

# Ultrashort-TE MRI longitudinal study and characterization of a chronic model of asthma in mice: inflammation and bronchial remodeling assessment

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Asthma is a chronic disease characterized by bronchial hyperresponsiveness (BHR), bronchial inflammation and remodeling. The great improvements in  $^1\text{H}$  MRI ultrashort-TE (UTE) sequences in the last decade have allowed lung images with high-resolution and good signal-to-noise ratio to be obtained in parenchymal tissues. In this article, we present a UTE  $^1\text{H}$  MRI high-resolution study of a chronic model of asthma in mice with the aim to longitudinally assess the main features of asthma using a fully noninvasive approach. Balb/c mice ( $n=6$ ) were sensitized with ovalbumin over a period of 75 days. The control group ( $n=3$ ) received normal saline on the same days. MRI acquisitions were performed on days 0, 38 and 78 to study the inflammatory volumes and bronchial remodeling (peribronchial signal intensity index, PBSI). Plethysmographic studies were performed on days 0, 39 and 79 to assess BHR to methacholine using the enhanced pause (Penh) ratio. The average inflammatory volume measured by MRI in the ovalbumin group ( $15.6 \pm 2.4 \mu\text{L}$ ) was increased significantly relative to control mice ( $-0.3 \pm 0.7 \mu\text{L}$ ) on day 38. The inflammatory volume was larger ( $34.2 \pm 3.1 \mu\text{L}$ ) on day 78 in the ovalbumin group. PBSI was significantly higher in the ovalbumin group on day 78 ( $1.53 \pm 0.08$ ) relative to the control group ( $1.16 \pm 0.10$ ), but not on day 38. After sensitization, asthmatic mice presented BHR to methacholine on days 39 and 79. Penh ratios correlated significantly with the inflammatory volume on day 39 and with the PBSI on day 79. This study shows, for the first time, that high-resolution UTE  $^1\text{H}$  MRI of the lungs may allow the noninvasive quantification of peribronchial eosinophilic inflammation with airways occlusion by mucus and of bronchial remodeling in a murine asthma model that correlates with functional parameters. Copyright © 2013 John Wiley & Sons, Ltd.

**Keywords:** lung  $^1\text{H}$  MRI; UTE; asthma; bronchial remodeling; inflammation

## INTRODUCTION

Asthma is a common chronic disease characterized by bronchial hyperresponsiveness (BHR), bronchial inflammation and remodeling (1,2). In asthma, bronchial inflammation involves the recruitment of a large range of inflammatory cells within the airways, such as eosinophils, mast cells and T lymphocytes. Bronchial remodeling includes a variety of structural changes, such as epithelial injury, subepithelial membrane thickening, mucous gland and goblet cell hypertrophy and hyperplasia, increase in smooth muscle mass, enhanced extracellular matrix deposition and neoangiogenesis (2–10). Such bronchial remodeling is associated with lung function decline and remains insensitive to current asthma treatments (11). Both bronchial inflammation and remodeling lead to an increased thickening of the bronchial wall, but the precise sequence of such histological changes in the natural course of the disease continues to be a matter of debate.

Many experimental murine models of sensitization (12–16) have been proposed to mimic the main features of human asthma in order to improve the understanding of the disease (17,18). Nevertheless, most of these models fall short of reproducing a number of human asthma hallmarks (18,19). Moreover, the evaluation of bronchial inflammation and remodeling often requires histological studies, and thus the sacrifice of animals.

In this context, current imaging-based techniques allow the noninvasive assessment of several features of asthma pathophysiology (20,21). For instance, micro-computed tomography ( $\mu\text{-CT}$ ), thanks to its high resolution, has provided important information on the structural and physiological changes in asthma (21,22). In particular, Lederlin *et al.* (23,24) have recently assessed bronchial remodeling in a validated model of murine asthma.

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**Abbreviations used:** BHR, bronchial hyperresponsiveness;  $\mu\text{-CT}$ , micro-computed tomography; ICC, intraclass correlation coefficient; LA, lumen area; OVA, ovalbumin; PBSI, peribronchial signal intensity index; Penh, enhanced pause; PS, peribronchial signal; ROI, region of interest; SEM, standard error of the mean; UTE, ultrashort TE.

However, MRI presents various advantages compared to  $\mu$ -CT, including the absence of ionizing radiation, strong tissue contrast and versatility in the wealth of information obtained (25). Nevertheless, the living lung is one of the most challenging organs to image with MRI because of its intrinsic properties (low proton density, sharp magnetic susceptibility gradients and motion artifacts), and special expedients are often necessary in order to fully exploit the MRI potential (22,25). In this sense, the use of hyperpolarized gas MRI has allowed the knowledge on asthmatic regional ventilation obstructions and defects over short and long time scales to be broadened (20,21,25–32). Although this information is of primary importance, the routine application of hyperpolarized gas MRI and its extension to pharmacological studies in rodents are difficult, in particular because of the high costs of the technique, the complex instrumentation required for the administration of gas and respiration control (often not commercially available) and the interference with the pathophysiology of the disease (as a result of artificial ventilation), which can hamper repeated measurements (20,25).

