Review Article

Understanding the Pathogenesis of Neuroinflammation using Magnetic Resonance Imaging

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Abstract

A non-invasive view of the brain with the aid of magnetic resonance imaging (MRI) is invaluable for studying pathological processes during autoimmune encephalomyelitis. Several MRI technologies are available that can be employed to study inflammation within the brain. These include labeling of inflammatory cells with paramagnetic contrast agents (such as USPIO/SPIO/VSOP iron-oxide or perfluoro carbon (PFC)-rich nanoparticles) and new tools that facilitate high resolution imaging particularly MR microscopy (μMRI, microscopic MRI; MR histology). In this review we will go into both MRI technologies, with a special focus on their applicability in studying brain inflammation in the experimental autoimmune encephalomyelitis (EAE).

Keywords

MRI; Fluorine; Nanoparticles; Microscopy; Experimental Autoimmune Encephalomyelitis

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Regarding cell labeling we will focus on PFC nanoparticles and fluorine (^{19}F) MRI since these have introduced a number of advantages over T2*-weighted MRI with paramagnetic iron-oxide nanoparticles. Another MRI technology that we will be discussing is high resolution μMRI with cryogenically-cooled RF coils. This technology will enable neuroscientists to achieve a comprehensive, detailed and non-invasive view of the brain within short acquisition times: an important practical consideration when conducting longitudinal studies on the kinetics and dynamics of immune cell infiltration into the brain.

Introduction

Inflammatory diseases of the central nervous system (CNS), such as multiple sclerosis (MS), involve a recruitment of immune cells during the early stages of pathogenesis, prior to the onset of clinical symptoms [1,3]. Normally the blood-brain barrier (BBB) restricts migration of immune cells to the CNS, but during inflammation its function becomes altered. Immune cells gain access to CNS parenchyma via a complex, multi-step process that involves crossing both the vascular endothelium and the glia limitans [4,5]. The experimental autoimmune encephalomyelitis (EAE) is an animal model that has been invaluable to shed further light on mechanisms underlying CNS autoimmunity. Indeed, the EAE model has been widely employed to examine the basic biology of CNS inflammatory processes, and to evaluate the effectiveness of nascent therapeutic approaches for MS [6,11]. The pathology of EAE – that is brought about via subcutaneous injection of myelin antigen (eg. PLP, myelin proteolipid protein; MOG, myelin oligodendrocyte glycoprotein; MBP, myelin basic protein) – varies between different animal species [12]. In mice (depending on the genetic background) pathology usually develops 1–2 weeks following immunization with myelin antigen. Lymphocytes (including antigen-specific T cells and B cells) and

antigen presenting cells (APC) accumulate mostly in the spinal cord but also in the cerebellum and less commonly in the cerebral cortex [13,14]. The pathology is in fact characterized by an ascending paralysis, commencing at the tail and moving rostrally to affect hind limbs and ultimately the forelimbs [15,16].

