmonary vessels in cases of fatal asthma, Saetta et al. (44) raised the possibility that pulmonary vascular inflammation may be in-

duced by "spillover" of proinflammatory mediators from adjacent bronchi.

To obtain information about the vascular system noninvasively using MRI techniques, two major types of DCE experiments may be used (25, 37, 41, 43). The first involves fast acquisitions at temporal resolutions ≤ 1 s/image aimed at quantitating blood flow and/or volume by analyzing the MR signal changes from the first pass through the vasculature of either an intravascular contrast agent (bolus tracking technique; Ref. 43) or of water spins with prepared magnetization (arterial spin labeling; Ref. 59). This kind of approach could be applied to derive information on angiogenesis in the lung. However, because of respiratory movements, it would be necessary to artificially ventilate and paralyze the rats. The ventilator could then be turned off for ~ 10 – 15 s, which would provide enough time for the measurement of the first passage of the contrast agent and therefore allow perfusion assessments. Since we wanted to avoid such invasive procedures in our study, we restricted ourselves to assessments of vessel permeability using a second type of DCE-MRI experiment. In this instance, images are acquired at slower time resolution (2–20 s/image) during the administration of contrast material [chelates of gadolinium(III), e.g., Gd-DOTA] to determine physiological parameters of the tissue vasculature and interstitial space (extracellular-extravascular space) by analyzing the contrast agent's leak into tissue. The signal changes due to residual contrast agent leaking into tissue after a bolus provides a measure for the permeability of vessels (52–54).

In conclusion, our data show that repeated allergen challenge at a dose sufficient to induce a response after a single instillation led to a reduced inflammatory status of the lungs of actively sensitized BN rats. The signals detected by MRI closely reflected the degree of inflammation as determined by BAL fluid analysis. We have also demonstrated that MRI can be used to monitor noninvasively vascular remodeling associated with chronic airway inflammation in this model, implied by reduced leakage due to increased vessel wall thickness following repeated allergen challenge. Our approach represents a useful means of profiling drugs with the aim being to reverse both inflammatory and structural changes in asthma (6–8). The MRI signals reflect a combination of responses of the distal and the proximal areas of the lung. As insufficient spatial resolution and specificity prevent a distinction between the proximal and the distal inflammation components, the technique provides an overall picture of the inflammatory response induced in the lung by allergen challenge.

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