In the past decade, Beckmann *et al.* (25,33–36) have shown that standard  $^1\text{H}$  MRI, combined with averaging approaches, can be used to detect and quantify accurately edematous signals in small rodent models of airway diseases without the need for respiratory or cardiac gating. Such studies were able to shed light on asthmatic inflammation mechanisms and evolution on a short time scale, taking advantage of the absence of background signal achievable using gradient echo sequences at TEs of a few milliseconds. Similarly, using gradient echo sequences with short TE ( $\sim 500\ \mu\text{s}$ ), Beckmann *et al.* (33) were able to show that lung parenchyma changes can be detected with standard approaches and related to pathophysiological structural changes in the lung tissue of diseased animals.

Nevertheless, gradient echo sequences are unable to provide good spatial resolution during the detection of the lung parenchyma signal, as spatial resolution needs to be sacrificed in order to reduce TE. However, the great improvements in ultrashort-TE (UTE) sequences in the last decade have made it possible to obtain lung images with high spatial resolution and good signal-to-noise ratio simultaneously in parenchymal tissues (37–39).

To exploit such advantages over standard sequences, we present here a study demonstrating the potential application of high-resolution UTE  $^1\text{H}$  MRI for the assessment, longitudinally and noninvasively, of peribronchial inflammation and bronchial remodeling in a chronic murine model of asthma in mice.

## MATERIALS AND METHODS

### Animals

Female Balb/c mice ( $n=9$ ), aged 6 weeks and weighing  $22.4 \pm 0.4\ \text{g}$ , were purchased from Elevage Janvier (Le Genest, Saint Isle, France). Before the start of the experiment, the animals were acclimatized in a temperature-controlled environment for 1 week. Experiments were carried out following the Institut National de la Santé et de la Recherche Médicale guidelines regarding the fair treatment of animals.

### Protocol

The general scheme of the study is shown in Fig. 1. Briefly, the protocol was composed of a sensitization procedure to induce the chronic asthma model, three MRI acquisitions to study

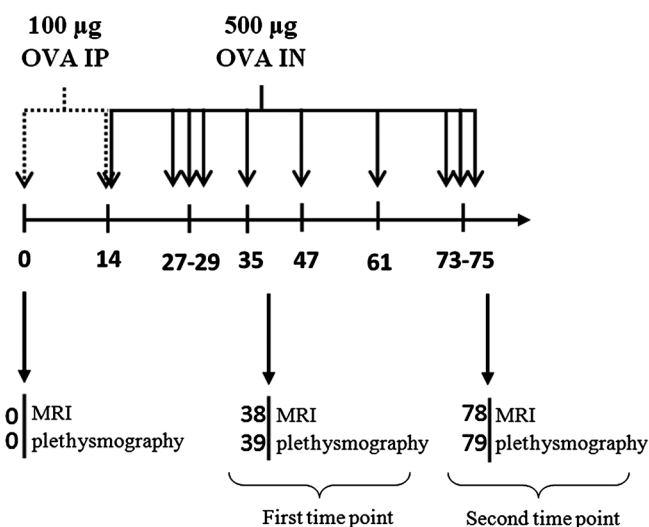


**Figure 1.** Scheme of the general protocol used in the present investigation, composed of a sensitization procedure to induce the chronic asthma model, three MRI acquisitions to study inflammation and bronchial remodeling, and three plethysmographic acquisitions to assess the bronchial hyperresponsiveness (BHR) of mice. The control group underwent the same protocol with saline instead of ovalbumin (OVA). Two time points have been identified and studied at days 38–39 and 78–79. IN, intranasally; IP, intraperitoneally.

bronchial inflammation and bronchial remodeling at different time points, and three plethysmographic acquisitions to assess BHR to methacholine after MRI measurements. The weight of the animals was measured at each time point.

### Chronic murine asthma model

Six mice (OVA group,  $n=6$ ) were sensitized by two intraperitoneal injections of 100 µg of ovalbumin (OVA; Sigma-Aldrich, Saint-Quentin-Fallavier, France) on days 0 and 14 in the absence of aluminum hydroxide. The mice were anesthetized using an intraperitoneal injection of 50 µg/g ketamine (Panpharma, Fougères, France) and 5 µg/g xylazine (Sigma-Aldrich). They were then challenged intranasally with 500 µg of OVA on days 14, 27, 28, 29, 47, 61, 73, 74 and 75, as described by Henderson *et al.* (12) and Lederlin *et al.* (23,24). The control group (controls,  $n=3$ ) received normal saline intraperitoneally or intranasally on the same days.

### MRI

The images were acquired on days 0, 38 and 78 with a 4.7-T Biospec 47/50 spectrometer (Bruker, Ettlingen, Germany), using a transmitter/receiver quadrature coil with an inner diameter of 25 mm (Bruker). The mice were placed prone in a custom-built plastic holder and kept anesthetized with 2% isoflurane in a mixture of  $\text{N}_2\text{--O}_2$  (80 : 20) via a facial mask. The body temperature was kept constant using warm circulating water, and the respiratory cycle was monitored continuously using a pressure sensor placed on the abdomen of the mice.

