Chronic inflammation in multiple sclerosis — seeing what was always there

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Key points

- Advanced MRI and PET methods enable visualization of features related to chronic inflammation in progressive and relapsing–remitting forms of multiple sclerosis (MS).
- Quantitative analysis of uptake of gadolinium contrast agent and ultra-small paramagnetic particles provide in vivo evidence of chronic, low-grade inflammation in people with progressive or relapsing–remitting MS.
- Lesions associated with activated macrophages/microglia (slowly expanding T2 hyperintense lesions and lesions with high susceptibility-weighted MRI signals at their rims) are more common in progressive MS than in RRMS.
- Persistent focal leptomeningeal inflammation, detectable with gadolinium contrast-enhanced T2 fluid attenuation inversion recovery MRI in many people with MS (particularly progressive MS), is associated with cortical lesions and accelerated cortical atrophy.
- Translocator protein (TSPO) PET can detect increased innate immune activation in brains of people with MS with typically greater activation in secondary progressive MS than in relapsing–remitting MS. Indirect evidence suggests that magnetic resonance spectroscopy measures of *myo*-inositol and some more recently introduced PET measures both can reflect contributions of astrocyte activation to brain innate immune responses.

Abstract | Activation of innate immune cells and other brain compartmentalized inflammatory cells in the brains and spinal cords of people with relapsing–remitting multiple sclerosis (MS) and progressive MS have been well described histopathologically. However, conventional clinical MRI is largely insensitive to this inflammatory activity. The past two decades have seen the introduction of quantitative dynamic MRI scanning with contrast agents that are sensitive to the reduction in blood–brain barrier integrity associated with inflammation and to the trafficking of inflammatory myeloid cells. New MRI imaging sequences provide improved contrast for better detection of grey matter lesions. Quantitative lesion volume measures and magnetic resonance susceptibility imaging are sensitive to the activity of macrophages in the rims of white matter lesions. PET and magnetic resonance spectroscopy methods also can be used to detect contributions from innate immune activation in the brain and spinal cord. Some of these advanced research imaging methods for visualization of chronic inflammation are practical for relatively routine clinical applications. Observations using these techniques suggest ways of stratifying patients with MS to improve their care. The imaging methods also provide new tools to support the development of therapies for chronic inflammation in MS.

{H1} Introduction

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating neurodegenerative disease[1]. The discovery in 1981 that MRI is sensitive to white matter lesions of MS [2] and subsequent, more detailed pathological correlations of the MRI signal with the acute inflammatory oedema, demyelination and gliosis associated with these MS lesions throughout the white matter of the CNS transformed the diagnosis and monitoring of relapsing–remitting MS (RRMS)[3,4]. The use of MRI as an outcome measure in early-phase clinical trials also reduced risks of drug development and accelerated the development of new treatments[5,6].

Radiological correlates of the inflammatory episodes particularly characteristic of RRMS have been the major focus for clinical MRI studies to date, but the neuropathology of MS also highlights chronic inflammation as a feature of all clinical stages of the disease. This chronic inflammation arises from activation of innate and adaptive immune responses that are relatively compartmentalized in the CNS. Many cell types contribute to the innate immune response in the CNS, but microglia, macrophages and astrocytes are numerically most important[7]. Sub-types of adaptive immune cells can become compartmentalized in the CNS as lymphoid-like leptomeningeal follicles[8,9] or as tissue-resident memory T cells[10]. Chronic CNS inflammation seems to play a major role in initiating the neurodegenerative process[11,12] in RRMS and

progressive forms of MS[13,14]. However, conventional clinical imaging is largely insensitive to this chronic inflammation, making it difficult to characterise and monitor in living patients.

The focus of this Review is on advanced imaging methods for assessing chronic inflammation in MS. I first review the major neuropathological features of these inflammatory processes, emphasizing that they have long been a well-recognized aspect of MS neuropathology. I then discuss advanced imaging methods and describe how they can be used to visualize this inflammation. These newer research imaging methods have the potential to define relationships between chronic inflammatory activity, measures of neurodegeneration and clinical progression in life. They also promise to enable better clinical management of MS through patient stratification for treatment and by facilitating the development of new therapies.

{H1} Chronic inflammation in MS

Most of what has been described as the neuropathology of MS comes from studies of brain or spinal cord tissue from patients with secondary progressive MS (SPMS). The neuropathology of MS traditionally has considered to involve neuroinflammation and demyelination with relative neuroaxonal sparing[15]. The characteristic, multifocal white matter demyelination lesions are peri-venular, with inflammatory activity that is associated with leukocytes, reactive astrocytes, microglia and myelin-laden macrophages in various combinations[16,17]. The leukocyte infiltrates are dominated by T cells, accompanied by a variable number of B cells, monocytes and occasional plasma cells. However, in addition to these classical features, substantial involvement of the grey matter now is recognized[18-20], as is clinically significant neuroaxonal injury and loss from the earliest stages of the disease[21-23].

{H2} The blood–brain barrier

Interactions between the peripheral immune system and any compartmentalized CNS immune responses depend on interactions between inflammatory cells or circulating signalling factors and the blood–brain barrier (BBB). The endothelial cells at the BBB form tight junctions and have low pinocytotic activity, enabling them to control the movement of peripheral inflammatory cells and molecules from the blood into the CNS[24]. The classical pathway of T cell migration into the CNS involves direct interactions between the T cells and the endothelium of post-capillary blood vessels.

