Cellular Imaging of Neuronflammation: A Novel Method for Unraveling the Roles of Macrophages Populations in EAE

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Introduction:

The use of superparamagnetic iron oxide (SPIO) based contrast agents for cell-specific imaging by MRI has now been demonstrated in a number of different disease applications. The detection of brain lesions in EAE models was one of the early applications of this imaging approach.[1] After intravenously (i.v.) administered SPIO, regions of signal void are typically observed in T2w images of the medulla and brainstem in acute EAE. Work in our lab has recently focused on the development of novel tools for cellular imaging at 1.5 T, using a clinical whole body scanner with a custom-built gradient coil insert. We have implemented this specialized MR hardware, together with an optimized 3D steady state free precession sequence, (called FIESTA) for cellular imaging with SPIO. The FIESTA sequence is highly sensitive to the presence of iron labeled cells; we have previously shown that discrete regions of signal loss, associated with very small numbers of iron labeled cells, and corresponding to individual perivascular cuffs, can be visualized in FIESTA images of EAE rat brain.[2]

We know little, however, of the identity of the cells that are responsible for the signal loss. All of the macrophage subsets (blood-derived, microglia, perivascular) have been implicated in the pathogenesis of EAE and all are phagocytic and so have the potential to take up SPIO. Several studies, including our experiments, have used immunohistochemical analyses with ED1, a general macrophage marker, and have shown that regions of signal loss correspond to cells that are ED1-positive macrophages. There is however no unique marker that will allow the distinction between the various macrophage populations in question. In EAE and other models of neuroinflammation, signal loss is often attributed to the accumulation of infiltrating blood-derived macrophages. This is because *in vivo* active labeling is based on the assumption that phagocytic cells in the blood during an immune response can be targeted with an iv injection of SPIO and, if the appropriate signals are present, these cells will migrate to the site of inflammation carrying the magnetic label with them. However, there is no unequivocal evidence that this is what happens. Here we describe an experimental model for addressing this issue; the selective depletion of blood-derived macrophages, with clodronate acid liposomes, prior to imaging with SPIO. Our specific hypothesis was that infiltrating macrophages are the main cell type contributing to the signal loss observed in MR images of EAE brain after Feridex administration. Therefore, we anticipated that treatment with clodronate would result in the reduction of regions of signal void in MR images of EAE brain after Feridex administration. Our results do not confirm this hypothesis, but rather indicate that other macrophage cell types involved in EAE are iron labeled after iv SPIO and contribute to the MR observation of signal loss.

Methods:

EAE was induced in two groups of Lewis rats. At 8 and 10 days post immunization (dpi) rats in Group I were administered clodronic acid liposomes iv, and rats in Group II were administered saline iv. Liposome encapsulated clodronate induces the selective apoptotic death of monocytes and macrophages. Depletion occurs rapidly and without directly affecting other peripheral leukocytes. A single iv injection of clodronate liposomes in rats results in the elimination of macrophages in bone marrow, spleen and liver within 2 days.[3]

All rats were administered Feridex iv, a SPIO, when hindlimb weakness was observed, which occurred at 12-14 dpi in all animals. Animals were sacrificed 24 hours after Feridex administration and the brain, liver and spleen were removed and prepared for *ex vivo* imaging. Imaging was performed at 1.5T using a customized microimaging protocol, which consisted of a high-powered gradient coil insert (peak slew rate 2000 T/m/s and maximum gradient strength 1200mT), custom built solenoidal RF coil. Images were obtained using 3DFIESTA with TR/TE 10/5ms, 30 flip angle, 21 kHz bandwidth, 100 micron in-plane spatial resolution, 200 micron slice thickness, 8 nex, 20 minute scan time. Histopathological analysis included hemotoxylin and eosin (H&E) to assess tissue and cell morphology, DAB enhanced Perl's Prussian Blue (PPB) to detect iron labeled cells and ED1 immunostaining to visualize macrophages.

