Gadofluorine M enhanced MRI reveals circumventricular organ involvement in CNS inflammation and facilitates occult lesion detection

E. Tysiak[1], and J. Wuerfel[1,2]

[1]Cecilie Vogt Clinic for Neurology, Charité University Medicine, Berlin, Germany, [2]Institute of Neuroradiology, University Schleswig-Holstein, Luebeck, Germany

Background: The central nervous system (CNS) may no longer be considered immune privileged but rather a site of selective immune activity[1,2]. Although the blood-brain barrier (BBB) covers most parts of the CNS, certain brain regions are devoid of it and are, therefore, in permanent contact to blood-born molecules and cells. These "exposed" areas include the choroid plexus and other small structures, which line the cavity of the third and of the fourth ventricle, and are known as circumventricular organs (CVO). CVO are characterized by a very dense capillary network with wide perivascular areas and provide an access route for immune cells into the brain parenchyma. Thus CVO might guide CNS immune surveillance. However, until recently no reliable method had been available to survey CVO *in vivo*. Assuming a crucial function as CNS "gate" for immune cells, the visualization of alterations in CVO might become of additional diagnostic and therapeutic value for the assessment of neuroinflammatory conditions.

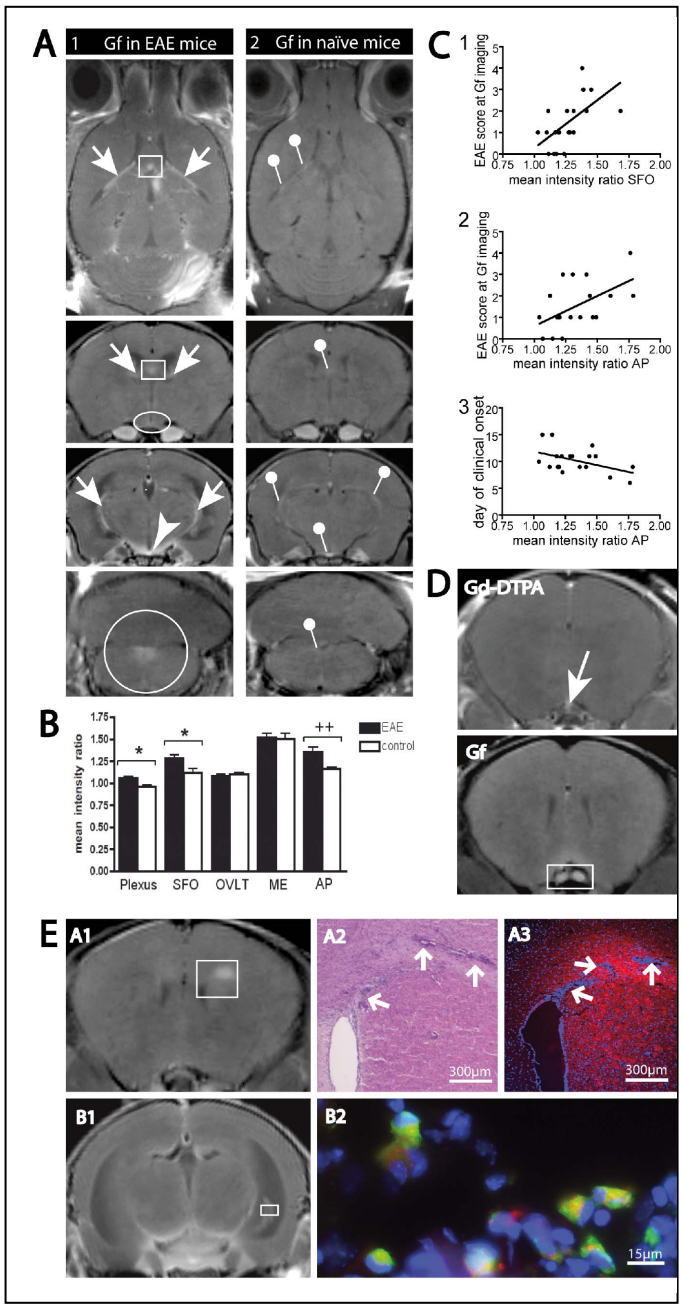

Figure legend: ***A*** Gf enhancement of the choroid plexus (arrows), the subfornicular organ (square), the organum vasculosum of the lamina terminalis (ovoid), the median eminance (arrowhead) as well as the area postrema (circle) are clearly marked in the EAE mice (column 1) and, much less pronounced, in naïve control mice (column 2). ***B*** Gf enhancement was quantitatively assessed computing mean intensity ratio of the CVO in T1-weighted images 24 hours after Gf application. The comparison between EAE and naïve control animals was performed using unpaired, two-tailed t-tests. Gf enhancement in EAE mice was significantly higher in the choroid plexus (*: p = 0,021), the subfornicular organ (*: p = 0,018) and the area postrema (++: p = 0,002 with Welch´s correction for significantly different variances). ***C*** The signal intensity (mean intensity ratio) of the subfornicular organ (SFO) (1) and the area postrema (AP) (2) on Gf enhanced MRI 24 hours post injection correlated to the corresponding EAE scores at the time of scanning. Additionally, the time needed from T cell transfer to disease onset correlated inversely with the mean intensity ratio of the area postrema (3). Each dot depicts the data point of one mouse. ***D*** Visualization of optic neuritis (square) was markedly improved by Gf, since neighboring intravascular signal prohibited the unambiguous determination on Gd enhanced images (arrow). ***E*** Pericallosal inflammatory focus (B1) and corresponding H&E stained (A2,) and fluorescence microscopy (A3; red: Gf, blue: Hoechst 33258) slices depict typical inflammatory plaques, including cellular infiltrations (arrows), diffusely and halo-like surrounded by Gf. After 72 hours, Gf uptake into macrophages/microglia was evident (B2). In the choroid plexus (B1), internalized Gf became visible in numerous IBA-1 positive cells (B2; red: Gf; green: IBA-1; blue: Hoechst 33258).

Methods: After induction of adoptive transfer EAE as previously described[3], 21 mice underwent cerebral Gadopentate dimeglumine (Gd; 0.2 mmol/kg) and Gf (0.1 mmol/kg bodyweight) enhanced MRI daily between day five and 16 post T cell transfer on a 7 Tesla rodent scanner (Pharmascan 70/16AS, Bruker BioSpin, Germany), applying a 20 mm RF-Quadrature-Volume head coil. Axial and coronal T1-weighted images (MSME; TE 10.5 ms, TR 322 ms, 0.5 mm slice thickness, matrix 256x256, FOV 2.8 cm, 8 av.) were acquired. Mean Gf intensity ratios were calculated in the choroid plexus, the subfornicular organ, the organum vasculosum of the lamina terminalis, the median eminence and the area postrema. Subsequently, the tissue distribution of fluorescence labeled Gf and the extent of cellular inflammation were assessed in corresponding histological slices (H&E, Hoechst 33258 nuclear stain, Fluoromyelin Red, IBA-1, GFAP and Gf-C3.5).

Results: Inflammatory plaques were widely distributed throughout the brain, with predominance to the brainstem and the periventricular region. In 15 mice that received both, Gd and Gf, a total number of 61 contrast enhancing lesions (CEL) could be visualized. Among these, 26 were exclusively detected after Gf administration, but not on Gd enhanced MRI. Gf signal intensity of the choroid plexus, the subfornicular organ and the area postrema increased significantly during EAE (A,B), correlating with disease severity and delay of disease onset after immunization (C). Furthermore, Gf improved the detection of EAE lesions, being particularly sensitive to optic nerve inflammation (D). In correlated histological slices, Gf initially accumulated in the extracellular matrix surrounding inflammatory foci, and was subsequently incorporated by macrophages/microglia (E).

Interpretation: Gf enhanced MRI provides a novel highly sensitive technique to study cerebral BBB alterations. We demonstrate for the first time *in vivo* the involvement of CVO in the development of neuroinflammation.

1.Ransohoff RM, Kivisakk P, Kidd G. Three or more routes for leukocyte migration into the central nervous system. Nat Rev Immunol. 2003;3:569-581. 2.Bechmann I, Galea I, Perry VH. What is the blood-brain barrier (not)? Trends Immunol. 2007;28:5-11. 3.Wuerfel J, Tysiak E, Prozorovski T et al. Mouse model mimics multiple sclerosis in the clinico-radiological paradox. Eur J Neurosci. 2007;26:190-198.