In the brains of people with primary progressive MS (PPMS) or SPMS, inflammatory markers and adhesion molecules are substantially upregulated in the microvascular endothelium[25]. This upregulation marks activation of endothelial cells at the BBB, a major mechanism facilitating

infiltration of peripheral immune cells into the CNS[26]. This complex process involves co-ordinated expression of multiple cell adhesion molecules on the luminal surface of endothelial cells[27]. Expression of the inflammatory activation marker human leukocyte antigen DR isotype and vascular cell adhesion molecule 1 (VCAM-1) can occur in as many as half of the cerebral microvessels with MS; only moderately fewer express intracellular adhesion molecule 1 (ICAM-1). Inflammatory cells also can enter the brain through the choroid plexus, which expresses particularly high levels of VCAM-1 in brains from people with progressive MS, even with later progressive disease[28].

Soluble forms of the adhesion molecules E-selectin, L-selectin, ICAM-1 and VCAM-1 can be measured in the blood of people with progressive MS[29] and are presumed to reflect their relative endothelial expression. Measurement of soluble VCAM-1has shown that levels in the blood of patients with PPMS are higher than those in patients with RRMS[29].

Post-mortem data also suggest that the integrity of the BBB is chronically impaired throughout the course of MS[30]. For example, tight junction abnormalities are only moderately less frequent in lesions that show some inflammatory activity but appear to be chronic than in lesions with active inflammation that appears to be of recent onset [31]. Evidence of chronic BBB impairment, such as abnormal tight junctions, also can be found in normal-appearing grey matter[32]. Furthermore, fibrinogen can be seen around vessels at the edges of mixed active–inactive lesions, an observation that, in association with histopathological evidence of ongoing demyelination and inflammation, suggests that blood proteins leak into the CNS and help to sustain chronic inflammation[33,34].

As important as understanding the pathways by which peripheral inflammatory cells enter the brain is defining how antigen-presenting cells leave the CNS to activate a peripheral immune response. Important insights into mechanisms for this came with discovery of a rudimentary CNS lymphatic system associated with the dural venous sinuses that allows migration of dendritic and some T cells for antigen presentation in deep cervical lymph nodes[35,36]. Memory B cells trafficking between cervical lymph nodes and the CNS parenchyma probably also use this route, which might become more functionally developed as a consequence of chronic CNS inflammation[26]. Reverse flow in the paravascular glymphatic system could also contribute to transport of brain antigenic proteins to the blood[37,38].

{H2} Leptomeningeal inflammation

Meningeal B cells, which can form ectopic lymphoid-like aggregates, could play major roles in cortical inflammatory demyelination and neurodegeneration[39] (Figure 1). Serum and CSF levels of C-X-C motif chemokine ligand 13 (CXCL13), which is believed to support the aggregation of the B cells[40], are elevated in patients with RRMS and SPMS[41,42]. Leptomeningeal inflammation observed in MS ranges from disorganized collections of immune cells in the meninges of patients with RRMS or PPMS to well-organized ectopic lymphoid follicles observed post-mortem in brains from some people with SPMS[39].

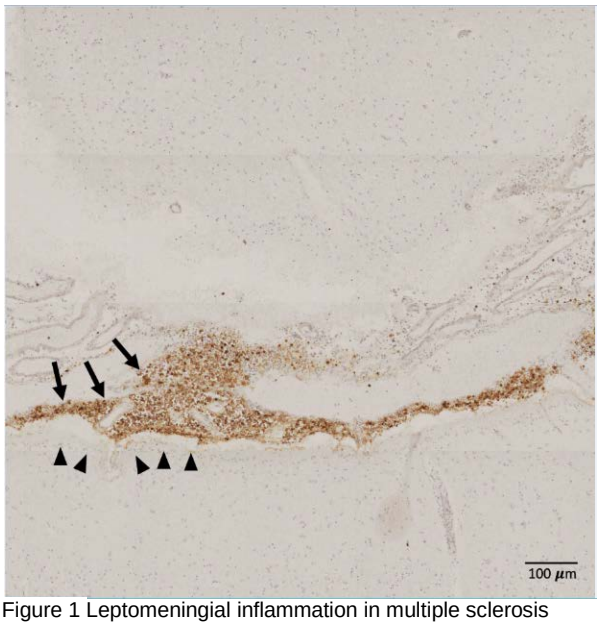

Figure 1 Leptomeningial inflammation in multiple sclerosis

Ectopic leptomeningeal lymphoid tissue is found at autopsy in the brains of ~40% of people with SPMS[9]. Its presence is associated with aggressive disease and more extensive cortical demyelination and neurodegeneration[8,43]. In addition to the almost complete demyelination in affected regions, loss of neurons in adjacent cortex also can be substantial. The post-mortem neuronal density in the cortex has been reported to be almost 40% lower in brains from people with MS than in those from matched controls (14.9 billion neurons per section in MS versus 24 billion per section in brains without evidence of neurological disease)[44]. Loss of synapses is even more marked than the loss of neurons[45,46].

{H2} White and grey matter

Lymphocytes in the brain parenchyma contribute to chronic inflammation[47,48]. Most of the T cells that are recruited to inflammatory foci have an activated (cytotoxic) phenotype and are presumed to be short-lived. However, some convert into tissue-resident effector memory T cells[10] that persist owing to loss of expression of the surface receptors that normally facilitate the egress of cells (the spingosine-1-phosphate receptor 1 (S1P1) and the C-C chemokine receptor type 7 (CCR7)). Upregulation of the integrin CD103 might also contribute to compartmentalization of these cells in the CNS[49]. An inflammatory trigger can cause these memory T cells to differentiate into cytotoxic T cells. B cells that can differentiate into plasmablasts and plasma cells also are found in the brain parenchyma of people with MS. These chronically tissue-resident leukocytes might be able to initiate a new inflammatory focus when inflammatory tissue injury exposes antigens that activate these cells[10].