### Inflammation study

For each animal, 12 consecutive axial slices, 1 mm thick, were acquired in an interleaved scheme to cover the whole lung volume. The acquisition was performed in free-breathing animals, using

a two-dimensional multislice UTE sequence (804 directions/128 points, four averages) with TE = 276  $\mu$ s, TR = 200 ms, bandwidth of 50 kHz, flip angle of 30° and field of view of 3 cm  $\times$  3 cm. The acquisition was preceded by 120 dummy scans. Free induction decay acquisition was started during read gradient ramping, immediately after the Gaussian-shaped radiofrequency slice excitation (duration, 0.3 ms) and the slice refocusing gradient. The total acquisition time was approximately 10 min.

#### Bronchial remodeling study

For each animal, 14 axial slices, 0.5 mm thick, were acquired in an interleaved scheme (with an interslice distance of 0.7 mm) to image the lungs with high resolution. The acquisition was performed in the free-breathing condition, using a two-dimensional multislice UTE sequence (804 directions/128 points, four averages) with TE = 343  $\mu$ s, flip angle of 30°, bandwidth of 50 kHz, field of view of 2.5 cm  $\times$  2.5 cm and TR = 200 ms. The acquisition was preceded by 140 dummy scans and the total acquisition time was approximately 10 min.

#### Image reconstruction and analysis

The images acquired with the UTE sequence were reconstructed with in-house software implemented in IDL (RSI, Boulder, CO, USA). Following the application of a regridding algorithm and a weighting function (40), the images were interpolated to a 512  $\times$  512 image matrix. Images were saved and analyzed with freeware medical image analysis software (MIPAV, National Institutes of Health, Bethesda, MD, USA).

#### Inflammation study

The volume of the peribronchial inflammation in the lungs was quantified with a semiautomatic segmentation procedure. In particular, a low-intensity threshold was applied to the images to exclude the noninflamed regions of the lung. The peribronchial inflammation was contoured with a semiautomatic tool able to generate contours whilst moving over the different structures of the lung, recognizing the level of intensity of each pixel and selecting regions according to the default preset parameters (MIPAV 'LevelSet' active-contour algorithm). The total volume of high-intensity signals was computed by multiplying the slice thickness by the sum of all the areas segmented in the 12 consecutive slices. As the signal intensity caused by OVA sensitization was

comparable with that produced by the vessels, a baseline was obtained on day 0 before the beginning of the protocol. The computed volume (corresponding to the vessels) was subtracted from that computed on days 38 and 78 (corresponding to vessels and inflammation) after OVA challenge (34,36). Typical images acquired in asthmatic mice at the different time points are shown in Fig. 2a–c, together with the semiautomatically segmented contours.

#### Bronchial remodeling study

For each mouse, the 14 slices were analyzed in random order by two independent observers to identify the visible bronchi. Only the bronchi identified by both observers were used in the subsequent study in order to avoid false positives. The bronchi were selected in areas sufficiently distant from the heart to avoid any possible effect of residual cardiac motion artifacts.

On average, nine bronchi per mouse were segmented at each time point. For each bronchus, the bronchial lumen was manually delineated and the lumen area (LA) was measured. Because the outer limit of the bronchial wall was not clearly identifiable in asthmatic mice, a ring-shaped region of interest (ROI) was drawn around the lumen in order to measure the peribronchial signal intensity normalized by the signal measured in the muscle (33) (PS for peribronchial signal), as shown in Fig. 3 for controls and asthmatic mice. The width (outer radius minus inner radius) of the ring-shaped ROI was chosen to be equal to the radius of the bronchial lumen, as peribronchial infiltration has been shown to grossly scatter just beyond this limit in a previous histological study using the same murine asthma model (12,23).

From LA and PS, a peribronchial signal intensity index (PBSI) was computed by assigning to each of the studied bronchi a weight depending on its LA [ $\text{PBSI} = \Sigma(\text{PS} \times \text{LA}) / \Sigma \text{LA}$ ].

The measurement procedure was performed on the selected bronchi by only one observer. Nevertheless, an intra- and interobserver agreement study was performed on the peribronchial signal and LA measurements to test the reproducibility. The measurements were performed by two observers (one repeating the analysis after 6 months) on  $n = 30$  randomly selected bronchi.

#### Plethysmography

BHR to methacholine (Sigma-Aldrich) was determined in unrestrained conscious mice by single-chamber plethysmography

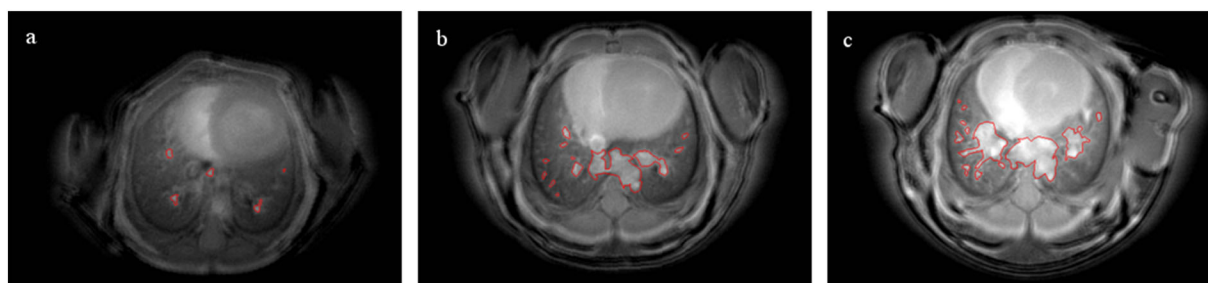


**Figure 2.** Typical ultrashort-TE (UTE) MRI axial slices acquired in asthmatic mice to quantify the inflammatory volume (804 directions/128 points; flip angle, 30°; TR/TE = 200/0.276 ms; four averages; slice thickness, 1 mm; field of view, 3 cm  $\times$  3 cm) at different time points. (a) The baselines (vessels) were acquired on day 0 before the sensitization protocol began, and the vessels and inflammatory areas were segmented from the images acquired on days 38 (b) and 78 (c). The differences between the computed volumes on days 38 and 78 and the baselines were obtained to compute the total inflammatory volumes in all the mice. The contours delineate the segmented areas (vessels and inflammation) at the different time points.

