Proton MRI as a non-invasive tool to characterize a murine model of allergic pulmonary inflammation.

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Introduction:

The detection by MRI of an edematous signal associated with allergic inflammation induced in the lung of actively sensitized Brown Norway rats challenged with ovalbumin (OVA) has been described before (Beckmann et al., 2001) This signal has been shown to significantly correlate with the inflammatory parameters in the broncho-alveolar lavage (BAL) fluid and with the perivascular edema quantified by histology. Thus, MRI represents a key noninvasive indicator of allergenic inflammation in this rodent model. The transfer of analogous procedures to the mouse would open perspectives on compound screening in this species and on transgenic applications.

Material and Methods:

Animal Model: OVA (250 µg/ml) mixed with aluminium hydroxide (10 mg/ml) was injected (0.2 ml per animal i.p.) on days 0 and 14 to female BALB/c mice. On days 18, 19, 20 and 21, mice were anesthetized with 2.0% isoflurane and OVA (5 mg/kg dissolved in saline) or vehicle (saline, 25 microliters per animal) was administered intranasally (i.n.).

MRI: 24h after the last challenge, measurements were carried out in anaesthetised and spontaneously breathing mice at 4.7 T (Bruker Biospec). Fifteen images (TE: 3.5 ms ; TR: 5.6 ms ; pixel size: 117x234 μm^2 ; thickness: 0.75 mm; acquisition time per image: 74 sec) were acquired sequentially using a gradient-echo sequence. Neither respiratory nor cardiac gating was applied for image acquisition.

BAL fluid and histology: Immediately after MRI, mice lungs were lavaged for cell count, total protein level and EPO activity assays or taken out and immerged in formalin for later histological analysis (Verhoeff's and alcian blue/periodic acid Schiff's staining).

Results and Discussion:

Twenty-four hours after last ovalbumin challenge, a volume of 37.8 ±5.4 µL (mean±sem, n=20) of fluid signals was detected by MRI in the anterior region of the lungs (fig.1 left). In saline-challenged mice, no fluid signal was detected by MRI (fig.1 right). BAL fluid cell analysis revealed a luminal infiltration of activated eosinophils (0.769.±0.04 resp 0.004±0.001 × 10^6 cells/mL of BAL fluid for OVA- and saline-challenged) and, in less proportions, neutrophils (0.014±0.005 resp 0.002±0.001× 10^6 cells/mL of BAL fluid for OVA- and saline-challenged) and lymphocytes (0.063±0.005 resp 0.004±0.0004 × 10^6 cells/mL of BAL fluid for OVA- and saline-challenged). High protein level (0.54±0.04 versus 0.21±0.01 mg/mL of BAL fluid), representative of plasma leakage from the lung vasculature to the airways, was observed in the BAL fluid of OVA-challenged mice, consistent with the development of an edematous component observed by MRI.

In agreement with the BAL findings, histological sections of the lungs from all OVA-challenged mice (fig.2 top left) showed marked peribronchial and perivascular edema accompanied by inflammatory cell infiltration. Furthermore, goblet cell hyperplasia and mucus plugging into the main airways was found in all OVA-challenged animals (fig.2).

The hallmarks of allergic inflammation revealed by BAL fluid analysis and histology in the present study are also observed in human asthmatic subjects. In analogy to earlier studies in rats, the model we developed opens perspectives for screening of anti-inflammatory compounds in mice and for transgenic applications aiming at target validation.

Figure 1 : Transversal sections through the thorax of balb/c mice, acquired 24 h after the 4th intranasal challenge of either saline (right) or OVA (left). Arrows indicate fluid signals.

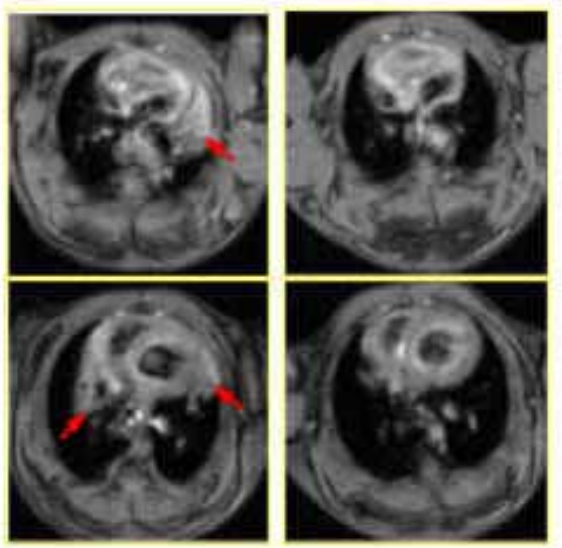

Figure 2 : Histological sections from the left lung of an actively sensitized OVA-challenged mouse (left), and saline-challenged (right), demonstrating perivascular edema and polynuclear infiltration (top), and mucus hypersecretion (bottom).