Despite these contributions of lymphocytes, white matter lesions in MS are classified neuropathologically on the basis of the nature and extent of microglial/macrophage-mediated inflammation and demyelination[50]. Lesions with signs of ongoing demyelination and with macrophages and microglia throughout their volume are classified as active lesions. Lesions with central demyelination, or thin myelin that is associated with remyelination[51], and with macrophages/microglia predominantly at their borders are classified as mixed active–inactive lesions (Figure 2). These macrophages in both types of lesions display a predominantly pro-inflammatory phenotype[52,53] (expression of markers such as CD68, CYBA, MHC class II antigens and ferritin; during active demyelination, myelin breakdown products, including iron, accumulate in these macrophages[54]. Microglia can also be found in the centres of mixed active–inactive lesions, but these microglia, activity of which might be facilitate remyelination[55], are relatively sparse and adopt a predominantly anti-inflammatory phenotype[56]. Activated astrocytes, which have functions intimately related to those of microglia, also are prominent[57] in mixed active–inactive lesions.

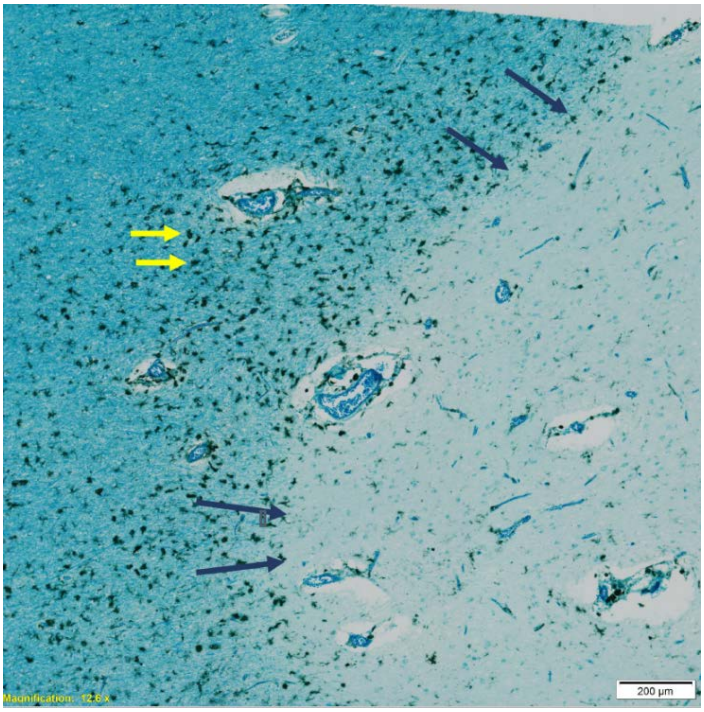

Figure 2 | Macrophages in a mixed active–inactive multiple sclerosis lesion

Chronic inflammation persists in white matter lesions throughout the course of MS. Although brains from patients with progressive forms of MS have fewer active lesions in the white matter than those from patients with RRMS, a range of active, mixed active–inactive and inactive lesions can be seen in post mortem brains of almost all people with MS[58]. Mixed active–inactive plaques are most abundant in brains from people whose disease duration was >10 years and who were >50 years of age at death[59,60]. Even in patients with the longest disease duration (42–64 years), one study reported that just over one-third of the lesions had an active or mixed active–inactive phenotype[60].

Microglia that express tumour microenvironment of metastasis 119 (TMEM119), which are neurodevelopmentally distinct from macrophages as they are derived from yolk sac progenitors[61], are found in the non-lesional white matter of brains from people with RRMS or progressive forms of MS. In the late stages of MS, these microglia have a phenotype that is intermediate between homeostatic and pro-inflammatory (which sometimes is described as ‘primed’), defined by co-expression of CD68 and MHC class II antigen[56]. They are often found in small clusters that are

diffusely scattered in the white matter[62]. Sometimes, these clusters of activated microglia are found in association with complement and degenerating axons, suggesting that the microglia have adopted a pro-inflammatory state[63,64], although microglia in the non-lesional white matter might also clear damaged neurons and other cellular debris[65].

{H2} Ageing and inflammation

The potential changes in the immunopathological mechanisms of MS with ageing are wide-ranging[66]. A comprehensive discussion of this complex and poorly understood area is beyond the scope of this Review, but two general and seemingly contradictory aspects are important to highlight. One is immunosenescence, which has been attributed, at least in part, to decreases in T cell responsiveness with ageing[67,68]. However, the other is an increase in inflammatory function with ageing; for example, microglia in the healthy human brain assume a more pro-inflammatory activation state with increasing age[69]. Broadly consistent with this observation, epidemiological evidence indicates an age-related increase in incident progressive disease and greater severity of disease with later onset[70].

The apparent contradiction between immunosenescence and relative inflammatory activation might be attributed to age-related changes in immune response network interactions. Circulating, adaptive immune cells that are activated by CNS dendritic cells and that are consequently trafficked into the CNS enhance CNS compartmentalized immune responses[56,71]. This process might be facilitated with even normal ageing of the immune system, such as decreased systemic regulatory T cell function and age-related lymphopenia[72]. Leukocyte trafficking to the CNS might be increased further by environmental exposures or co-morbidities (for example, neurovascular inflammation associated with small vessel disease)[73,74] that lead to endothelial activation[75-77].