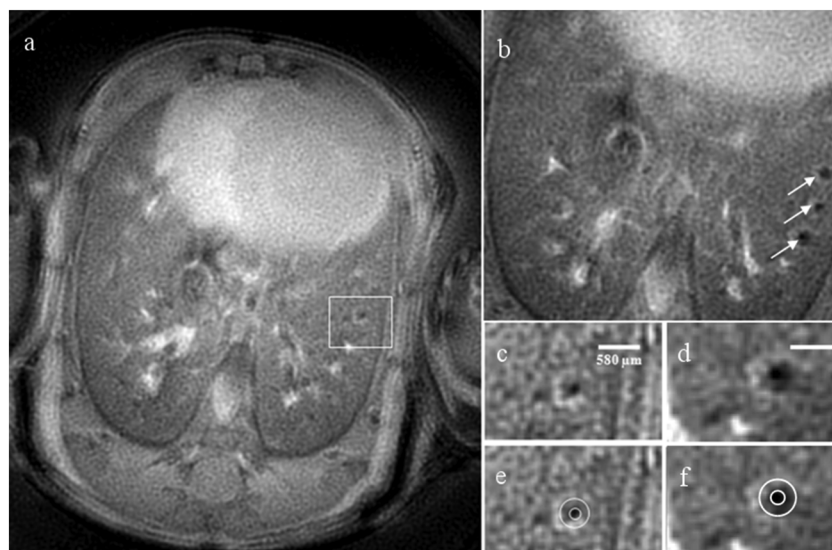


**Figure 3.** Typical ultrashort-TE (UTE) MRI axial slices acquired in asthmatic (a) and control (b) mice to quantify the bronchial remodeling on day 78 (804 directions/128 points; flip angle, 30°; TR/TE = 200/0.343 ms; four averages; slice thickness, 0.5 mm; field of view, 2.5 cm × 2.5 cm). The arrows in (b) underline the three bronchi selected for the analysis in a typical procedure of bronchi identification. The zoom on asthmatic (c) and control (d) mouse bronchi and the respective typical segmentation procedure used (e, f) to measure the lumen area (LA) and peribronchial signal (PS) [from which the peribronchial signal intensity index (PBSI) is computed] are shown. The scale bars correspond to 580 μm.

(Emka Technologies, Paris, France). The enhanced pause (Penh) parameter (41) was measured and averaged for 3 min, 90 s after each successive inhalation of a precise dose of aerosolized methacholine (8 mg/mL) (23,42,43). These measurements were performed for all mice after MRI acquisitions on days 0, 39 and 79. The ratio of Penh measured in response to 8 mg/mL methacholine to that with normal saline was used to quantify the BHR. This ratio is referred to as the 'Penh ratio' or 'plethysmographic index' hereafter.

### Statistical analysis

The comparison of functional data between the OVA and control groups for inflammatory volumes, BHR and bronchial remodeling was performed using Wilcoxon's signed-rank test. The comparison of functional data between the same groups (OVA or controls) at different time points was performed using the Kruskal–Wallis nonparametric test with Dunn's multiple comparison. Correlations between inflammatory volumes or PBSI and functional hyperresponsiveness were performed using Spearman's correlation test.

The intra- and interobserver agreements of LA and PS were evaluated by Spearman correlation coefficient, intraclass correlation coefficient (ICC) and Bland–Altman plot analysis (means of the two measurements plotted against their differences). The bias was estimated from each Bland–Altman plot as the mean of the differences, and the 95% limits of agreement were computed from the standard deviation of the differences between the measurements.

Significance was fixed at the 5% probability level. All analyses were performed using GraphPad Prism (GraphPad Software Inc., La Jolla, CA, USA). All data are presented as the mean value ± standard error of the mean (SEM), except for the Bland–Altman analysis (mean value ± standard deviation).

## RESULTS

All the animals survived until the end of the protocol. The weights of the animals were statistically identical in the two populations (OVA and control groups) at all time points.

### MRI inflammation study

As shown in the bar plot of Fig. 4a, the average volumes of inflammation measured in the OVA-challenged group ( $15.6 \pm 2.4 \mu\text{L}$ ) and in the control mice ( $-0.3 \pm 0.7 \mu\text{L}$ ) on day 38 differed significantly in the two populations ( $p < 0.024$ ). A larger inflammatory volume ( $34.2 \pm 3.1 \mu\text{L}$ ) was measured on day 78 in asthmatic mice, resulting in a significant difference relative to the inflammatory volume measured in the controls on the same day ( $2.8 \pm 1.1 \mu\text{L}$ ). No statistically significant difference in the inflammatory volume was measured in the control mice at the different time points ( $p > 0.11$ ). However, a significant increase in the inflammatory volume in the OVA group was observed from day 38 to day 78 ( $p < 0.001$ ).