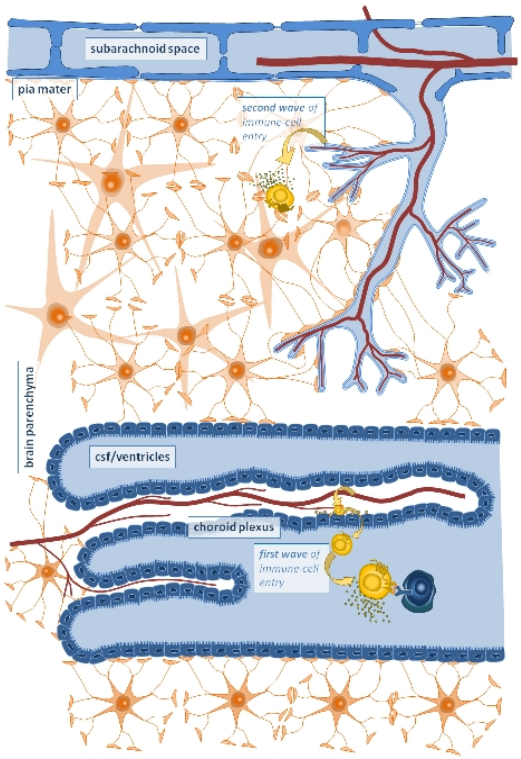

Figure 1. *Current view of immune cell infiltration during autoimmune encephalomyelitis.*
The invasion of myelin-specific T cells into the CNS occurs in two phases. The first wave of immune-cell entry involves the passage of a first group of surveying myelin-specific T cells through the blood–CSF barrier (BCB) into the non-inflamed brain. The second wave of immune-cell entry involves an activation of the blood–brain barrier (BBB) and transmigration of myelin-specific T cells into the CNS parenchyma via the BBB following reactivation by myelin-loaded antigen-presenting cells (APC) in the perivascular spaces (or Virchow Robin Spaces, VRS)

Pathophysiology of neuroinflammation

EAE studies focusing on the early stages of brain inflammation have revealed new insights on the mechanisms involved in the different waves of immune cell entry during evolution of CNS autoimmunity [17]. The current paradigm is that the invasion of myelin-specific T cells into the CNS occurs in two phases (**Figure 1**). The first wave of immune-cell entry involves a chemokine-dependent passage of a first group of patrolling myelin-specific T cells through the blood–CSF barrier (BCB) into the non-inflamed brain [10]. These cells cross the choroid-plexus blood vessels accessing the choroid tissue stroma and then migrate through the tight junctions between the chemokine-rich ependymal cells to enter the cerebral ventricles, from which they migrate to the subarachnoid space.

The second wave of immune-cell entry involves an expansion of myelin-specific T cells in the perivascular spaces and activation of the blood–brain barrier (BBB). The activated BBB that expresses increased amounts of adhesion molecules promotes the rolling, tethering and crawling of T cells to the inner wall of activated cerebral blood vessels (usually against the blood flow) to cross into the subarachnoid space, where they are presented with specific antigen by local resident antigen-presenting cells [11]. Following restimulation, myelin-specific T cells produce large quantities of cytokines and enter the CNS parenchyma – following a disruption of the BBB – to initiate the destructive chain of events in the brain tissue.

To gain a comprehensive and longitudinal view of brain inflammation – particularly during the early stages of EAE – methods employing high resolution brain imaging would be advantageous. In MS patients the detection of contrast-enhancing lesions (CEL) by Magnetic Resonance Imaging (MRI) at the site of BBB disruption as a result of contrast agent leakage into the CNS parenchyma has been used for several years as a correlate of active inflammation [18,19]. However, this does not provide direct evidence of immune cell trafficking into the CNS [20,21], and may occur independently of the formation of new lesions [22]. There is therefore a need to pursue supplemental MRI techniques, to gain a more accurate and comprehensive view of the pathogenesis of CNS inflammation.

Boosting the effective spatial resolution

Scientists performing MRI on small animals are faced with one common dilemma: to observe sufficient detail in anatomical structures analogous to those in the human brain. There has been indeed an intense effort to maximize the effective spatial resolution of the MR images, beyond that required by clinical MRI.

Microscopic MRI (μMRI or MR histology) – defined as MRI with a spatial resolution <100 μm [23,24] – is one means of amplifying image detail in order to observe even minor changes in brain pathology during the course of disease. MRI resolution depends on several factors including magnetic field strength, gradient strengths and digital resolution, but the main limiting factors are coil sensitivity and signal-to-noise ratio (SNR) [25]. Upon reducing voxel size to amplify spatial resolution, a loss in SNR is to be expected. This loss can be considerably compensated for by increasing signal averaging: this produces images with

an impressive level of microscopic detail as shown in *ex vivo* fixed brain tissue samples [26]. However, increased signal averaging comes at a cost in scan time, and is hence not practical for studies with anesthetized animals. This along with the presence of movement artifacts makes it inherently difficult to achieve sufficient resolution to visualize brain pathology *in vivo*.

