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Advanced MRI and staging of multiple sclerosis lesions

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Abstract

Over the past few decades, MRI-based visualization of demyelinated CNS lesions has become pivotal to the diagnosis and monitoring of multiple sclerosis (MS). In this Review, we outline current efforts to correlate imaging findings with the pathology of lesion development in MS, and the pitfalls that are being encountered in this research. Multimodal imaging at high and ultra-high magnetic field strengths is yielding biologically relevant insights into the pathophysiology of blood–brain barrier dynamics and both active and chronic inflammation, as well as mechanisms of lesion healing and remyelination. Here, we parallel the results in humans with advances in imaging of a primate model of MS — experimental autoimmune encephalomyelitis (EAE) in the common marmoset — in which demyelinated lesions resemble their human counterparts far more closely than do EAE lesions in the rodent. This approach holds promise for the identification of innovative biological markers and next-generation clinical trials that will focus more on tissue protection and repair.

Introduction

More than a third of a century has elapsed since the publication of the first *in vivo* MRI study of multiple sclerosis (MS) lesions, a period during which the identification of such lesions has become our single most important diagnostic test for MS. Remarkably, the in-depth study of MS lesions by means of contemporary imaging techniques continues to uncover fundamental aspects of the pathophysiology of the disease[1]. Multimodal imaging at high and ultra-high magnetic field strengths has yielded biologically relevant insights into lesion structure and blood–brain barrier (BBB) dynamics that are gradually narrowing the gap between the macroscopic view of the radiologist and the microscopic view of the pathologist.

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Competing interests statement

The authors declare no competing interests.

Author contributions

All authors researched data for the article, made substantial contributions to discussions of the content, wrote the article, and reviewed and/or edited the manuscript before submission.

In this Review, we explore how imaging findings can mirror the pathological development of the demyelinated MS lesion. We describe the patterns by which gadolinium leaks into the parenchyma, either centrifugally (outward from the central vein) or centripetally (inward from the lesion edge), highlighting the dynamism of the BBB in veins and capillaries as it responds to inflammatory stimuli. The introduction of ultra-high-field 7 T human MRI scanners, and of MRI techniques that are highly sensitive to paramagnetic substances, has enabled visualization of prominent central veins within most demyelinated MS lesions, and of paramagnetic rims at the lesion margin in some cases. We also review progress in the imaging of demyelination and remyelination.

Throughout this article, we parallel the results in humans with recent advances in 7 T imaging of a primate model of MS, experimental autoimmune encephalomyelitis (EAE) in the common marmoset. The demyelinated brain and spinal cord lesions resemble the human counterpart far better in this model than in the rodent EAE model[2]. Animal models offer the possibility to image at baseline and frequently after disease induction, thereby enabling investigation of early, subtle radiological changes within normal brain tissue before lesion formation[3]. Another important advantage of animal models is that the pathology underlying the MRI findings can be accessed once the experiment is ended. Lesions in the marmoset brain are often small, so high-resolution (≤100 μm isotropic voxels) scans of the fixed brain, performed prior to processing the tissue for histology, can be very useful (Figure 1). Customized 3D-printed tissue holders and slicers facilitate matching of the histopathology with MRI, which greatly improves the interpretability of the data[4]. This technology, combined with serial MRI scanning before and after lesion development, can elucidate the cellular basis of development and repair of EAE lesions and, by extension, the histopathologically similar lesions in MS itself.

Taken together, the advances described in this article hold considerable promise for deepening our understanding of the biology of MS while opening up the possibility of next-generation proof-of-concept clinical trials mainly focused on tissue protection and repair.

Initiation of MS lesion development

Immunopathology

Breakdown of immunological tolerance to CNS myelin or myelin-like antigens is generally accepted to be the basis of MS pathogenesis. Routes of physiological immune surveillance have been recognized to be involved early in the pathogenesis of both MS and EAE. Peripheral lymphocytes undergo trafficking, participate in immune surveillance and interact with CNS-resident antigen-presenting cells in the meninges (at the blood–leptomeningeal barrier), along ventricular ependymal surfaces (blood–choroid plexus barrier), within perivascular spaces (BBB), and in cervical lymph nodes.[5] Once they escape peripheral tolerance, autoreactive lymphocytes can be further activated and become hostile effectors ($CD8^+$, $CD4^+$ T-helper 1 (T_H1), $CD4^+$ T_H17, and B lymphocytes),[6] thereby precipitating demyelination and axonal injury, which are the typical features of MS.

In early disease, the most dramatic immunopathological events leading to focal areas of demyelination — the ‘lesion’ of MS — tend to occur at the level of the BBB in small or

medium-size parenchymal veins, although early subpial cortical demyelination has also been recognized.[7] As they follow the venous vasculature, demyelinated MS lesions are commonly found around the ventricles (deep venous system, subependymal and medullary veins), at the grey–white matter junction (superficial venous system, especially cortical veins), within the optic nerve (branches of the central retinal vein), and in the brainstem (anteriorly and transversely oriented deep veins) and spinal cord (often posterior deep spinal veins). The spatial predilection and timing of a particular vein's involvement remain fascinating research topics. In general, however, the process of rapid demyelination, which progressively spreads from and along a prominent parenchymal vein to involve the surrounding tissue, directly influences the macrostructure and shape (ovoid, ellipsoidal, triangular or U-shaped) of MS lesions (Figure 2)

