Anti-IL17 treatment reduces clinical score and VCAM-1 expression in EAE-ABH mice detected by in vivo magnetic resonance imaging

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Background: Recent data have established that IL-17-producing CD4+ T cells, driven by IL-23 and referred to as Th17 cells, play a pivotal role in the pathogenesis of EAE. We have previously demonstrated that anti-VCAM-1 conjugated to 1μm Microparticles of Iron Oxide (MPIO) enables detection of *in vivo* VCAM-1 expression in mice following the unilateral injection of interleukin-1β into the brain [1] as well as early EAE in pre-symptomatic mice (Mardiguian *et al*, **British Neurosci. Assoc. Abstr., Vol 20, P66.13, 2009**). Also, anti-IL-17 therapy has been previously demonstrated to have therapeutic benefit [2].

Methods: Chronic Relapsing EAE was induced in Biozzi ABH mice (6-10 wks) by subcutaneous injection in the abdominal flanks with 500μg of mouse spinal cord homogenate emulsified in 0.15ml PBS + 0.15ml of complete Freud's adjuvant. Animals were weighed daily and assessed for clinical signs according to the following guidelines: 0-healthy, 1-limp tail, 2- incomplete hind limb paralysis, 3- complete hind limb paralysis and 4-forelimb paralysis/ moribund. We used myOne tosylactivated MPIO (1-μm diameter; iron content 26%) with *p*-toluenesulphonyl (tosyl)-reactive surface groups (Invitrogen) for antibody conjugation as described [1]. ABH mice were administered either with 400mL of anti-IL-17 (32 mg/kg, subcutaneously) or IgG (32 mg/kg, subcutaneously) following the acute phase of EAE at days 17, 24, 31 and 38 post EAE induction. At days 28 and 42 following immunisation, animals were anaesthetised and injected intravenously with either VCAM-MPIO or control IgG-MPIO (4 x 108; 4.5mg iron / kg body weight; n = 3-4 per group). After 1h animals underwent MRI at 7T and a T2*-weighted 3D gradient-echo dataset was acquired (acquisition ~1h; isotropic resolution 88mm). Post-gadolinium T1-weighted images were acquired to assess BBB integrity. T2*-weighted images were processed into a 3D isotropic dataset and converted into tiff images. For each image, the brain was manually masked to exclude extracerebral structures. Quantification of VCAM-MPIO binding (defined as focal hypointensities) was performed by observers blind to the identity of the dataset. Volumes are expressed as raw volumes in μL.

Results: A significant reduction in the clinical score was observed in the relapse phase in the anti-IL-17A-treated animals compared with IgG treated animals. During remission, VCAM-1 expression was significantly higher in IgG-treated mice than in mice treated with anti-IL-17A with more number of Gd-DTPA enhanced lesions on T_1-weighted images. However no significant difference in the number of Gd-DTPA enhanced lesions was found. During relapse at day 42, no significant differences in VCAM-1 expression and in the number of Gd-DTPA enhanced lesions were observed between the groups

Conclusion: Our results show that therapeutic dosing initiated at peak of disease was able to block the ongoing disease processes in the anti-IL-17A-treated animals, and reveals that the remission phase is still associated with activation of the brain endothelium, which can be suppressed with anti-IL-17A-treatment. We also re-established that our targeted MRI contrast agent VCAM-MPIO is more accurate and successful to detect early therapeutic effects than the clinical Gd-DTPA enhancing MRI.
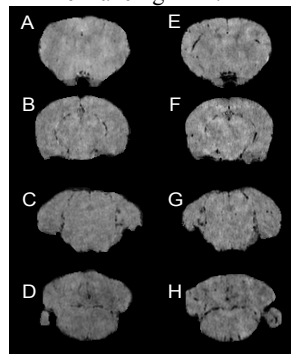

Fig 1: Detection of early inflammatory events by VCAM-1-targeted MRI contrast agent.
Images showing the interaction of VCAM-MPIO with VCAM-1 expressed on the endothelial surface detected as focal hypointensities on T2*-weighted images. At day 28, the presence of MPIO-binding in the forebrain (A), the hippocampus (B) and the cerebellum (C) and (D) of CR-EAE ABH mice treated with IgG (control) is more abundant than in the forebrain (E), the hippocampus (F) and the cerebellum (G) and (H) of CR-EAE ABH mice treated with anti-IL-17A.
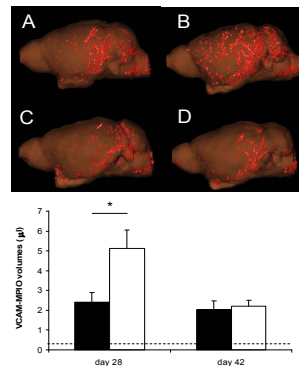

Figure 2: Anti-IL-17A treatment decreases VCAM-MPIO binding in CR-EAE ABH mice.
3D reconstruction of the brain shows that anti-IL-17A treated CR-EAE ABH mice (A) exhibit less MPIO binding during remission (day 28) than IgG- treated mice (B). At day 42, however, no significant differences in MPIO binding were observed in anti-IL-17A- and IgG-treated mice (C and D, respectively). Graph showing the significant reduction in VCAM-MPIO binding in anti-IL-17A treated CR-EAE ABH animals compared with IgG-treated controls at day 28. No significant difference was found at day 42.

References:
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