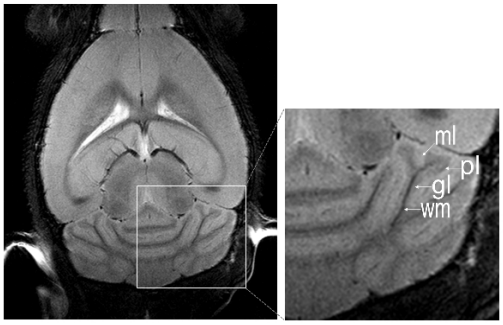

Figure 2. *Horizontal T_2-weighted (T2W) image of a healthy mouse brain.*

A TurboRARE MRI sequence (TR/TE 3000/36ms; 47 μm^2 in plane resolution, 16 slices of 400µm thickness) provides good contrast between grey and white matter boundaries (especially in the cerebellum). These horizontal scans that cover the entire mouse brain were perfumed in 5 minutes and delineate the three cortical layers of the cerebellum (ml, molecular layer; pl, Purkinje cell layer; gl, granular cell layer) surrounding the white matter (wm) of the arbor vitae.

Our group is employing a method that uses cryogenically-cooled RF detection devices on a 9.4-Tesla animal MRI to increase signal-to-noise ratio (SNR) and maximize effective spatial resolution in mouse brains [27]. The RF coils used are made of superconducting material that helps reduce coil resistance and thermal noise and therefore increase SNR by up to a factor of 2.9 [28]. This system increases the virtual field strength by a factor of at least 2 (circa 19 Tesla) without the disadvantages associated with higher field strengths, such as stronger susceptibility artifacts and B_0 inhomogeneities. We performed high spatial resolution µMRI of mice brain during the course of EAE in an attempt to visualize early inflammatory pathology *in vivo* prior to and during commencement of disease. The quality of the images achieved with these RF coils is depicted in **Figure 2**. Our ultimate aim is to employ µMRI to follow the evolution of lesions in longitudinal studies. We have demonstrated that brain pathology can be detected prior to disease manifestation – even without the use of contrast agents – and showed excellent correspondence between µMRI findings and conventional histology (unpublished observations).

Non-invasive tracking of different cell populations

As mentioned in the previous section, the main strength of µMRI in studying brain autoimmunity is that it gives the opportunity to follow the lesion evolution non-invasively over the course of disease. However to gain a comprehensive understanding of the trafficking of different immune cell populations during EAE, a reliable cell tracking method that employs high resolution imaging would be advantageous. Over the past decade several developments have been made within the MRI field that has enabled scientists to track cells *in vivo*. The prospect of being able to non-invasively track immune cells *in vivo* is not only fascinating for scientists studying the pathophysiology of inflammation but also for clinical researchers administering immune cell therapies such as dendritic cell vaccines in the clinic [29].

Labeling of cells for MRI detection has been primarily achieved by incorporating contrast agents into the cell (via mechanisms of pinocytosis or phagocytosis) or by tagging them to cellular surface entities [30,31]. The labeling of cells in this manner renders them distinguishable from surrounding cells by MRI. Such cell labeling strategies make use of contrast agents that modulate the nuclear magnetic resonance (NMR) relaxation times (T1, T2, T2*) of the labeled cells. Shortening of T1 or T2/T2* on these sequences results in an increase or decrease in signal, respectively. T1 agents include gadolinium (Gd) chelates such as Gd-DTPA (diethylene-triamine pentaacetic acid) or manganese (Mn) chelates such as Mn-DPDP (mangafodipir trisodium). On the other hand iron oxide nanoparticles are employed as T2* enhancement agents. The latter contrast agents have been commonly employed to study immune cell infiltration in EAE [21,32–34]. A variety of iron oxide–based labels are available including superparamagnetic iron oxide particles (SPIO), ultrasmall iron oxide agents (USPIO) and micrometer-sized iron oxide particles (MPIO). The latter are particularly applicable for the labeling of pathological cells since they are practical for long-term experiments [35] and are also taken up rapidly and without any adverse effects by these cells [36]. SPIO T2* contrast agents have been employed for labeling various cells including macrophages [37] and oligodendrocyte progenitors [38] and clinical SPIO have been approved by the FDA to label dendritic cells that are applied as cancer vaccines in melanoma patients [39].