### MRI bronchial remodeling study

LA was not significantly different between the OVA-challenged and control groups at any time point analyzed. As shown in Fig. 4b, PBSI was significantly higher ( $p < 0.024$ ) for the asthmatic group on day 78 ( $1.53 \pm 0.08$ ) relative to the controls ( $1.16 \pm 0.10$ ). No difference between the groups was observed on day 38.

No statistically significant difference in PBSI was measured in the control mice at the different time points ( $p > 0.83$ ). Contrastingly, a significant increase in PBSI in the OVA group was observed from day 38 to day 78 ( $p < 0.038$ ).

LA and PS measurement agreement over time and across observers was excellent, as shown in Table 1 and Fig. 5 (always  $p < 0.001$ ). The intraobserver bias was minimal and the interobserver bias was negligible (Table 1).

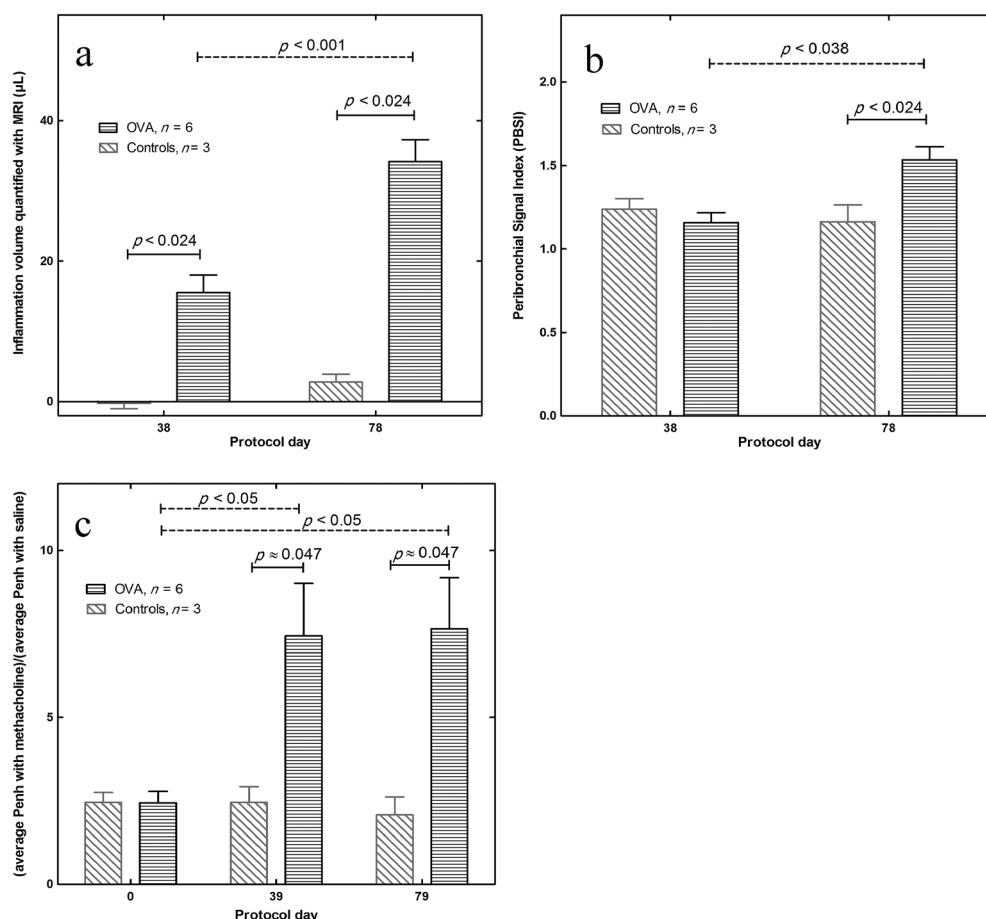


**Figure 4.** Bar plots (mean  $\pm$  SEM) showing the different computed indices for ovalbumin (OVA) and control groups at the different time points of the protocol. (a) Bar plot showing the inflammatory volumes. The Wilcoxon's signed-rank test showed a statistically significant difference between asthmatic mice and controls on days 38 and 78 ( $p < 0.024$ ). A statistically significant increase in the inflammatory volumes measured in the OVA group from day 38 to day 78 ( $p < 0.001$ ) was observed. Such a difference was not present in the control group. (b) Bar plot showing the peribronchial signal intensity index (PBSI). The Wilcoxon's signed-rank test showed a statistically significant difference between asthmatic mice and control mice on day 78 ( $p < 0.024$ ), but not on day 38. A statistically significant increase in the PBSI in the OVA group from day 38 to day 78 ( $p < 0.038$ ) was observed. Such a difference was not present in the control group. (c) Bar plot showing the plethysmographic index. The Wilcoxon's signed-rank test showed a statistically significant difference between asthmatic mice and control mice on days 39 and 79 ( $p \approx 0.047$ ). The Kruskal–Wallis test showed a statistically significant difference between the plethysmographic index measured on day 0 and on days 39 and 79 in the OVA group. Such a difference was not present in the control group.