Repeated systemic inflammatory episodes (e.g., with severe infections) increase the relative abundance of pro-inflammatory microglia. Experiments with a rodent model have provided novel evidence for a long-lasting, epigenetically mediated microglial memory of recurrent systemic inflammation[78]. Interactions between microglia and astrocytes might contribute to this phenomenon, as functional changes in astrocytes relevant to their roles in innate immune responses are regulated by miRNA changes with ageing[79]. One goal in the use of advanced imaging in MS is to monitor not just the extent of chronic brain immune activation and the associated cellular phenotypes, but also the trafficking of inflammatory cells from the blood into the CNS that contribute to modulation of this immune activation.

{H1} Imaging of chronic inflammation in MS

The absence or low frequency of clinical relapses in SPMS and PPMS has often been interpreted clinically as evidence that CNS inflammation is relatively absent. However, this view does not reflect the neuropathological evidence for persistent inflammation discussed above and also neglects early longitudinal observations with gadolinium contrast MRI that described an average of more than one enhancing lesion per patient each year in patients with SPMS, although fewer in patients with PPMS [80]. While the frequency of gadolinium enhancing lesions amongst people with SPMS is significantly lower than for those with RRMS, it is sufficiently high to enable plausible clinical study designs for highly effective treatments intended to suppress this inflammation [81]. These imaging studies might not fully represent observations for the most commonly encountered patients today, but they highlight the potential for inflammatory lesion activity even in progressive forms of MS.

Conventional clinical MRI has unquestionably revolutionized the diagnosis and monitoring of MS[3,4], but is relatively insensitive to the chronic inflammation in grey and white matter that is so prominent on post mortem neuropathology. The past two decades have seen the introduction of new research imaging methods to address this challenge. Quantitative dynamic MRI with contrast agents is sensitive to the reduction of BBB integrity associated with inflammation and to trafficking of inflammatory myeloid cells into the brain parenchyma from the blood. MRI imaging sequences[82-84] provide greater contrast for grey matter lesions. Quantitative lesion volume measures, T2* MRI and susceptibility imaging are sensitive to the activity of macrophages in the rims of white matter lesions. PET and magnetic resonance spectroscopy methods also can be used to assess innate immune activation in the brain (or spinal cord). Together, these methods are beginning to make the major neuropathological features of chronic CNS inflammation observable in vivo (Box 1). By using them in large numbers of patients, direct correlations can be made between changes in chronic inflammation and clinical states.

{H2} Impairment of the blood–brain barrier

Gadolinium contrast enhancement (of acute and active white matter lesions) is usually assessed qualitatively in the clinic, but in a research setting, quantitative measures of change over time after injection of the contrast agent can be made from serial, rapidly acquired images[85]. Quantitative analyses enable detection of the disruption to BBB integrity that accompanies chronic, low-grade inflammation, which is less severe than that associated with acute inflammation. After careful spatial alignment of the serially acquired images, small brain signal changes over time after

injection of contrast agent into the blood can be fitted to a diffusion model that quantitatively reflects the permeability of the BBB to the contrast agent. These measures are sensitive to small impairments of venule barrier function that are believed to lead to the leakage of fibrinogen and other proteins seen in neuropathological studies[33,34]. In this way, multiple } lesions in a single brain can be differentiated on the basis of differences in permeability to gadolinium contrast agent[85]. Chronic inflammation in non-enhancing lesions in people with either RRMS or progressive MS is associated with persistently increased permeability to gadolinium contrast agent[86]. Similar small, chronic increases in contrast agent permeability can be found in the deep grey matter[87].

Quantitative gadolinium measures are not yet useful or practical as clinical tools for assessing chronic inflammation in MS. To become useful, clinically meaningful differences in contrast agent permeability and how they reflect changes in the associated inflammatory pathology need to be defined. Another important consideration is the benefit of gadolinium contrast relative to its potential harm; concerns among doctors and regulators include the rare adverse event of nephrogenic systemic sclerosis, which can occur in people with reduced renal function, and the potential long-term adverse effects of gadolinium accumulation in tissues (including the brain) with repeated contrast administration[88,89].