Human imaging

As demyelinated lesions in the brain and spinal cord are easy to detect on long-echo-time MRI pulse sequences, such as T2-weighted and T2-fluid-attenuated inversion recovery (FLAIR), these techniques have been implemented in everyday clinical practice and are integral to current MS diagnostic criteria[8,9]. Though recognized in pathological studies as early as the 19th century[10], the perivenular topography of MS lesion development has only recently been directly visualized *in vivo*, using high-resolution susceptibility-sensitive imaging at a variety of magnetic field strengths (Supplementary information S1 (movie))[11–18]. The reasons for the characteristic prominence of the central vein within MS demyelinated lesions in comparison to surrounding brain parenchyma are still open to debate, and include an elevated concentration of deoxyhaemoglobin (higher oxygen extraction at the site of inflammation) and changes in vessel diameter (slower venous flow, postinflammatory scarring processes of the vein wall)[19–21].

Far from being a mere morphological feature of MS lesions, the 'central vein sign' has potential relevance for the diagnostic work-up[9,22], as it is consistently described as atypical in small vessel disease[15,16,23,24] and migraine[25], and is rarely observed in other immunological conditions characterized by brain lesions (for example, vasculitis and neuromyelitis optica spectrum disorder)[26]. Large population studies will be required to ascertain the true positive predictive value of the central vein sign.

Marmoset imaging

Various EAE immunization protocols have been described in marmosets[27–30], but in this Review we will focus primarily on disease induced by subcutaneous injection of human white matter homogenate with an adjuvant[3,27], which in our hands produces a disease that bears strong similarities to relapsing–remitting MS. As in MS, MRI can detect demyelinated lesions that form in the CNS of marmosets with EAE[31–33]. EAE lesions in the marmoset brain present with similar features to MS lesions, including distribution throughout the CNS with a preferred location in the subcortical white matter[3,34,35], uptake of gadolinium contrast agent[3,36], and perivenular topography[37] (Figure 1). Interestingly, the veins around which most of the lesions form are already visible on scans prior to disease induction, supporting the notion that the inflammation associated with EAE affects previously normal veins. These superficial medullary veins drain the white matter lesions and pass through the cortex into

the subarachnoid space. The close relationship between these veins and cerebrospinal fluid within their perivascular spaces is likely to have an important role in lesion development.

Before abrupt opening of the BBB

Immunopathology

A prevalent adaptive immune response to myelin-like antigens triggers an aggressive, scavenger-type response of the innate immune system against CNS myelin. This response is characterized by a classic triad: first, dramatic opening of the BBB; second, blood-derived inflammatory cell (monocyte–macrophage) invasion; and third, macrophage-mediated stripping of myelin sheaths from axons[38,39] The temporal sequence of the earliest pathological events has not been completely unravelled, and remains the subject of active investigation in animal studies. Perivascular inflammatory cuffing is the most prominent pathological feature associated with the newly forming lesion, and is characterized by aggregates of lymphocytes and monocytes confined within the perivascular space of the postcapillary venule and not yet breaching the glia limitans of the BBB (parenchymal basement membrane and astrocytic end-feet). Other notable events include variable immunoglobulin and complement deposition (the distinguishing feature of immunopattern II compared with immunopattern I)[40]; parenchymal microglial and astrocytic activation; perivascular swollen or apoptotic oligodendrocytes with still-intact myelin (the so-called prephagocytic or preactive lesion)[41,42]; and early axonal and synaptic dysfunction[38,39,43,44].

Human imaging

The early pathological findings in both MS and EAE are consistent with the *in vivo* detection of subtle radiological changes in the normal-appearing white matter days to weeks before the parenchymal gadolinium enhancement that defines the radiological onset of the impending MS lesion[45–52]. These data, derived mostly from quantitative or semiquantitative MRI studies that are pathologically nonspecific and only statistically significant at the group level, support the concept of a short-term temporal dissociation between the earliest immunological events in lesion development and the dramatic opening of the BBB (captured on MRI as parenchymal leakage of gadolinium) and subsequent demyelination. Recently, taking advantage of high-resolution imaging and frequent scanning, we have directly visualized focal alterations of MRI signal, on T2-FLAIR and T2*-weighted images, around the central vein of the future MS lesion[18]. Specifically, in some cases, before the overt onset of the radiologically defined demyelinated lesion, the ‘inflamed’ central vein enhances in isolation, perhaps owing to higher BBB permeability with entrapment of the contrast agent within the perivascular space[18].

Marmoset imaging

In a study published in 2014[3], we monitored the status of BBB permeability by mapping the longitudinal relaxation time (‘T1’) before and after injection of a triple dose of gadolinium-based MRI contrast agent. We performed weekly MRI scans, including T1 mapping, as well as proton density-weighted and T2-weighted imaging, after induction of EAE in marmosets. We showed that the BBB becomes focally and increasingly permeable starting approximately 4 weeks prior to lesion appearance. We also demonstrated that areas of

abnormal BBB permeability observed at the time of sacrifice corresponded to inflammatory nodules without associated demyelination. Those nodules consisted of small clusters of perivascular and parenchymal, IBA1-positive, activated microglia and macrophages in association with variable numbers of perivascular lymphocytes. Our results suggest that early changes in vascular permeability, associated with perivascular inflammatory cuffing and parenchymal microglial activation, might precede the formation of new lesions.