**Table 1.** Measurement agreement of ultrashort-TE (UTE) MRI bronchial lumen areas (number of pixels) and peribronchial signals. The reproducibility study was performed on  $n = 30$  bronchi randomly chosen among the two time points (days 38 and 78) and the two groups (ovalbumin, controls)

|                                                  | Over time (intraobserver) agreement | Across observers (interobserver) agreement |
|--------------------------------------------------|-------------------------------------|--------------------------------------------|
| Peribronchial signals                            |                                     |                                            |
| Spearman correlation coefficient ( $p$ value)    | 0.962 ( $<0.001$ )                  | 0.867 ( $<0.001$ )                         |
| Bias $\pm$ standard deviation                    | $-0.007 \pm 0.078$                  | $0.05 \pm 0.12$                            |
| Intraclass correlation coefficient               | 0.981                               | 0.944                                      |
| Bronchial lumen areas                            |                                     |                                            |
| Spearman correlation coefficient ( $p$ value)    | 0.573 ( $<0.001$ )                  | 0.612 ( $<0.001$ )                         |
| Bias $\pm$ standard deviation (number of pixels) | $-1.3 \pm 5.1$                      | $-0.7 \pm 3.8$                             |
| Intraclass correlation coefficient               | 0.652                               | 0.760                                      |

## Plethysmography

As shown in the bar plot of Fig. 4c, the Penh ratio measured before the sensitization protocol was not statistically significantly different

between the OVA and control groups ( $p > 0.90$ ). Conversely, after sensitization, asthmatic mice showed significant increases in the Penh ratio at days 39 and 79 ( $p \approx 0.047$ ) of about 204% and 269%, respectively, relative to the controls.

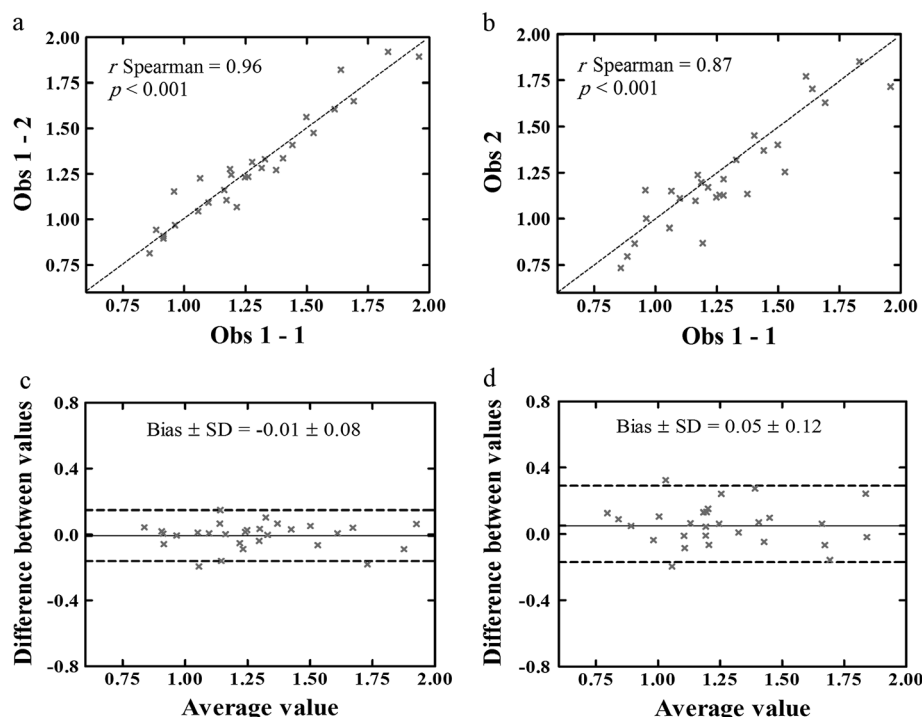


**Figure 5.** Intra- and interobserver reproducibility study for the peribronchial signal. Correlation over time (intraobserver) (a) and across observers (interobserver) (b) of the peribronchial signal measurements. The diagonal lines correspond to the lines of equality. Bland–Altman plots showing the means of the measurements over time (c) or across observers (d) against their differences. Broken lines correspond to the 95% limits of agreement (mean difference  $\pm$  1.96 standard deviation) and full lines correspond to the mean differences (bias). Obs 1 - 1, first measurement obtained by observer 1; Obs 1 - 2, second measurement obtained by observer 1; Obs 2, measurement obtained by observer 2. *r*, correlation coefficient.