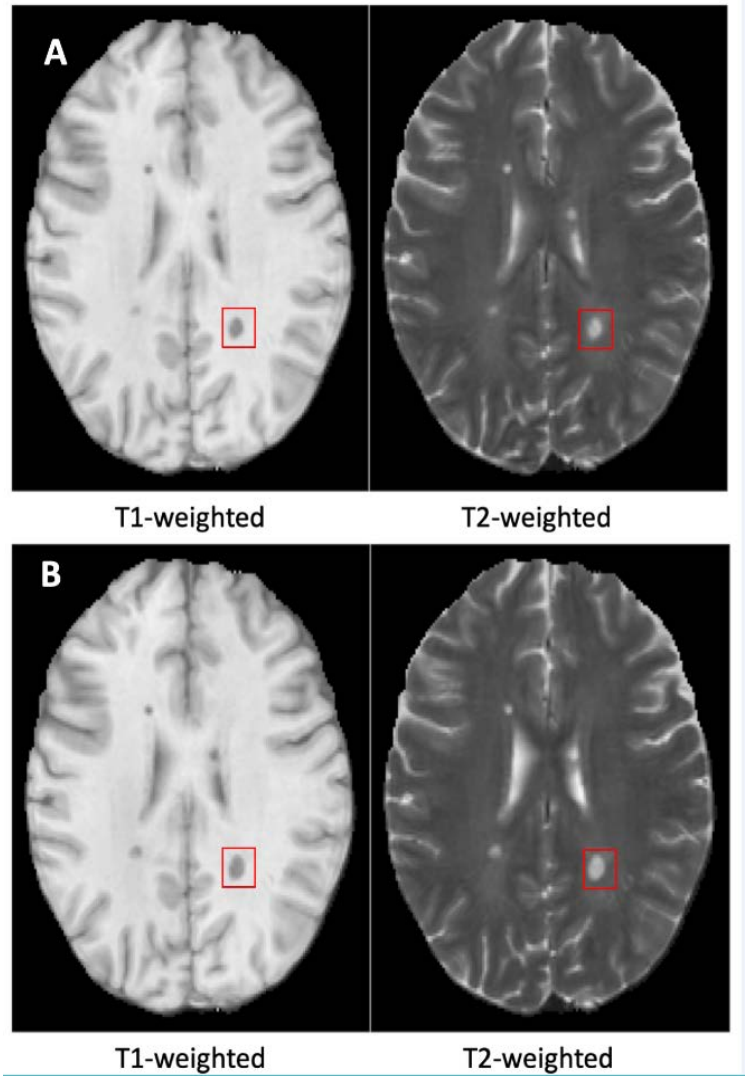

Figure 3 | A slowly expanding multiple sclerosis lesion

{H2} Lesion-associated inflammation

Quantitative measurements of longitudinal changes in the sizes of individual white matter lesions provide in vivo evidence for chronic inflammation that could be more widely available for routine clinical assessments than quantitative gadolinium enhancement measures. Precise assessment of lesion volumes with sensitive, computer-based analyses of paired, serial MRI scans enables monitoring of the slow expansion of some T2 hyperintense lesions[90] (Figure 3). These slowly expanding lesions do not typically show conventional gadolinium enhancement and tend to have a lower

intensity than non-expanding lesions on T1 images, probably reflecting greater parenchymal rarefaction owing to axonal loss[91]. These lesions are more common in PPMS than in RRMS[90]. The neuropathological correlates of these expanding lesions have rims enriched with macrophages/microglia that contain iron (which is released inside the cells upon phagocytosis of myelin)[92,93]. Consequently, magnetic resonance susceptibility imaging provides an indirect approach to their identification in vivo[94]. Changes in myelin and iron content alter the local magnetic susceptibility and, as a result, the so-called T2* relaxation time. On R2* (1/T2*) images, the core of most white matter lesions is hypointense, predominantly reflecting the loss of iron-rich myelin, which normally appears as enhancement on R2* images. Upon myelin breakdown, paramagnetic iron that is phagocytosed by macrophages/microglia at the borders of demyelinating lesions increases the local R2* signal at the borders. Lesions with an isointense core and a hyperintense border on $R2^*$ images are found more commonly in the brains of people with progressive MS than in those with RRMS[95].

With serial imaging, lesions that have hyperintense borders on R2* images are more likely to expand over time than are lesions without such borders. These lesions with border-associated magnetic susceptibility changes have been characterized best with 7T MRI, as the differences in magnetic susceptibility are easier to detect with higher magnetic fields, but the smaller magnetic susceptibility differences also can be detected with clinically available 3T MRI[94]. Detection of R2* hyperintense lesion borders enables identification of patients with active inflammation defined in this way, even with a single scan. Identification of R2* hyperintense borders and slowly expanding lesions[90] provide (at least partially) independent markers of chronic inflammation. When used together, they could improve sensitivity for chronic inflammation and enable stratification of people with progressive MS based on this evidence for active, chronic inflammation.

A different MRI-based approach to identification of chronic lesion-associated inflammation involves detection of brain MRI signal enhancement after intravenous administration of ultra-small paramagnetic iron oxide nanoparticles (USPIO)[96]. The nanoparticles are phagocytosed by activated, tissue-resident phagocytes and circulating myeloid effector cells that migrate from the blood to white matter lesions. Enhancing lesions then show high signal intensities on T1-weighted images (and low signal intensities on T2-weighted images) with both gadolinium and USPIO if both contrast agents are used. However, gadolinium and iron oxide nanoparticles reveal different types of BBB impairment: lesions can be found that enhance only with gadolinium, only with USPIO or with both[96]. Lesions that enhance with both contrast agents are larger and more likely to persist in a 6-month follow-up scan[97,98]. These lesions might, therefore, be those in which BBB

impairment is most severe and myeloid macrophage infiltration, which is associated with proinflammatory macrophages that directly attack myelin, is greatest[99].

USPIO contrast enhancement thus provides another novel potential tool for assessment of chronic inflammatory activity in MS. Uniquely among the imaging methods for monitoring of MS, USPIO contrast enhancement also provides a measure of inflammatory cell trafficking from the blood into the CNS. However, several reports have described serious, life-threatening anaphylactic reactions to USPIO administered for iron supplementation[100,101] and the nanoparticles are taken up and retained in several organs, the long-term health risks of which cannot be assessed easily. The FDA has issued a black box warning regarding these risks[102]. Consequently, the current generation of USPIO agents does not offer a favourable risk–benefit balance for routine clinical use as an approach for stratification based on disease activity in MS. However, potentially safer, less immunogenic USPIO nanoparticles and related agents are under development[103].