One drawback of contrast agents that modulate the NMR relaxation times is a general difficulty to distinguish negative contrast created by contrast-

labeled cells from other susceptibility-related T2* effects such as paramagnetic deoxygenated blood (blood clots or air bubbles) [40]. Furthermore, the background signal from flowing water makes it difficult to identify labeled cells/tissue especially if the approximate localization is not known a priori.

These problems are overcome with the application of fluorine (^{19}F)-rich nanoparticles since it is possible to track cells labeled with these particles very selectively *in vivo* [41]. Fluorine is distinct from any other NMR-active atom; its negligible endogenous presence in the body provides essentially a ^{19}F background free signal. One other advantage of ^{19}F MRI is the possibility to overlay cells labeled with ^{19}F-nanoparticles with the anatomic proton (^{1}H) image (^{1}H MRI). Thus ^{19}F-MRI provides an important counterpart to ^{1}H-MRI. The potential applications for ^{19}F-rich compounds in magnetic resonance spectroscopy (MRS) and MRI have long been recognized [42,44]. Fluorine compounds that are commonly used in biomedical applications are chemically inert and synthetically derived perfluorocarbons (PFCs) that consist primarily of carbon and fluorine atoms. These fluorine-rich compounds are insoluble in water and must therefore be emulsified for clinically relevant applications such as intravenous, intraperitoneal or intraparenchymal injections. The particles obtained by emulsification typically have a size of approximately 200 nm. The size of the PFC particles employed in recent studies to label cells for *in vivo* ^{19}F-MRI tracking ranged from 100 nm to 230 nm [41,45,47]. We have recently shown that the efficiency in labeling dendritic cells with perfluoro-15-crown-5-ether (PFCE) particles – as shown from the ^{19}F spectral signal – increases with increasing particle size (560>365>245>130 nm) [48].

Increasing evidence suggests that the physico-chemical properties of nanoparticles could modulate the immune system [49]. In our study we could show that the larger fluorine-rich particles (560 nm) promote the maturation of DC and as a result the priming of naïve T cells [48]. A previous report on the physical properties of nanoparticles also demonstrated a skewing of the T cell response: particles larger than 1 µm induced a Th1 response *in vivo*, whereas particles smaller than 500 nm were associated with a Th2 response [50]. In contrast to smaller particles, larger particles (>500 nm) alter DC biology by precipitating different uptake mechanisms. Particles larger than 500 nm are commonly taken up by phagocytosis, in contrast to particles smaller than 500 nm that are usually taken up by endocytosis via structures such as caveolae or clathrin-coated vesicles. However, apart from particle size, it is possible that other biologically-relevant changes in particle characteristics – such as shape and surface topology – could also alter DC biology. Scientists are indeed studying such parameters to deliver solutions for controlling biological responses [52]. It would therefore be interesting to study the influence of other physical parameters on DC immunogenicity. Such studies are important to perform in preclinical studies since any undesirable modulation of the transplanted immune cells might pose potential hazards in certain clinical settings especially in those where the immune response should be kept under control e.g. in autoimmune disease and transplantation medicine.

In summary, the visualization of lesion development by *in vivo* µMRI and cell tracking by ^{19}F-MRI provides an opportunity to closely follow the autoimmune process throughout disease progression in the EAE. Thus, in parallel to conventional histological examination and in combination with other contrast agents, µMRI and ^{19}F-MRI will be invaluable tools for longitudinal studies investigating immune cell infiltration during brain inflammation and for the evaluation of novel therapeutic strategies for MS.

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