

# In vivo quantification of inflammation burden in an experimental allergic encephalomyelitis rat model using fluorine-19 MRI

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## Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) affecting millions of people worldwide. Experimental allergic encephalomyelitis (EAE) is a widely studied animal model of MS. EAE is a T cell-mediated inflammatory and demyelinating disease that displays perivascular and parenchymal infiltrates in the CNS. In this study, we evaluated the use of perfluorocarbon (PFC) emulsion imaging probes infused i.v. and <sup>19</sup>F MRI detection to quantitatively evaluate the inflammatory burden in an EAE rat model (1). Prior work has shown a strong linear correlation between macrophage burden and <sup>19</sup>F MRI signal magnitude in tissue samples from EAE rats (2). In this EAE model, as expected, we observed significant <sup>19</sup>F signal in the spinal cord; we also observed pronounced <sup>19</sup>F signal accumulation in the vertebral bone marrow proximal to the CNS lesions. In a treatment group receiving cyclophosphamide, a dramatic reduction in the PFC (i.e., leukocyte) accumulation was observed.

## Materials and methods

**Animal model of EAE:** Female DA rats (n = 15), 11 weeks old, were inoculated with emulsified syngeneic spinal cord (2). The inoculant was prepared by homogenizing frozen DA spinal cord (50 mg/rat) with incomplete Freund's adjuvant (IFA, Difco, MI) at 200 µL/rat and mycobacterium tuberculosis (Difco) at 2 mg/mL. A treatment group (n = 5) received cyclophosphamide (20 mg/kg body weight, 20 mg/ml, Baxter, IL) i.p. every five days from Day 0. A control group (n = 5) were also prepared by inoculating with IFA, but no spinal cord homogenate. After induction, rats were monitored daily for body weight and clinical symptoms of EAE. Symptoms were scored according to the convention: 0 = normal, 1 = limp tail, 2 = paraparesis with a clumsy gait, 3 = hind-limb paralysis, 4 = hind- and fore-limb paralysis, and 5 = moribund. Upon the first signs that an animal reached clinical stage 2, rats received a single i.v. injection (0.5 mL, 20% v/v) of PFC emulsion (VS-1000, Celsense, Inc., PA) via the jugular vein. **In vivo <sup>19</sup>F MRI:** Two days after PFC injection, animals underwent <sup>19</sup>F/<sup>1</sup>H MRI using a 7 T horizontal bore scanner (Bruker, MA). A reference capillary with diluted PFC emulsion in agarose was placed alongside the animal's torso. Two sets of sagittal <sup>19</sup>F RARE images were acquired to cover the entire spinal cord. The parameters were TR/TE=1000/12 ms, RARE factor 8, NA=800, and resolution 1.4×0.7×2 mm<sup>3</sup>. For anatomical reference, T<sub>2</sub>-weighted <sup>1</sup>H images were acquired using MSME in the same slices as <sup>19</sup>F; imaging parameters were TR/TE=1000/11 ms, NA=4, and resolution 0.23×0.23×2 mm<sup>3</sup>. The <sup>19</sup>F/<sup>1</sup>H images were fused and quantified offline using Voxel Tracker software (Celsense, Inc., PA). **Ex vivo <sup>19</sup>F MRI:** After *in vivo* experiment, animals were sacrificed for *ex vivo* MRI. Fixed, thoracic 5-7 vertebrae were imaged using an 11.7 T vertical bore scanner (Bruker). For <sup>19</sup>F, 3D RARE was used with parameters TR/TE=900/12 ms, RARE factor 8, NA=2,400, and resolution 0.2×0.2×0.4 mm<sup>3</sup>. Co-registered high-resolution anatomical <sup>1</sup>H images were acquired using 3D RARE with parameters TR/TE=800/11 ms, RARE factor 8, NA=36, and resolution 0.05×0.05×0.1 mm<sup>3</sup>. **Histological analysis:** The thoracic 5-7 vertebrae were decalcified prior to H&E staining; immunohistochemistry (IHC) staining of CD68+ macrophages was performed using the ED1 antibody (AbD Serotec, Inc., NC).

