The lys-EGFP-ki transgenic mouse: a novel model system for studying the cells involved in neuroinflammation in EAE

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Introduction

Multiple sclerosis (MS) is an inflammatory, demyelinating disease of the central nervous system (CNS). The major pathological hallmark of MS is the presence of sclerotic plaques in the brain and spinal cord. Experimental allergic encephalomyelitis (EAE) is an animal model for immune-inflammatory diseases of the CNS in general, and for MS in particular. The inflammatory infiltrates in acute EAE and MS include a diverse accumulation of lymphocytes and macrophages, primarily in white matter regions of the brain tissue. The importance of T cells in EAE has been extensively documented, particularly the ability to initiate the disease and the autoimmune specificity for myelin provided by these cells. However, other cell types, specifically the macrophages, are also implicated in the pathogenesis of EAE. Both infiltrating hematogenous macrophages and activated resident brain microglia are phagocytic and both macrophage populations have been implicated in the pathogenesis of MS and EAE.

The detection of inflammatory lesions in EAE models was one of the early applications of cellular imaging with superparamagnetic iron oxide (SPIO) agents.[1] Intravenously (iv) injected SPIO nanoparticles are internalized into macrophages and other phagocytic cells. This approach has been termed "active labeling" of cells. The presence of iron oxide is indicated by abnormal signal hypointensities in T2 and T2* weighted images. Our previous work has shown that discrete regions of signal loss, corresponding to the presence of iron labeled cells in individual perivascular cuffs, can be detected using a 3DFIESTA imaging sequence and a customized microimaging system at 1.5T.[2]

In EAE, and other studies of inflammation, it is generally believed that signal loss is associated with the accumulation of infiltrating hematogenous macrophages. This is because 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, unequivocal evidence that the cells responsible for the signal loss are labeled in the peripheral blood, and subsequently infiltrate the CNS, is hard to come by. There is no unique histological marker that will allow the distinction between hematogenous macrophages and activated microglia. A key issue in understanding the pathogenesis of MS is the reliable identification of phagocytes capable of degrading myelin and presenting autoantigen to T cells. Therefore, the identity of the cells in brain lesions is as important as the presence of the cells.

Here we describe the use of a novel experimental EAE model which allows us to differentiate between macrophages of hematogenous and microglial origin: the transgenic *lys*-EGFP-*ki* mouse. The *lys*-EGFP-*ki* model was created using an enhanced version of green fluorescent protein (EGFP) that was knocked into the C57Bl/6 genome replacing the lysozyme M (*lys*-M) gene.[3] The *lys*-M promotor drives the expression of EGFP specifically in mature myelomonocytic cells, which include monocytes, macrophages and neutrophils (but not microglia). EGFP is an easily detectable marker that naturally fluoresces making it easy to detect by fluorescence microscopy. In other words, the blood derived monocyte/macrophage cells in this animal are 'green' and microglia are not.

Methods

EAE was induced in female *lys*-EGFP-*ki* mice at 6-8 weeks of age. Mice received Feridex (200 µl diluted in heparinized saline; dose = 2.24mgFe; total volume 500 µl iv tail vein) during the first relapse phase (40 days post immunization) of this EAE model. Controls animals were non-EAE lys-EGFP-*ki* mice that received Feridex. Twenty-four hours after Feridex administration mice were anesthetized with ketamine/xylazine for 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. After imaging mice were perfusion-fixed. The brains were removed and processed for histopathology. Immunostaining was performed to visualize (i) EGFP expression in hematogenous macrophages, using an ALEXA anti-GFP antibody, and (ii) macrophages/microglia, using rat anti-mouse F4/80 (rhodamine red) a Pan macrophage marker.

Results and Discussion

In vivo images of *lys*-EGFP-*ki* EAE mice obtained 24hrs after Feridex administration show focal regions of signal loss in periventricular regions within the brain (Figure 1A). No signal loss was detected in the brains of normal *lys*-EGFP-*ki* mice administered Feridex. In Figure 1A signal loss is shown in the *lys*-EGFP-*ki* mouse brain near the WM of the corpus callosum and external capsule (blue arrow). Immunostaining of contiguous sections from the same brain shows that EGFP positive hematogenous macrophages can be detected in the same region as the signal loss (1C). The pan macrophage marker F4/80 shows both hematogenous and microglial macrophages as red (1B). The blue arrow indicates the location that corresponds to the area of signal loss and where the accumulation of cells is visible as a dense red signal.

The results of this experiment demonstrate that hematogenous macrophages take up iv administered iron oxide particles in the peripheral blood and arrive at inflammatory brain lesions. Understanding the mechanisms for in vivo cell labeling is important for the advancement of the application of these imaging tools to study the cellular events in EAE and will provide information about the roles of various macrophages in EAE. These cellular imaging techniques have the potential to provide information about the specific cells involved in the initiation and progression of disease in MS, and to allow the imaging and detection of pre-disease states at a time when intervention may provide more effective therapy.

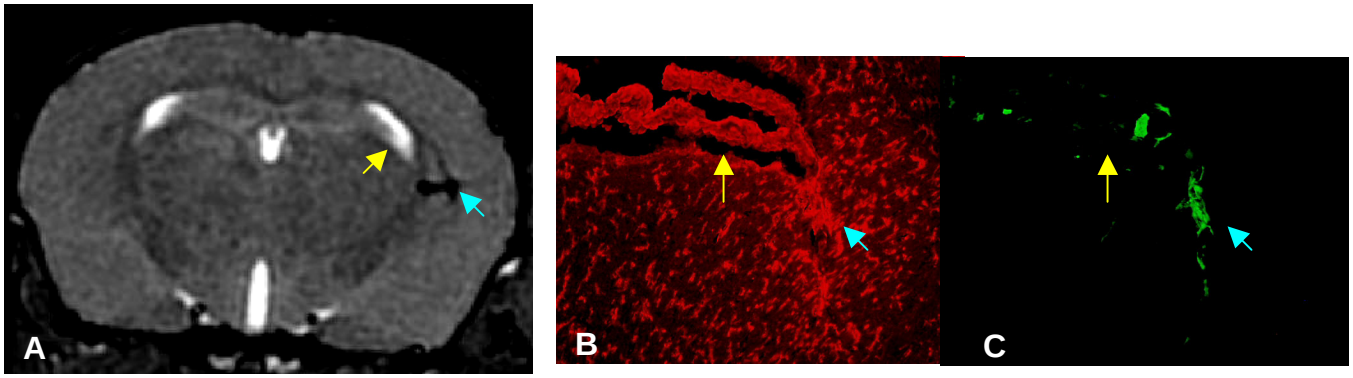

Figure 1. (A) FIESTA image of lys-EGFP-ki EAE mouse brain 24 hrs after iv Feridex shows a localized region of signal loss near white matter tracts (blue arrow), the fluid filled ventricles appear with high SI, (B) anti F4/80 rhodamine red fluorescent image depicts all macrophages/microglia as red, denser red signal is observed in the vicinity of the signal loss, (C) anti-GFP fluorescent image depicts the green fluorescence of hematogenous macrophages in an area corresponding to the region of signal loss in the FIESTA images. Yellow arrowhead indicates location of edge of lateral ventricle.

References: 1. Dousset et al. MRM , 1999; 2. Oweida et al. Molecular Imaging, 2004; 3. Faust et al. Blood, 2000