BBB dynamics in early MS lesions

Immunopathology

The BBB is recognized to be a highly organized structure, composed of endothelial cells, pericytes and astrocytic end-feet that partially isolate the peripheral blood circulation from the CNS parenchyma[53]. The endothelial and parenchymal basement membranes, consisting of collagen IV, fibronectin and laminin, enclose the 'perivascular' space, in which — as stated above — normal and pathological immune surveillance takes place. In early MS lesions, autoreactive and activated lymphocytes directly contribute to impairment of the BBB of the postcapillary venules, characterized initially by moderately increased permeability and eventually by dramatic structural disruption[38,39,44,54]. Activated $CD4^+$ lymphocytes can secrete the cytokines IFN-γ and tumour necrosis factor (TNF) which upregulate endothelial adhesion molecules (VCAM1 and $\alpha 1$ integrin) and chemokines, thereby inducing massive recruitment of monocytes and allowing transendothelial migration of these cells into the perivascular space and, subsequently, the parenchyma. Structural disruption of the BBB is mediated by the secretion of matrix metalloproteinases that can damage endothelial tight junctions and basement membranes, causing leakage of serum proteins and water (vasogenic oedema) into the white matter[55,56]. Impaired breakdown of leaking fibrinogen, and its breakdown product fibrin, might also contribute to the inflammatory demyelinating cascade[57,58]. This highly inflammatory environment eventually leads to disorganization of the BBB's outer shell, that is, the astrocytic end-feet that comprise the glia limitans.

Human imaging

The striking radiological appearance of newly forming MS lesions is commonly accepted to be related to leakage into the parenchyma (and, therefore, visualization on T1-weighted images) of intravenously injected contrast agents, which are usually gadolinium-based[59,60]. This leakage reflects the short-term structural opening of the BBB — specifically, damage to tight junctions and basement membranes — that occurs at lesion onset.

Dynamic contrast-enhanced MRI (DCE-MRI) at high magnetic field strength is a technique in which T1-weighted images are quickly acquired before, during, and at various time points after gadolinium injection. Through use of this technique, the spatiotemporal dynamics of BBB opening in active MS lesions can be dissected *in vivo*, thereby providing complementary insights to the immunopathological studies. In this dynamic view, in essentially all early active demyelinating lesions, gadolinium leaks initially from the central vein and flows outward, filling the lesion within a few minutes. On static images acquired 5–10 min after contrast injection — the typical situation in clinical practice — such lesions

would be characterized as having ‘nodular’ enhancement. From a dynamic point of view, this process has been termed centrifugal (outward) contrast enhancement[61,62].

After a few days to a few weeks, many of the larger lesions exhibit a shift in the pattern of gadolinium enhancement. In such cases, the expanding inflammatory edge enhances first, and gadolinium then flows centripetally (inward) toward the demyelinated lesion centre[17,61,62] (Supplementary information S2 and S3 (movies)). When this shift occurs, the appearance of the lesion on static images might be either a nodule or a ring, depending on several factors. Especially important among these factors is the time elapsed between contrast injection and MRI acquisition, but the location of the lesion is also important in determining whether the ring is complete or open: an open ring is typically seen when the lesion abuts grey matter or the ventricles. Centrifugal enhancement clearly supports the centrality of perivenular pathogenic events at lesion onset. The significance of centripetal enhancement is less certain, and we hypothesize that this feature might be part of the physiological — though detrimental if unrestrained — wound-healing inflammatory reaction of the CNS parenchyma to focal demyelination.

Marmoset imaging

The dramatic opening of the BBB can also be visualized in active EAE lesions, using T1-weighted images obtained after intravenous injection of gadolinium-based contrast agents. Like MS lesions, active EAE lesions usually display nodular enhancement on static postcontrast T1-weighted images. To our knowledge, open-ring enhancement has not been reported in marmoset EAE. The spatiotemporal dynamics of the gadolinium leakage in active EAE lesions have not yet been fully characterized, mainly due to the limitations imposed by the smaller dimensions of EAE lesions and the higher cardiac output of marmosets compared with humans. Nonetheless, a recent quantitative MRI study has provided some insights into the dynamics of BBB leakage over the span of a few weeks[3]. Following the early gradual increase in permeability that occurs prior to lesion development, the BBB reaches its maximum permeability once the lesion becomes visible on all types of images (T1-weighted, T2-weighted, T2*-weighted and proton density-weighted, as well as T2-FLAIR). After the lesion appears, the permeability of the BBB gradually decreases back to near-normal levels, signifying a restored BBB, in approximately 2–4 weeks[3].

Macrophage-mediated demyelination

Immunopathology

Stripping of myelin from axons and phagocytosis of myelin debris are mediated by blood-derived macrophages and activated CNS-resident microglia, which together constitute the prevalent cellular population within the active demyelinating lesion[38,63–65]. These cell types are difficult to distinguish even at the tissue level (although progress has recently been made on this front[66]), and here we will use the term ‘macrophage’ for simplicity.