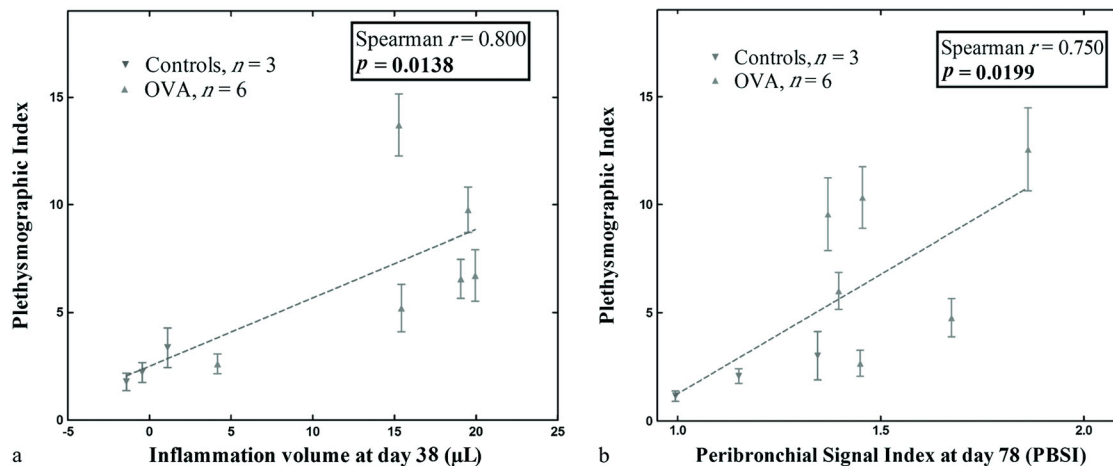


**Figure 6.** (a) Correlation plot between the plethysmographic index and the inflammatory volumes measured at day 38. (b) Correlation plot between the plethysmographic index and the peribronchial signal intensity index (PBSI) on day 78. OVA, ovalbumin.

There was no statistically significant difference in the Penh ratio measured in the control mice at the different time points ( $p > 0.93$ ). However, there was a significant increase in the Penh ratio in the OVA group at days 39 and 79 relative to day 0 ( $p < 0.014$ ).

## Correlations

The inflammatory volumes and PBSI were correlated with the Penh ratio measured in the asthmatic and control mice for the different time points. The inflammatory volume was significantly correlated with the Penh ratio at days 38–39 ( $p < 0.014$ , Fig. 6a),

but not at days 78–79 ( $p = 0.18$ ). By contrast, PBSI correlated significantly with the Penh ratio at days 78–79 ( $p < 0.020$ , Fig. 6b), but not at days 38–39 ( $p = 0.44$ ).

## DISCUSSION

Among the large number of murine asthma models described in the literature, that chosen for the present MRI investigation is known to include the main characteristics of asthma, i.e. BHR, inflammation and remodeling (12). The two main differences from

the more common models of OVA-induced inflammation are the absence of aluminum hydroxide in the sensitization procedure and the span of the protocol over a much longer period of time (about three times longer) (27,28,33–36,44–46). Although these changes make the sensitization protocol more complex, the resulting inflammation appears to mimic better the slow allergen-induced development of asthma in humans. Indeed, histological analyses of this model show a significant eosinophil infiltration into the lung interstitium, goblet cell hyperplasia with airways occlusion by mucus (inflammatory phase, without edema signs), airway smooth muscle hyperplasia and increased collagen deposition around airways and blood vessels (bronchial remodeling process) (23,24).

The images obtained with the present sequence in free-breathing mice, because of the ultrashort echo time, allow the visualization of the signal coming from interstitial tissue and lung parenchyma. The signal-to-noise ratio in the healthy lung parenchyma is greater than 30 and the motion artifacts are negligible. Furthermore, the resolution achieved is higher than 150  $\mu\text{m}$  and allows the detection of the main generations of bronchi (up to the fourth). Consequently, such improvements over typical lung images permit the simultaneous visualization and accurate quantification of the peribronchial inflammation, together with the study of the peribronchial changes that characterize this murine model of asthma.

With regard to the quantification of the extent of inflammation, the method used to compute the inflammatory volumes has been adapted from that proposed and validated by Beckmann *et al.* (34), simplifying the adaptive Lloyd–Max histogram quantification with a fixed low threshold. While the models studied by Beckmann *et al.* were of an acute nature, primarily investigated for the characterization of the early stages of intense inflammation, that studied in this work is chronic, with a slow development of eosinophil infiltration. For this reason, in contrast with the large localized edema previously assessed in refs. (34,36,45–47), the images presented here show a slowly increasing inflammation (from the first to the second time point) diffused in both lungs, rather than specifically situated in one lobe. The increase in the average inflammatory volumes from days 38 to 78 is consistent with the increasing number of albumin intranasal challenges given to the mice between the two time points, which is in agreement with the histological findings of Lederlin *et al.* (24) on the same asthma model.

The second part of the study is related to the assessment of the bronchial remodeling component of this murine model of asthma (3,12,23,24). Working on the same murine model of asthma, Lederlin *et al.* (23) have recently shown that a peribronchial density index measured with  $\mu\text{-CT}$  (defined similarly to PBSI used in the present investigation) correlates well with the bronchial smooth muscle size measured on histological slices, whose hypertrophy and hyperplasia are two of the main components of bronchial remodeling. The choice of the PBSI parameter computation is the result of different considerations. Although, in asthmatic patients, it has been shown that histological changes are entirely limited to the bronchial wall (48), previous studies on the same asthma murine model as used in this investigation have demonstrated that cell infiltration is largely scattered in the peribronchial space (12,23,24). As a result, a peribronchial ring-shaped ROI (whose width is equal to the bronchial radius) was preferred to a wall thickness/area measurement, in order to include most of the remodeling elements into the selected region and to avoid inaccuracies. In addition, as no distinction has been made among the different generations of bronchi, the weighted average of the normalized peribronchial signal by LA can be considered as a

way to minimize the influence of the different size of bronchi selected for the measurement.

