Elucidating the Involvement of Spino-Olivocerebellar Pathways in Relapsing-Remitting EAE Using USPIO MRI

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Introduction Multiple sclerosis (MS) is an inflammatory and demyelinating CNS disease that affects ~400,000 adults in the US; however, currently available treatments remain unsatisfactory [1]. To better understand the underlying pathophysiology [2-5] or to evaluate the efficacy of new therapeutic agents for MS [6-7], experimental autoimmune encephalitis (EAE) is widely used as a preclinical animal model of MS. Recently, ultrasmall superparamagnetic iron oxide (USPIO)-enhanced T_2-weighted MRI was described as an invaluable tool that allows *in vivo* monitoring of infiltrating macrophages, the predominant effector cells in EAE allowing visualization of new lesions during disease development [5-7]. However, the relationship between the spatio-temporal pattern of uptake of USPIO by macrophages and the corresponding behavioral signs has not been fully elucidated. Here, we sought to investigate the occurrence of lesions in relapsing-remission EAE and its implications on observed neurological deficits. Toward this goal, EAE was induced in rats with myelin oliodendrocyte glycoprotein (MOG). During the disease course, behavioral symptoms were scored daily, and rats were imaged during acute, remission, and relapse phases. USPIO-enhanced T_2-weighted imaging was used to image macrophage infiltration; MRI-detected lesions were validated with histological and immunohistochemical staining.

Materials and Methods To induce EAE, female Dark Agouti rats (~200 g, n = 8) were immunized with an intradermal injection of 100µl of MOG/IFA (incomplete Freud adjuvant, Sigma, St. Louis USA). During the disease course, behavioral scores were recorded: 0-no signs; 1-loss of tail tone; 2-abnormal gait; 3-unilateral hind limb paralysis; 4-bilateral hind limb paralysis/unilateral front limb paralysis; and 5-morbund/bilateral front limb paralysis/urinary retention/blood in urine. To detect USPIO-labeled infiltrated macrophages *in vivo*, rats were infused with SH U 555 C (Bayer Schering Pharma AG, Germany) at 500 µmol Fe/kg iv, 24 hrs prior to imaging. Imaging experiments were conducted on a 7T MRI scanner (Bruker Biospin, Karlsruhe, Germany) using a RARE sequence with imaging parameters: TR / TE = 3200 / 75 ms, RARE factor = 8, pixel size = 125 × 250 µm^2, slice thickness = 1.25 mm, and average = 16. To validate our MRI findings, histological staining was performed with diaminobenzidine (DAB)-enhanced Perl's Prussian blue (for USPIO/iron content) and CD_{68} immunohistochemistry (for identification of macrophages) in the same animals at predetermined times.

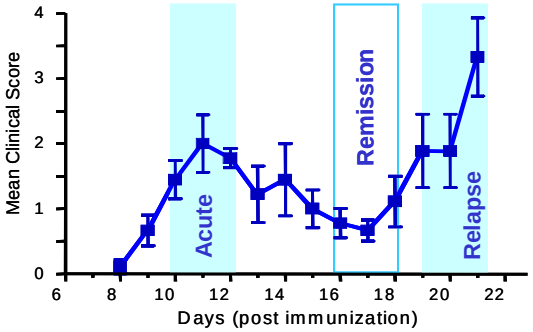

Fig. 1 The mean clinical score recorded from EAE rats showing the acute, emission and relapse phases.

Results and Discussion Figure 1 illustrates the group mean clinical score over the disease course, indicating the acute (~Day 10-12), remission (~Day16-18), and relapse (~Day 19-21) phases. USPIO-labeled lesions occurred in distinct CNS regions at various phases: brainstem and spinal cord (acute), no lesion (remission, not shown), and cerebellum and spinal cord (relapse) (Figure 2). Histopathology and immunohistochemistry confirmed that the hypointense regions shown in USPIO-enhanced T_2-weighted images delineate the uptake of USPIO by infiltrated macrophages. Interestingly, the distinct spatio-temporal pattern of lesions reveals the involvement of spino-olivocerebellar pathways in EAE. In the acute phase, areas of USPIO uptake were observed mainly at the inferior olives (brainstem) (score = 1~2), the origin of the olivocerebellar system [8], and occasionally in the vestibular nucleus or spinal cord (score > 2). In the relapse phase, lesions were found in the cerebellum (scores = 2~4), and the lateral columns of spinal cord (score = 5). These findings were observed consistently across all animals. Notably, the inferior olives are known to project afferent innervations, or climbing fibers, to Purkinje cells in the cerebellar cortex, which are critical for the coordination of movement [8, 9]. Previously, Kennedy *et al* demonstrated that the olivocerebellar pathways play an essential role in the cerebellar control of the limb posture and movement [10], which might well explain the motor dysfunction observed in EAE rats (loss of tail tone, abnormal gait, or paralysis). Therefore, the involvement of spino-olivecerebellar pathways in EAE elucidated by USPIO-labeled lesions provides important insights into further understanding of this disease model.

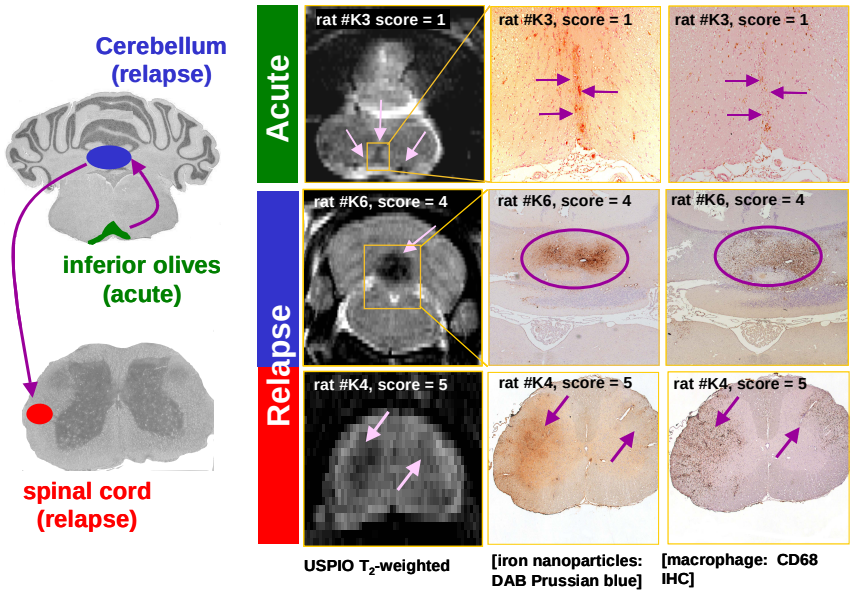

Fig 2. Spatio-temporal pattern of USPIO uptake in macrophages at the acute and relapse phases in relapsing-remitting EAE rats, illustrating the role of the spino-olivocerebellar pathway in the underlying pathophysiology of the observed neurological deficits. Notably, the histological staining and immunohistochemistry data correspond well with MRI findings.

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