The proinflammatory status of macrophages probably contributes to further tissue damage via free radical formation and secretion of proteases and cytokines, including IFN-γ and TNF. Macrophages track the spatiotemporal pattern of BBB opening and demyelination

described above. Thus, they initially distribute throughout the lesion, starting from the centre, and they subsequently become more concentrated at the lesion margin (so-called 'late active' lesions). Interestingly, the efficiency by which macrophages clear myelin debris seems to decrease with age[67]. In addition, to limit amplification of oxidative stress and mitochondrial energy failure, an excess of free iron, probably released from oligodendrocytes during the process of demyelination and/or mediated by endothelial transferrin receptor upregulation, is buffered mainly by macrophages and astrocytes[68–70] Persistently demyelinated axons are more prone to degenerate in an inflammatory environment[71,72], which might explain the varying degrees of axonal injury and loss that coexist with demyelination in MS lesions.

Human imaging

A persistent issue in imaging of MS lesions *in vivo* is related to the fact that inflammation, demyelination and axonal loss within lesions are not cleanly distinguishable on T2-weighted and T1-weighted images. This lack of specificity has prompted a number of attempts to develop MRI approaches that are more pathologically interpretable, such as quantification of the short T2 component in multi-echo T2 relaxation experiments, which seems to correlate with water selectively enclosed between myelin bilayers (so-called 'myelin water imaging')[73–76].

Complementary work on the spatiotemporal dynamics of the BBB has shown that the front of demyelination and inflammation can be captured by coupling DCE-MRI with susceptibility-based MRI at 7 T[17]. These experiments have shown that in centripetally enhancing lesions, the initial opening of the BBB at the lesion edge clearly colocalizes with a paramagnetic rim on phase images — a novel endogenous marker of inflammation[17] (Supplementary information S2 (movie) and Figure 3). Other investigations showed that on 3 T phase images, visualization was better overall in young lesions in early disease than in older lesions[51,77–79]. Properties of the younger lesions that might explain this observation include a greater propensity to demyelination than to axonal loss, and high inflammatory activity with attendant paramagnetic substances, such as free radicals and iron.

Marmoset imaging

By combining serial conventional imaging and histopathology, we have demonstrated that <1-week-old EAE lesions surround a central inflamed blood vessel that is cuffed with T cells and macrophages[3]. Perivascular B cells and blood-derived MRP14$^+$ early activated macrophages are also observed, albeit less frequentlyand macrophage–microglia-associated demyelination and disrupted axons are seen throughout the lesion. Lesions 1–5 weeks old continue to enlarge, and active demyelination is observed at the lesion edges. Interestingly, early activated macrophages are only found at the leading edge of demyelination[3].

Conventional MRI lacks specificity toward tissue pathology in EAE lesions, as in MS lesions, and inflammation, demyelination and axonal loss cannot be differentiated *in vivo*[31]. In one study, quantitative MRI findings correlated with macrophage infiltration, but demyelination was a potential confounder[33]. Recently, an advanced MRI technique, based on complex analysis of the T2* decay curve, was used for *in vivo* detection of the myelin

water content in white matter of healthy marmosets[75]. However, the application of this technique to track demyelination and remyelination remains challenging, as T2* decay curves can be strongly affected by oedema in the extracellular space, iron accumulation in macrophages, and the orientation of white matter tracts relative to the magnetic field of the MRI system. To date, a paramagnetic rim on phase images has not been observed in marmoset EAE lesions, and whether this is due to lack of sensitivity of the applied MRI techniques or to biological differences remains uncertain.

Lesion evolution after BBB restoration

Immunopathology

The process of BBB closure and inflammation resolution is recognized as a potential source of new therapeutic targets in MS, but the underlying mechanisms have not been completely unravelled. An emerging idea is that inflammation in MS, while clearly driving demyelination and tissue destruction, can also trigger healing, albeit dysregulated and, hence, either incomplete or ineffective. In this context, an analogy has been drawn between extra-CNS wound healing and the process of repair within MS lesions[80]. The initiation of healing seems to reside in the extreme plasticity of macrophages, the functional phenotypes of which run the gamut from destructive (‘classically activated’) to restorative (‘alternatively activated’) in the face of environmental stimuli that may vary over time. In the realm of remyelination[81–85], recent data indicate that in early lesions, classically activated macrophages (sometimes called ‘proinflammatory’ macrophages) can induce the recruitment of oligodendrocyte precursors, whereas alternative (‘anti-inflammatory’) activation is required for differentiation of these precursor cells into myelinating oligodendrocytes[67]. Remyelination remains the subject of extensive investigations that are outside the scope of this Review[84–89].

Human imaging

After the resolution of gadolinium enhancement, usually 2–8 weeks following its emergence, MS lesions are radiologically defined as ‘chronic’. The lesions remain clearly visible on both T2-weighted and T1-weighted images, and the extent of T1 hypointensity seems to correlate with axonal degeneration in the white matter[90,91]. Unfortunately, as discussed above, clinical MRI sequences are unable to directly image remyelination. After oedema resolves, typically 1–3 months after lesion onset, tracking of longitudinal signal intensity on T1-weighted, T2-weighted, proton density-weighted, and magnetization transfer-weighted sequences, as well as through lesion volume measurement, remains the established strategy to assess short-term lesion repair[92,93].