In this investigation, the measurements have shown that, at the second studied time point (day 78, i.e. in the presence of bronchial remodeling), the PBSI increases significantly in the asthmatic group relative to the controls. Such an increase in the index is coherent with the main changes that take place following the remodeling mechanism. The increase in the number of mucous-secreting goblet cells in the epithelium, the increase in the thickness of the subepithelial collagen layer, the greater number and size of the vessels and elastic fibers within the inner airway wall and a generalized increase in the extracellular matrix and airway smooth muscle mass (3,12,23,24) can explain the increase in the peribronchial MRI signal of asthmatic mice, relative to the lower signal measured in healthy mice. Such a difference between control and asthmatic groups is not present at the first time point (day 38). This finding is consistent with several studies which have shown that long protocols of 8–12 weeks are needed to initiate the bronchial remodeling process (17,19), and with the histological results presented in ref. (24) on the same chronic asthma model as studied in this work. As, at the second time point, the remodeling features are also accompanied by eosinophil inflammation, it may be argued that the PBSI could reflect peribronchial inflammation rather than the remodeling itself. Nevertheless, Lederlin *et al.* (23), in a  $\mu\text{-CT}$  counterpart experiment, have shown that such a parameter is not correlated with the number of inflammatory cells, despite the fact that an intense peribronchial cell inflammation is found in asthmatic mice. Furthermore, in this study, no difference in the PBSI between asthmatic mice and controls was observed on days 38–39, when only inflammation was present. In this sense, the extension of the present model to a third time point, characterized by remodeling without inflammation, may shed further light on the subject.

One of the clear advantages of the <sup>1</sup>H UTE MRI investigation presented in this work is that no cardiorespiratory gating is needed. Preliminary studies have shown that the quality of gated images is not significantly superior to those acquired in spontaneous respiration. Such observation is in agreement with previously published studies on UTE sequence properties, e.g. ref. (37), showing that cardiorespiratory gating acquisitions and averaging approaches with radial acquisitions can lead to equivalent spatial resolutions. The consequent advantages are not only restricted to the almost two-fold reduction in the acquisition time (23,24), but also include the simplification of the protocols, as no invasive endotracheal intubation or mechanical ventilation is required. Similarly, the absence of gating permits the TRs to be kept constant in the different acquisitions, ensuring the possibility to perform a precise and accurate comparison between each mouse and each day, respectively. However, it should be noted that the spatial resolution achieved with  $\mu\text{-CT}$  is still superior to that obtained in the present study (23,24), which notably facilitates the analysis of bronchial remodeling. Nevertheless, the reproducibility study performed on UTE MRI data has demonstrated very good intra- and interobserver agreement. Overall, the absence of ionizing radiation (compared with  $\mu\text{-CT}$ ) and of invasive respiratory control techniques [which have been proven to lead to severe lung injuries (49)] together make the UTE MRI longitudinal studies in mice a concrete and valuable possibility. In addition, potential applications to humans can also be conceived.

BHR is the third hallmark of the asthma syndrome which is reflected in the studied murine model. Although the physical mechanisms inducing this phenomenon are known (2,3), the exact contributions of inflammation and remodeling are still disputable (50). The use of Penh as a measure of BHR is still an open debate. Although it has been shown that it does not represent the airway resistance *per se* (51), several studies have re-evaluated the validity of unrestrained whole-body plethysmography in mice to assess BHR (17,41). In particular, it has been shown that, in Balb/c mice, Penh correlates well with lung resistance (24,42), especially if specific criteria are followed during the measurements (17,52). In this study, BHR assessed by the Penh ratio resulted in significant differences between the asthmatic and control populations at both time points after the beginning of the sensitization protocol. The significant correlation of the inflammatory volumes with the Penh ratio at days 38–39 and the relative absence of correlation with PBSI provide further confirmation that, in the absence of remodeling, the hyperresponsiveness is related directly to eosinophilic inflammation. In comparison, at days 78–79, remodeling becomes relevant and the correlation between the hyperresponsiveness and inflammatory volumes is no longer significant. Similarly, the positive correlation between PBSI and the plethysmographic index at the second time point strongly reinforces the hypothesis that structural remodeling plays a central role in the exaggerated airways narrowing in asthmatics, and can be added to the bulk of evidence which suggests that peribronchial changes in diseased animals act more like foes than like a defensive mechanism (3,53).

The bulk of evidence shows that the first time point (days 35–36) is characterized only by a reduced peribronchovascular eosinophilic inflammation and a significant BHR, whereas the second time point (days 78–79) presents an extended eosinophilic inflammation together with a strong peribronchial remodeling and BHR. To the best of our knowledge, this is the first MRI study of peribronchial wall modifications and the correlation of inflammatory volumes and remodeling parameters with functional data in a longitudinal model of murine asthma. The measurements in the present investigation and the histological analysis performed on the same asthma model in previous studies (12,23,24) together suggest that PBSI can be used to assess bronchial remodeling in mice and may be a translational concept in humans.

The immediate consequence is that UTE MRI could be a reliable tool to monitor noninvasively the evolution of bronchial remodeling in a therapeutic drug delivery preclinical study. Such results in mice can be seen as a first step towards human studies with the aim of identifying MRI markers of asthma.

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