{H2} Cortical MS lesions

Cortical MS lesions that have been seen using histopathological methods post mortem can be identified with MRI, but they cannot be defined with high sensitivity because the cortex is thin relative to conventional imaging voxel dimensions and the contrast of the lesions relative to normal-appearing cortical grey matter is low[82,104]. An early study suggested that <10% of cortical lesions that can be seen histopathologically could be identified with clinical MRI — juxtacortical lesions were more frequently detected[105]. Even with side-by-side review of the magnetic resonance images and post mortem histopathology, many of the intrinsic cortical lesions could not be identified on the images[104]. Intracortical lesion detection can be improved by use of 3D double inversion recovery[82], the related 3D magnetization-prepared rapid gradient echo method[83] or phase sensitive inversion recovery MRI[84], as they provide greater contrast between lesions and healthy appearing grey matter. Cortical lesions also can be detected with similar sensitivity by co-registration and multiplication of conventional 3D T2-weighted and 3D-fluid attenuation inversion recovery (FLAIR; for CSF suppression) images[106]. However, the major challenge is spatial resolution. The higher resolution of 7T MRI promises to provide future opportunities for improved cortical lesion detection[107].

Despite these limitations, the numbers of cortical lesions visible with 3T MRI can be counted and related to the clinical course of patients with MS. Generally, cortical lesions identified with MRI seem to be most frequent in the hippocampus and the temporal and frontal lobes (particularly the motor and cingulate cortices)[108,109]. These cortical lesions progress independently of white matter lesions[110] and cross-sectional data indicate that they are associated with greater local cortical

atrophy[111], more severe clinical presentations, more rapid progression and greater disability (particularly disability related to cognitive performance)[112,113]. Nevertheless, on average, the rates at which new cortical lesions develop is similar in people with RRMS and people with SPMS[114], and the rate in PPMS also is comparable[115].

Post mortem studies have identified leptomeningeal inflammation that is associated with extensive subpial cortical demyelinating lesions and neurodegeneration[9,116]. Related observations have been made with MRI in vivo. One patient with MS who died soon after a routine MRI scan showing persistent leptomeningeal enhancement had a pattern of demyelination that was typical for a type III subpial lesion in the adjacent cortex[104]. In patients with RRMS, focal leptomeningeal contrast enhancement is associated with reduced thickness of the adjacent cortex[117]. Long-lasting, focal gadolinium contrast enhancement of the meninges can be seen with MRI in a considerable proportion of patients with MS[118]. its presence in people with MS does provide evidence for active inflammatory pathology. Reliable detection of this phenomenon is challenging because the contrast enhancement is relatively small and needs to be distinguished from the transient increase of signal in meningeal veins as the contrast bolus transits through. With 3T MRI, detection is improved by use of a T2-weighted FLAIR (T2-FLAIR) MRI acquisition sequence, which can increase sensitivity by as much as an order of magnitude relative to T1-weighted imaging[118]. In one study, multifocal leptomeningeal enhancement was seen in a greater proportion (86%) of people with SPMS than of people with RRMS (18%)[119]. A higher frequency of leptomeningeal contrast enhancement in scans of people with progressive MS relative to those with RRMS has been confirmed in larger studies, which also have provided evidence for an association between leptomeningeal contrast enhancement and long-term cortical atrophy[119].

With the important caveat that the use of gadolinium contrast agent must be justified by the clinical benefits of the study (because of potential adverse effects of gadolinium contrast described above), T2-FLAIR with contrast is practical to include in a clinical MS scanning assessment[119,120]. Post-processing in which the pre-contrast image is subtracted from the post-contrast image can minimize false positive interpretations of the scans and increase the accuracy of detection of leptomeningeal contrast enhancement. Use of T2-FLAIR contrast leptomeningeal enhancement in conjunction with counts of MRI-visible cortical lesions could provide a way of assessing new inflammatory activity that complements assessment of white matter lesions, and might better predict future disability[121].

{H2} CNS innate immune activation

Gadolinium contrast-based methods assess inflammation through secondary effects of the inflammation on BBB permeability. Chronic brain inflammation can be evaluated more directly by imaging molecules that are associated with microglia and astrocytes of the innate immune system. This can be done with PET using radioligands that bind to molecules that are specifically expressed in these cells.

{H3} PET of microglia

In vivo evidence for chronic microglial activation in MS has come from translocator protein **}** (TSPO) PET studies (Figure 4). TSPO is relatively highly expressed in microglia (although, in the brain, also is found in endothelial cells and in some activated astrocytes), although it cannot be used to distinguish between microglia with pro-inflammatory and homeostatic activation phenotypes, as it is associated with both[122]. Most TSPO-expressing inflammatory cells in active MS lesions or at the borders of mixed active–inactive lesions seem to be microglia[123].