Marmoset imaging

The subacute EAE lesions described above show a decrease in BBB permeability in their core area over approximately 4 weeks[3]. After 5 weeks, the lesions display markedly reduced BBB permeability and inflammation, and the macrophages disappear. These lesions are still visible on MRI but become smaller over time — a finding that is consistent not only with a reduction in inflammation but also with potential repair, including remyelination. As most marmoset experiments end within weeks to months of lesion development, the natural

history and pathology of chronic lesions has not been extensively studied, and is an enticing topic for future research.

Chronic MS lesions

Immunopathology

On histological sections, chronic lesions appear hypocellular and demyelinated, with a varying degree of axonal loss. However, an inflammatory infiltrate, wherein macrophages and activated microglia contain early or late myelin degradation products, characterizes the margins of a variable percentage (20–40%) of demyelinated lesions (variously termed ‘chronic active’, ‘slowly expanding’, or ‘smouldering’)[63,64,68,94,95]. On the basis of autopsy studies, this lesion category is most common in people at the transition to clinically progressive disease and those with long disease duration, supporting the notion that these lesions contribute to long-term and clinically important tissue damage[68,94,95].

Human imaging

Chronic inflammation is trapped behind an intact BBB and, hence, remains invisible to current gadolinium-based MRI. Through the use of high-resolution, noncontrast, susceptibility-based imaging, a paramagnetic rim has recently been detected at the margin of some nonenhancing MS lesions. Initial MRI–pathology investigations suggested that this radiological finding might aid identification of chronic active/smouldering lesions *in vivo*. Steady accumulation of macrophages at the lesion edge, in association with paramagnetic inflammatory species related to free radical production and/or intracellular iron accumulation, might explain the paramagnetic signal observed *in vivo*[12,17,78,96–100] (Figure 3).

In the context of early lesion evolution, the subset of centripetally enhancing lesions with a phase rim that persists after the closure of the BBB tend to exhibit poor lesion recovery in terms of lesion volume and T1 hypointensity at year 1, suggesting impairment of early repair and/or permanent tissue injury (unpublished data, Figure 3). If confirmed, this feature will constitute a valuable imaging biomarker, not only to better stage MS lesions *in vivo*, but also to uncover specific components of MS-related hidden inflammation. Similarly, peripheral macrophages experimentally labelled with ultrasmall superparamagnetic iron oxide particles (USPIO) have been tracked at the edges of demyelinated lesions with an apparently intact BBB (that is, lesions with no observable leakage of traditional gadolinium-based contrast agents)[101–103]. A direct comparison between USPIO-based and susceptibility-based imaging is currently lacking.

Marmoset imaging

Bert ‘t Hart and colleagues applied conventional imaging (T2-weighted and T1-weighted with gadolinium injection) to a group of marmosets reaching the chronic stage of EAE disease[31]. Among the chronic lesions, late active demyelinating lesions (with macrophages containing myelin proteolipid protein degradation products located at the edge) were observed together with inactive demyelinated and remyelinated lesions, as confirmed using histopathology[31]. However, the MRI scans performed in this study could not differentiate

these chronic active lesions from their inactive counterparts. USPIO-based MRI, which has been successful in detecting infiltrated macrophages in rodent EAE[104–106], could prove useful for identifying chronic active lesions in marmoset EAE. Another approach would be to perform serial susceptibility-based imaging to detect phase rims in those chronic lesions. These strategies represent enticing targets for future research.

Caveat

Four different initial immunological patterns are associated with acute myelin damage and BBB opening, supporting the idea that MS is a collection of different pathophysiological entities[40,107,108]. What has been described in this Review — the staging of white matter MS lesions and its MRI correlates — probably refers to immunological patterns I and II (myelin-autoimmune hypothesis), which together are the most common types of acute MS lesions[40]. No imaging correlate has yet been shown to definitively identify lesions with immunological pattern III, in which the prime mover is thought to be toxic and/or hypoxic injury to mature oligodendrocytes[40].

Cortical and leptomeningeal involvement

Immunopathology

Cortical and leptomeningeal involvement has recently been recognized as a key feature in MS pathogenesis (early disease with clinical relapses and remission)[7,109] and related neurodegeneration (thought to be largely responsible for clinical progression)[110]. Like the white matter, the cortex can display demyelination, but with much less pronounced inflammation and, importantly, without abrupt opening of the BBB[7,111–113]. Remyelination may be enhanced within the cortex in comparison with the white matter[84,114].

Topographically, cortical lesions develop along the pial surface (‘subpial lesions’) or around cortical venules that drain into the subarachnoid space (small intracortical and leukocortical demyelinated lesions) [111,115]. This topography supports the hypothesis that leptomeningeal inflammation, involving effector lymphocytes, cytotoxic diffusible mediators and immunoglobulins, is strictly related to cortical lesion development via direct damage of the subpial layers of the cortex and/or inflammation spreading into the parenchyma[7]. Subpial cortical demyelination has been characterized as specific to MS-related pathological processes[116]. Of note, aggregates of lymphocytes resembling lymphoid follicles have been recognized in the leptomeninges in patients with progressive MS, and have been judged to reflect an extreme form of compartmentalized, long-lasting inflammation[7,117–122]. The interplay between leptomeningeal inflammation, cortical demyelination and neurodegeneration, as well as more generally between tissue damage in white and grey matter, is still under investigation[110,123,124].