## Results

Fig. 1 shows representative <sup>19</sup>F/<sup>1</sup>H MRI images of the EAE rat, where <sup>19</sup>F is rendered in the hot-iron scale. The <sup>19</sup>F signal is visible in the spinal cord, presumably in the EAE lesions, as well as in the bone marrow of the vertebral cavities proximal to the spinal cord lesions. The PFC emulsion droplets preferentially co-localize within macrophages, but not glial cells (4). The total amount of apparent <sup>19</sup>F atoms in different tissues was quantified (Fig. 2) in Voxel Tracker with the aid of the external <sup>19</sup>F emulsion reference capillary. With prophylactic cyclophosphamide treatment, the <sup>19</sup>F signal in the spinal cord exhibited a significant reduction compared to the untreated EAE group (Fig. 2, p<0.05). The <sup>19</sup>F signal reduction with treatment was observed both in the spinal cord and bone marrow (Fig. 2). H&E staining of the spinal cord shows inflammatory infiltrates (Fig. 3a-b) which is absent in the treated and control animals (data not shown). IHC staining of CD68+ cells confirms the infiltration of macrophages/monocytes into white matter regions of the EAE rat (Fig. 3c-d) but not into the treated and control animals (data not shown). Additionally, IHC staining of CD68+ cells displays a visible increase in macrophages/monocytes in the vertebral bone marrow of the EAE rat (Fig. 3e).

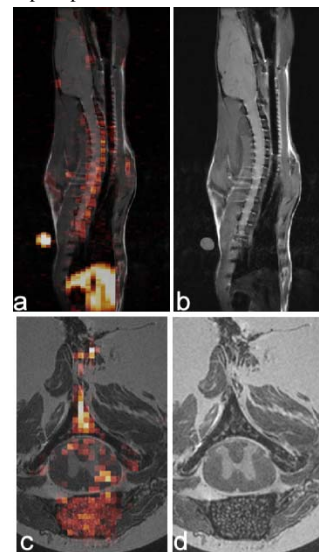
## Conclusions

In this study, we showed that intravenously infused PFC emulsions and <sup>19</sup>F MRI is effective in the quantitative analysis of EAE lesions *in vivo* and visualizing the effects of a therapeutic. Interestingly, our findings indicate that EAE progression displays elevated leukocytes in the bone marrow in proximity to the CNS lesions, which is significantly suppressed by the treatment with cyclophosphamide. Because of the positive signal provided by the PFC agents and the absence of <sup>19</sup>F background, we were able to visualize changes in inflammatory cell burden in the bone marrow, a region that is difficult to analyze using conventional <sup>1</sup>H imaging and super-paramagnetic nanoparticle contrast agents. Overall, these results suggest <sup>19</sup>F MRI may serve as a sensitive biomarker for discovery and preclinical assessment of novel MS treatments.

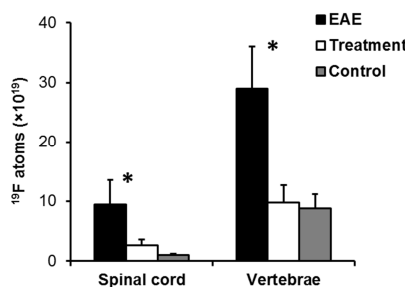
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## References

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**Fig. 1.** *In vivo* and *ex vivo* <sup>19</sup>F/<sup>1</sup>H MRI of EAE rat showing <sup>19</sup>F labeled macrophage accumulation in the spinal cord and proximal vertebral bone marrow. (a) Shows an *in vivo* <sup>19</sup>F/<sup>1</sup>H image of an EAE rat. (b) is the anatomical image of (a). (c) is the *ex vivo* <sup>19</sup>F/<sup>1</sup>H image of thoracic-6 vertebrae of the EAE rat. (d) is the anatomical image of (c).



**Fig. 2.** *In vivo* quantification of inflammation load in the spinal cord and vertebral bone marrow in EAE rats. (\*) is p<0.05 with treatment and control groups.

**Fig. 3.** *Ex vivo* analysis shows macrophage burden in the spinal cord and vertebral bone marrow of EAE rats. Panels (a-b) are H&E staining of ventral and dorsal infiltrates (\*) in the spinal cord. Panels (c-d) are IHC staining of CD68+ cells. Panel e is the IHC staining of CD68+ cells in the vertebral bone marrow.