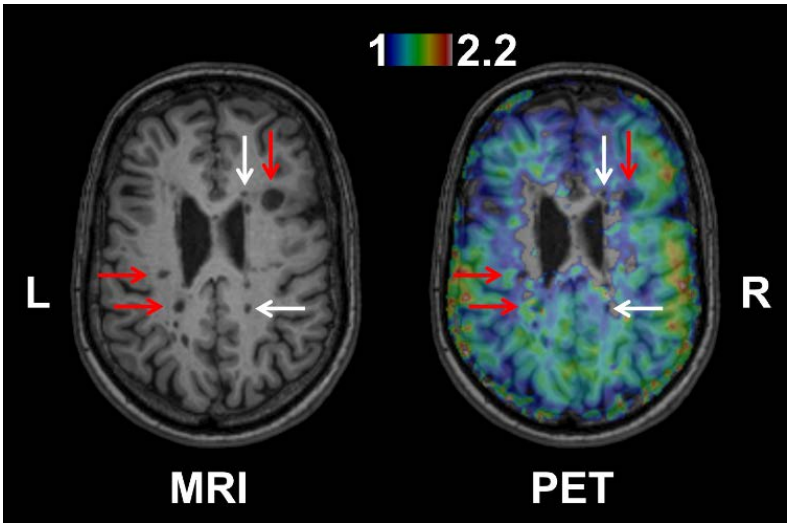

Figure 4 | PET imaging of microglial activation in secondary progressive multiple sclerosis

Use of the “first-generation” TSPO radioligand ^{11}C-PK11195 first identified an association between TSPO radioligand up suggesting foci of inflammation and some T2 hyperintense lesions in the white matter of people with MS[124,125]. Ex vivo autoradiography and histology provided confidence in the relative specificity of the TSPO radioligand binding to microglia/macrophages[125]. Subsequent studies have extended these observations across different patient groups and demonstrated associations of increased TSPO PET radioligand uptake with brain atrophy in progressive MS[126]. Similar findings have been produced with use of two second-generation TSPO PET radioligands[127]. In people with SPMS, increased TSPO PET radiotracer uptake is seen in the non-lesional white matter, as well as in lesions[128]. Greater global brain uptake of the second-generation TSPO radioligand ^{11}C-PBR28 has been associated with a longer duration of disease, and uptake is greater in brains of people with SPMS than in people with RRMS[127].

TSPO PET can also be used to characterize heterogeneity of radioligand uptake between individual lesions that is broadly consistent with the differences in microglial/macrophage density

between lesions that have been described histopathologically.[50] TSPO PET reveals considerable heterogeneity in radioligand uptake between lesions in the same person. Heterogeneity is apparent even among T2 hyperintense lesions that are not distinguishable with conventional clinical MRI. Classification of T2 hyperintense brain lesions according to their radioligand uptake shows that the proportion of lesions in which TSPO radioligand uptake is low relative to surrounding non-lesional white matter (which are considered to be relatively non-inflammatory and presumed to be inactive) is higher in people with SPMS (35%) than in those with RRMS (23%), but lesions in which TSPO radioligand uptake is high (which are presumed to be active) are found in both groups. Lesions defined as active on the basis of their high TSPO radioligand uptake can be numerous even in people who are receiving the disease-modifying treatments that are currently most effective for RRMS (e.g., natalizumab or alemtuzumab)[127,128]. Diffuse white matter uptake of TSPO radioligand that is consistent with widespread microglial activation in the non-lesional white matter also can be seen in RRMS and SPMS[128]. TSPO radioligand uptake increases with greater disability measured with the Multiple Sclerosis Severity Scale (MSSS).[129] The magnitude of the diffuse uptake in the non-lesional white matter can explain much of the variance in disability[126].

The potential to see chronic inflammatory changes in living patients with MS using TSPO PET allows the clinical significance of these changes to be assessed prospectively. For example, in a group of people who were followed-up over 1 year after PET imaging, TSPO radiotracer uptake in the normal-appearing white matter (which reflects the diffuse microglial activation seen in post mortem studies) at baseline correlated well with the number of enlarging T2-hyperintense lesions that developed over the subsequent year[126]. In people with SPMS, microglial activation in the normal-appearing white matter at baseline was strongly correlated with subsequent progression of brain atrophy[126].

Less data have been collected from PET imaging of innate immune activation in the grey matter (in part because the effective resolution of cortical grey matter with PET is lower than that of conventional MRI). An initial report described increased ^{11}C-PK11195 TSPO PET radioligand uptake in subcortical grey matter of the thalamus.[125] However, as the thalamus and brainstem have higher microglial densities than other regions even in the healthy brain, interpretation of this observation is uncertain and needs quantitative correlations between histopathology and TSPO radioligand binding in tissue post mortem. Subsequent work has shown that TSPO radiotracer uptake in the cortical grey matter correlates with disability, as assessed by the Expanded Disability Status Scale and the Multiple Sclerosis Impact Scale (MSIS-29), and with cognitive performance[129,130], suggesting that TSPO PET identifies grey matter inflammatory activity that is relevant to disability in MS. This study exploited the spatial localization made possible by high-

resolution PET and 7T MRI to elegantly demonstrate localized increases in ^{11}C-PBR28 uptake to the cortex[130].

To date, increased TSPO radioligand uptake associated with the cerebral cortex in MS has been interpreted as an indication of inflammation in the grey matter, distinguishing confidently between chronic meningeal and chronic cortical inflammation[9,43] is problematic given the relatively poor spatial resolution of PET and the uncertainties inherent in co-registration of PET and MRI scans[10,43]. The strongest general argument that the peripheral grey matter signal arises from activated cortical microglia in the cortex is that lymphocytes express lower levels of TSPO than do microglia, making even clusters of meningeal lymphocytes less likely to be a major source of signal observed. Further ex vivo studies are needed to explore this question.