Human imaging

Cortical lesions have been reported *in vivo* at all stages of MS (though more frequently in progressive MS), but subsequent autopsy findings indicate that their numbers are profoundly underestimated on MRI[125–127]. Of note, cortical lesions do not enhance after gadolinium administration, which at present precludes their staging in the living brain. Double inversion

recovery[125], phase-sensitive inversion recovery[128], and high-resolution T2*-weighted[129–131] and magnetization-transfer-weighted sequences[132,133] can detect some of these lesions, especially those with transcortical involvement and/or demyelination of the underlying white matter ('leukocortical lesions'). Subpial demyelination affecting only the superficial cortical layers, and small intracortical lesions have been visualized more reliably on high-resolution post-mortem images (Figure 4), but are only sporadically reported *in vivo*[129–131]. Cortical demyelination shows positive correlations with disability and cognitive impairment scores, thereby underlining the clinical importance of improved detection of this feature[130,134–138]. Together with periventricular, infratentorial, spinal cord, and optic nerve lesions, cortical/juxtacortical lesions contribute to the recently proposed revised 'dissemination in space' MRI criteria for the diagnosis of MS[9].

With respect to leptomeningeal inflammation, a recent study, in which *in vivo* and post-mortem MRI findings were correlated with pathology, reported the prevalence of subarachnoid-space abnormalities in a cohort of ~300 people with MS on both 3 T and 7 T postcontrast T2-FLAIR images[139]. Spatially confined disruption of the blood–leptomeningeal barrier, manifesting as foci of gadolinium enhancement, was identified in ~35% of individuals with progressive MS and ~25% of all patients studied (Figure 4). The presence of leptomeningeal enhancement correlated with whole-brain and cortical atrophy[139]. In autopsy cases, these foci colocalized with perivascular inflammatory infiltrates — specifically, lymphocytes and macrophages clustered around leptomeningeal vessels and along intracortical perivascular spaces — and adjacent subpial cortical demyelination[139].

Marmoset imaging

Demyelinated cortical lesions similar to those in MS (leukocortical, intracortical and subpial) are known to affect the marmoset EAE brain, as shown in several histopathological studies[140–143]. Clusters of T lymphocytes and plasma cells have recently been observed in the meninges overlying subpial cortical demyelination[144], suggesting that local meningeal lymphocytic and plasma cell infiltration also contributes to the pathogenesis of subpial demyelination in marmoset EAE. However, *in vivo* MRI visualization of cortical demyelination and meningeal inflammation has not yet been reported in this animal model.

Conclusions

In this Review, we have summarized recent research efforts to narrow the gap between pathology and imaging in MS lesion development and staging. We have also presented parallel efforts in marmoset EAE, an animal model bearing strong immunological, pathological and radiological similarities to MS. In marmoset EAE, the pathological basis of developing and repairing lesions can be investigated using a combination of serial *in vivo* MRI imaging and histology, and the results of these investigations have strong relevance for our understanding of similar processes in MS. Some observations from human imaging, such as open-ring enhancement, phase rims, meningeal enhancement and cortical lesions, have not been consistently reported in marmoset EAE, and certain observations from marmoset imaging, such as consistently increased permeability of the BBB in the weeks

preceding focal demyelination, have not been described in humans. These differences may be attributable in large part to technical factors, but biology could also contribute substantially.

The parallels reviewed here suggest that the combination of human MS and marmoset EAE research is a promising pathway to achieve a deeper understanding of the pathobiology of BBB dynamics, chronic inflammation, and mechanisms of repair and remyelination, together with the development of new MRI techniques to detect and monitor their evolution *in vivo*. The ability to image both marmosets and humans at the same field strength (7 T) enables ready investigation and translation of new MRI markers from bench to bedside, and vice versa. Finally, this unique approach holds promise for the identification of novel biological targets and next-generation clinical trials that will focus on tissue protection and repair — a major unmet need in MS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Biographies

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Daniel S. Reich — a neurologist and neuroradiologist — directs the Translational Neuroradiology Unit in the National Institute of Neurological Disorders and Stroke, part of the NIH (Bethesda, Maryland, USA). In his clinical practice, he cares for patients with multiple sclerosis (MS) and other neurological diseases, and he also leads several clinical studies focusing on MS. Research in his laboratory focuses on the use of advanced MRI techniques to understand the sources of disability in MS, and on ways of adapting these approaches for research trials and patient care. He is particularly interested in harnessing noninvasive imaging modalities to dissect biological mechanisms of tissue damage.

Key points

- Recent research efforts have narrowed the gap between *in vivo* MRI and pathology with respect to multiple sclerosis (MS) lesion development and staging
- Demyelinated lesions in marmoset experimental autoimmune encephalomyelitis (EAE) resemble their human counterparts far better than do lesions in rodent EAE models
- The perivenular topography of MS lesions might facilitate the radiological work-up
- Smouldering lesions and meningeal inflammation are important features of chronic inflammation that should be targeted for *in vivo* imaging

