Assessment by MRI of brain infiltration by peripheral blood macrophages in anti-VLA-4 antibody treatment of experimental autoimmune encephalomyelitis

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Synopsis
The treatment of an experimental allergic encephalomyelitis (EAE) with anti-VLA-4 antibody was monitored by the development of classical signs and the consequences for both rupture of the blood brain barrier (BBB) and macrophage migration into the brain using MRI with gadolinium and USPIO. We show that although in almost all animals clinical EAE is suppressed by the anti-VLA-4 antibody treatment these animals present ongoing macrophage infiltration into the CNS parenchyma shedding some new light on the role of macrophage brain infiltration in an EAE disease process with relevance for the understanding and therapy of MS.

Introduction
The formation of large infiltrates by mainly T-cells, macrophages and some B-cells in the central nervous system (CNS) contributes to the pathogenesis of multiple sclerosis (MS) [1]. The process by which T cells cross BBB to form perivascular infiltrates has been characterized as sequential events mediated in part by alpha4 integrins (VLA-4) expressed on leukocyte cell surface. Antibodies against the adhesion molecule VLA-4 reduce clinical and histological signs in rodent EAE models of MS by blocking T-cell migration and disease development [2]. Anti-VLA-4 antibodies are under testing in MS patients (Natalizumab). In the acute non relapsing-remitting EAE in the Lewis rat, migration of macrophages in the CNS can be monitored in vivo using the ultra-small-particle-iron-oxide (USPIO) contrast agent by magnetic resonance imaging (MRI) [3,4]. Since treatment with inhibitory anti-VLA-4 (CD49d) monoclonal antibodies (mab) reduces the clinical development of various EAE by blocking T-cell migration into the brain, we asked whether the inflammatory process such as macrophage migration and rupture of the BBB are altered by the antibody treatment which can be monitored by MRI.

Methods:
Acute EAE was induced in 16 female Lewis rats. For treatment, eight rats received the TA2 anti-rat VLA-4 mab (TA2, gift from Biogen, Cambridge, MA) by a single injection at day 6. The remaining 8 EAE induced rats were used as positive controls. The USPIO contrast agent was AMI-227 (Sinerem®, Guerbet laboratory, France). For gadolinium enhanced imaging we used Gd-DOTA at the dose of 0.5 mmol/kg. The contrast agents were administered by i.v. injection. Rat MRI studies at 1.5 T were performed within 12 to 16h after appearance of the clinical signs, before and after Gd-DOTA infusion, and 20 to 24 hours after USPIO injection allowing the accumulation of USPIO in macrophages and their infiltration to active inflammatory sites in the CNS. Imaging sequences included sagittal and coronal SE T1-weighted images, coronal turbo SE T2 and T2*-weighted images. Histological examination included sagittal and coronal SE T1-weighted images, coronal turbo SE T2 and T2*-weighted images. Histological examination included hematoxylin-eosin staining to assess inflammatory infiltrates, mab ED1 for macrophages and activated microglia and anti-rat pan T cell mab for T cell CNS infiltration.

Results
TA2 anti-VLA-4 antibody treatment reduces clinical EAE : 3 out of 8 anti-VLA-4 antibody treated rats (37%) and 7 out of 8 placebo treated rats (88%) developed EAE (p<0.03).

MR findings : None of the animals independently of treatment presented alterations of BBB with gadolinium. The MRI performed 24 hours after USPIO infusion to determine macrophage infiltration into the brain parenchyma showed abnormalities in all the rats (100%) treated by placebo and in 6 of the 8 (75%) rats treated by anti-VLA-4 antibody. In the treated group, the positive USPIO signal was not correlated with clinical signs.

Histology : Inflammatory sites as determined by HE staining were significantly reduced in rats treated by TA2 anti-VLA mab (8.2± 7.5 vs control 25.9±19.3). In all the control animals a very important T cell infiltration was revealed. In animals treated with TA2 antibody only one animal presented a minor T cell CNS infiltration. In the brains, ED1 positive macrophages and activated microglia were significantly different in animals treated by placebo (11 cells/unit ± 3.2) compared to TA2 anti-VLA4 antibody treated animals (2.9± 2.8 cells/unit; p=0.02).

Discussion
In EAE the beneficial effects of anti-VLA-4 treatment which prevents and reduces the development of clinical EAE was confirmed in this study. However, most of anti-VLA-4 antibody treated animals presented with positive monocytes/macrophages brain infiltration as shown by MRI with the USPIO contrast agent, although four of these animals presented no clinical signs of EAE. There was no evidence for the rupture of the BBB. This indicates that at the early stage of clinical EAE disease macrophage infiltration into the brain parenchyma is an event distinguishable in time and space that independently occurs from the rupture of the BBB. Our observations raise important implications concerning the role of T cells and the role of macrophages and their interaction in the development of brain lesion in EAE and MS. One implication is on the physiopathology of the blood cells interacting with the brain : monocytes/macrophages may behave at least partially independently from the T-cells. The other implication is on therapy : although the clinical symptoms are significantly reduced, an inflammatory reaction is still present in the brain, meaning that the disease may silently affect the CNS. All these processes can be monitored in vivo by MRI and are relevant for the understanding and therapy of MS.

References