Results and Discussion:

Depletion of blood-derived macrophages, prior to the administration of the cellular imaging label Feridex, did not inhibit the observation of signal loss as hypothesized. Signal loss caused by the presence of iron labeled cells was detected in both EAE groups. This result suggests that blood derived macrophages are not the only cell type that are responsible for the observation of signal loss ED1+ positive cells associated with iron-induced signal loss include other phagocytic cells involved in the pathogenesis of EAE. There were striking differences in the extent and spatial location of the signal loss in brain images of the two EAE groups (figure 1). In brains from clodronate treated EAE rats prominent regions of signal loss were observed throughout the brain in various locations including the brainstem, medulla, cerebellum and midbrain. In contrast, in brains from non-treated EAE rats signal loss was more sparse and only observed in the brainstem and medulla.

Clodronate acid treatment did not affect the clinical course of EAE; onset of clinical signs occurred between day 12-14 post immunization for all animals.

ED1 staining showed that the degree of inflammation was similar in both groups of EAE rats (figure 2). However, PPB staining revealed that inflammatory lesions in brains of clodronate treated rats contained many more iron labeled cells than did lesions in brains from untreated EAE rats. Furthermore, PPB+ cells were located predominantly in perivascular regions, whereas ED1+ cells were located in perivascular regions and in the parenchyma.

An important point to consider when interpreting these results is the fact that clodronate treatment has blocked the uptake of iron by the liver and spleen, dramatically prolonging the blood circulation time of Feridex and therefore enhancing the opportunities for cellular uptake of SPIO by other immune cells. Signal loss caused by the uptake of iron by resident liver macrophages was observed in liver images from non-treated EAE rats but not in livers from rats that had received clodronate acid liposomes. Since PPB+ cells were mainly perivascular and since Feridex has been shown not to cross the BBB, we propose that activated perivascular macrophages are preferentially labeled in this experiment, with cellular iron uptake substantially enhanced by clodronate liposome treatment. While the uptake of SPIO by circulating hematogenous macrophages may also occur in EAE with an *in vivo* active labeling approach, this study shows that signal loss observed in MR images after administration of iv SPIO cannot be attributed solely to this cell population.

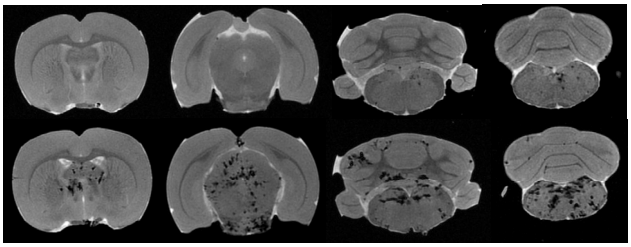

Figure 1. 3DFIESTA images of EAE rat brain (100x100x200 microns), 24 hrs after *in vivo* iv administration of Feridex. Top row: control EAE rat brain. Bottom row: brain images of EAE rat brain treated with clodronate acid liposomes to deplete hematogenous macrophages.

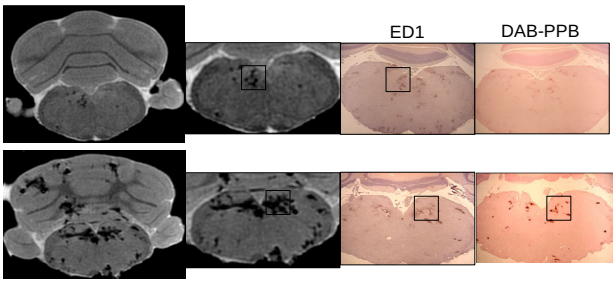

Figure 2. MR and histology comparison. Top row: control EAE rat brain. Bottom row: EAE rat brain of clodronate treated rats. ED1 stains monocytes/macrophages/microglia and shows that the degree of inflammation in both groups is similar. DAB-PPB stains iron. Many more ED1+ cells are also PPB+ in clodronate treated rat brain. PPB+ cells are mainly perivascular. Stained tissue sections are 8 microns; MR is 200 microns slice thickness.

References: 1. Dousset et al. MRM, 1999
2. Oweida et al. Molecular Imaging, 2004
3. van Rooijen N and Kesteren-Hendrikx E. Methods Enzymol, 2003.