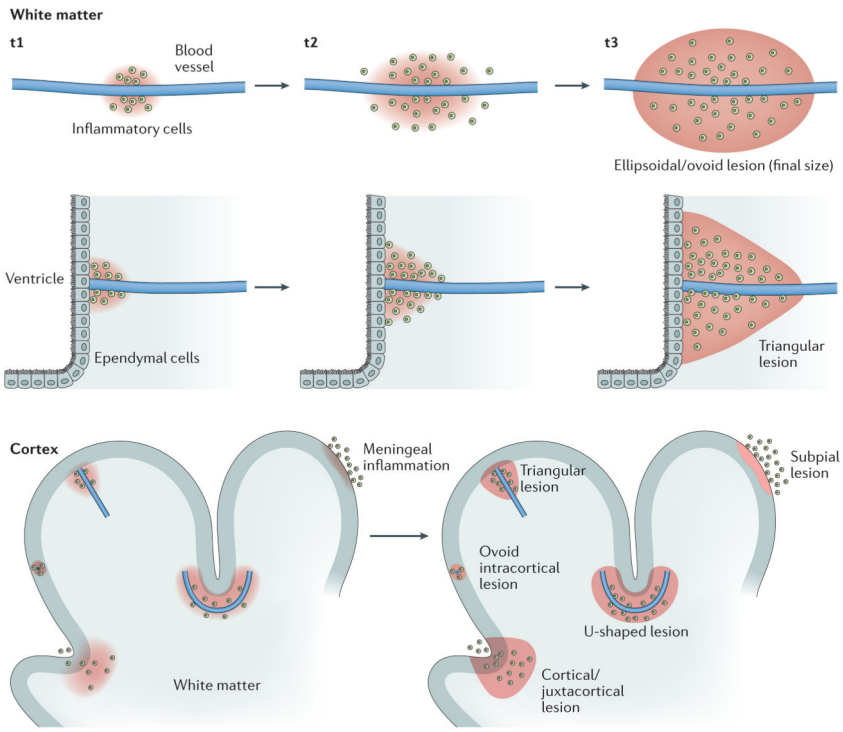

Figure 1. MRI and histology of the marmoset brain

A common marmoset housed at the NIH Intramural Research Program facility was studied in accordance with the standards of the American Association for Accreditation of Laboratory Animal Care and the National Institute of Neurological Disorders and Stroke's Animal Care and Use Committee. **a,b** | Baseline 7 T MRI showing a healthy brain on proton density-weighted (**a**) and T1-weighted (**b**) images. After MRI, the animal was immunized with 200 μg of fresh-frozen human white matter homogenate, and started developing white matter lesions soon after. **c,d** | These lesions are visible on follow-up proton density-weighted (**c**) and T1 weighted (**d**) MRI scans performed before termination of the experiment. Note that a central vein within the lesion (red arrow) can be discerned on the proton density image (**c**). **e,f** | 7 T post-mortem 100 μm isotropic MRI after necropsy (**e**) was used to guide tissue processing for histology (**f**), allowing precise correlation between MRI and histopathology (red arrows). Complete demyelination, as seen on Luxol fast blue staining (**f**), matches the radiological appearance of the lesion both *in vivo* and post mortem (**c, d, e**).

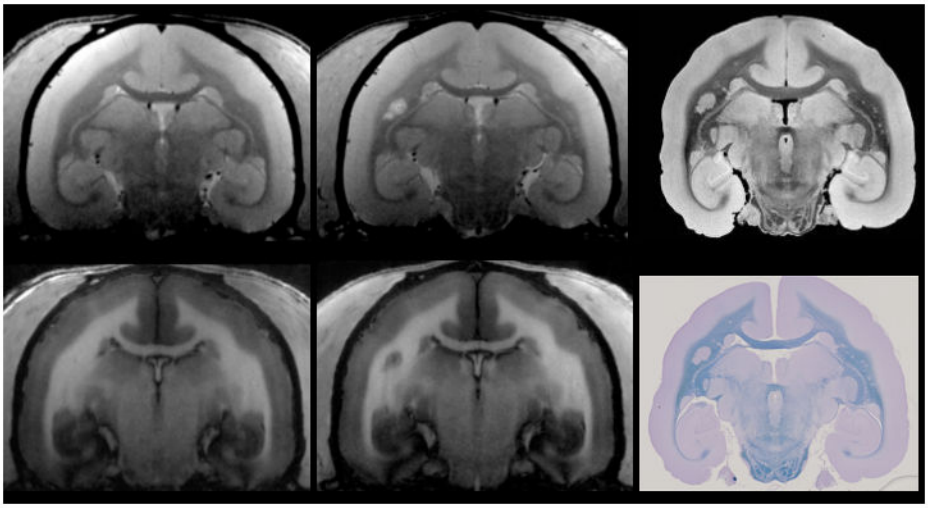

Figure 2. Perivenular topography of demyelinated MS lesions in white and grey matter

In the newly forming MS lesion, the distribution of myelin-scavenging inflammatory cells, spreading along and outward from an inflamed central vein, largely dictates the final lesion configuration. An ellipsoidal and/or rounded configuration is typical of MS lesions in the white matter. Of note, the shape of MS lesions can be also affected by the macrostructure of the surrounding tissue. For example, both the ependymal wall of the ventricles and the cortex may act as barriers to the spread of myelin-scavenging inflammatory cells. Depending of the trajectory of the vein and its position relative to these structures, lesions might assume a triangular shape, for periventricular and leukocortical–juxtacortical lesions, or a U shape, for leukocortical–juxtacortical lesions. The lower panel shows the various types of cortical demyelination. In addition to vasculocentric demyelination, which occurs in purely intracortical and leukocortical lesions, plaque-like demyelination can affect the subpial layers of the cortex and is thought to be directly triggered by leptomeningeal inflammation. MS, multiple sclerosis; t, time point.

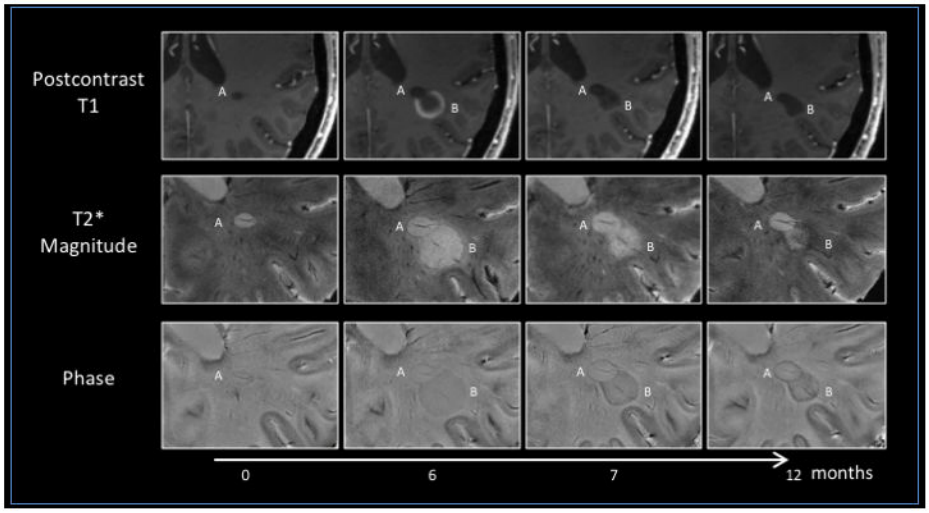

Figure 3. Serial MRI evaluations in progressive MS

An untreated 49-year-old woman with progressive MS and radiological relapses underwent serial MRI evaluations at 7 T under an institutional review board-approved natural history protocol at the NIH. Lesion A is a pre-existing, non-enhancing lesion with a paramagnetic rim visible on both T2*-weighted magnitude and phase images, unknown onset, lesion A). Lesion B, a new centripetally enhancing MS lesion (open ring on postcontrast T1-weighted images; Supplementary information S2 (movie)), appears adjacent to lesion A at month 6. Both lesions exhibit a central vein on T2*-weighted magnitude and phase images. In lesion B, the peripheral T2*-weighted magnitude and phase rim persists after resolution of contrast enhancement (1 and 6 months after lesion onset). Of note, lesion B shrinks over time after enhancement resolves, whereas lesion A nearly triples in size, even in the absence of visible contrast enhancement on postcontrast T1-weighted images. In both lesions by month 12, the persistent T2*-weighted magnitude and phase rim has become darker and thicker, suggesting accrual of paramagnetic substances at the lesion edge. Pathologically, the rim possibly results from macrophages loaded with iron, and lesions with this radiological feature might, therefore, correspond to the so-called ‘chronic active’ or ‘smouldering’ lesions. MS, multiple sclerosis.

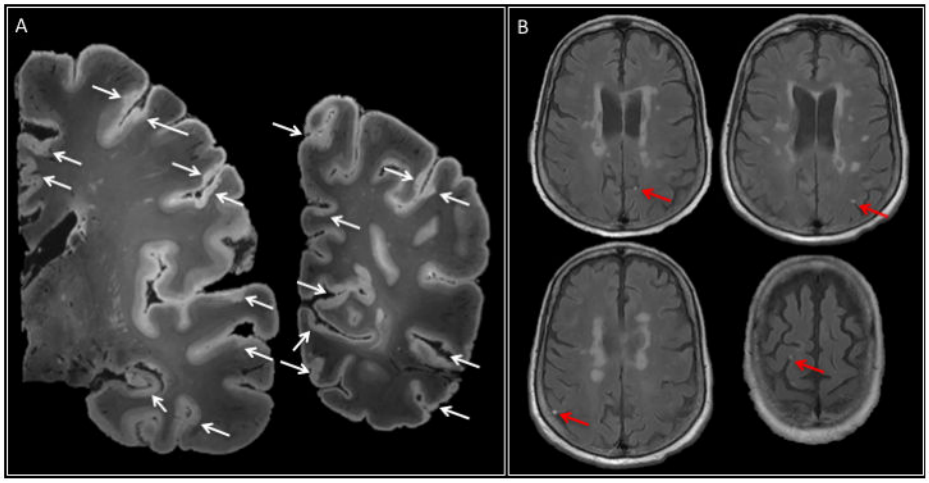

Figure 4. Cortical and leptomeningeal lesions

a | 7 T post-mortem MRI (two representative coronal slices of the left hemisphere, T2*-weighted gradient-echo sequence, 64 nl isotropic voxels) in a 66-year-old woman with progressive multiple sclerosis and disease duration (time from symptom onset to death) of 20 years. The arrows indicate widespread involvement of the cortex in the demyelination process, including subpial cortical and leukocortical lesions. Cortical lesions often face each other across sulci, consistent with the idea that leptomeningeal inflammation has a direct pathogenic role in cortical lesion development. **b** | Several foci of leptomeningeal contrast enhancement (arrows) were apparent in this patient on postcontrast 3 T MRI *in vivo*.