The various TSPO radioligands available each have specific advantages and disadvantages (Box 2), the overall value of any TSPO radioligand is limited for some applications by the lack of specificity of TSPO as a marker of microglia; TSPO is expressed in vascular endothelia and high levels are expressed in some activated astrocytes[131]. Interpretation of the TSPO PET signal therefore depends on the neuropathological context. To date, the neuropathological information available for MS has been limited; more quantitative immunohistological data are needed, particularly to facilitate interpretation of differences in radioligand uptake between lesions with different radiological and neuropathological features[127]. Another general problem with use of TSPO PET is that TSPO expression does not clearly distinguish between microglia with pro-inflammatory, anti-inflammatory or homeostatic phenotypes. In human microglia (unlike in rodent microglia), expression of TSPO is not upregulated in the transition from a homeostatic to an activated state[122].

These limitiations of use of TSPO as a radioligand target have stimulated the search for alternative, microglia-specific PET radioligands. Preclinical studies published in 2019 have suggested potential for ^{11}C-CPPC, a high-affinity ligand that binds specifically to the macrophage colony-stimulating factor 1 receptor (CSF1R), expression of which is limited to microglia in the brain[132] — results in rodents and non-human primates support further development of this ligand for human use. Another promising alternative is ^{18}F-JNJ-64413739, a radioligand targeting the P2X7 receptor[133], which is strongly expressed in microglia/macrophages, although also in B cells. Radioligands that target other candidate targets, including cyclo-oxygenase 1, cyclo-oxygenase 2[134] and the CB2 cannabinoid receptor[135] are being developed and evaluated. None of these newer radioligands have yet been used for the study of MS, but the emerging range of potential PET radioligand targets suggests that future studies could employ different, molecularly

specific radioligands serially in the same subject to better characterize chronic immune responses. However, extension of this rationale with the development of radioligands that discriminate between homeostatic, activated neuroprotective and proinflammatory microglial phenotypes is a challenge yet to be addressed.

{H3} PET of astrocytes

In addition to microglia, astrocytes also contribute to the innate immune response — evidence suggests that they are principle effectors of neuronal and oligodendroglial cell death upon proinflammatory microglial activation[136]. Acetate is a potential ligand for astrocyte PET, as it is relatively selectively metabolized by astrocytes in the CNS. In a small pilot application, ^{11}C-acetate uptake in the bilateral thalami and diffusely in the white matter was shown to be greater in the brains of people with MS than in the brains of healthy controls[137]. The numbers of T2 lesions and T1 black holes correlated with the ^{11}C-acetate uptake, suggesting that this measure is a relevant index of inflammatory pathology.

Other PET radioligands target proteins that are believed to be relatively selectively expressed in astrocytes, although these radioligands have yet to be applied to the study of MS. ^{11}C-DED binds to mitochondrial monoamine oxidase B, which is expressed predominantly in astrocytes. The PET signal with this ligand is increased in regions of dense astrocytosis in Creuzfeldt–Jakob disease[138] and in Alzheimer disease[139], suggesting potential for its use in MS. An alternative PET radioligand characterized in humans is ^{11}C-BU99008, which targets the imidazoline-2 binding site that is thought to be found predominantly in astrocytes and is implicated in regulating expression of glial fibrillary acidic protein[140]. The usefulness of this radioligand for MS has not yet been explored, although one potential disadvantage now is that the specific target protein (or proteins) has not been identified.

A more widely explored index of astrocytosis is magnetic resonance spectroscopy (MRS) measures of brain *myo*-inositol, a metabolic marker associated with glial cell activation and proliferation[141]. Concentrations of *myo*-inositol are greater in astrocytes than in neurons and can be expected to increase with neuroinflammation[141]. *Myo*-inositol levels are increased in brains of people with MS relative to healthy controls[142]. MRS has advantages over PET in that it does not involve radiation, it is available with clinical MRI scanners, and the cost is relatively low. Direct evidence to support the use of MRS-measured *myo*-inositol levels as an index of astrogliosis largely comes from a biopsy study of brains from three patients with unusual, acute inflammatory lesions[143]. A study in which MRS was used to determine relative brain *myo*-inositol concentrations in a small group of people with MS who also were studied TSPO PET suggested that the two

techniques measure at least partially independent phenomena (despite their general correlation with higher levels of inflammation) [144]. This evidence is consistent with the notion that they report on different aspects of neuroinflammation in MS. However, the specificity of *myo*-inositol as a biomarker for astrocytes is unclear because anabolic and catabolic pathways for *myo*-inositol are also expressed in other cell types[145].

{H1} Conclusions

Current clinical management of MS relies on conventional MRI measures of lesion count and distribution, and of BBB breakdown detected by gadolinium contrast[4,146-149]. However, these methods are relatively insensitive to the chronic inflammation that has been identified neuropathologically in the CNS in MS. A growing body of data suggests that this chronic inflammation is a major factor in the progressive neurodegeneration and disability characteristic the disease[20,43,113,119,126,130,150].

New MRI and PET tools are becoming available that enable researchers to visualize chronic brain inflammation in vivo. A general hypothesis that is gaining acceptance is that the balance between chronic inflammation and acute inflammatory episodes changes with ageing and disease progression[70]. The availability of the kinds of advanced imaging techniques reviewed above means that this hypothesis can now be tested with clinical studies. Important questions can be addressed. For example, which aspects of the neuroinflammatory responses contribute to homeostatic resilience to neural or glial injury and which accelerate neurodegeneration? What explains the variation in chronic immune responses between individuals? In conjunction with additional imaging tools for assessment of demyelination and remyelination, neurodegeneration and synaptic loss[151-154], the impact of chronic neuroinflammation and its modulation in the CNS can now be studied directly.

The fact that most current disease-modifying treatments for MS seem to have modest or no clinical efficacy in progressive forms of the disease has been disappointing. These therapies might not optimally modulate the complex balance of adaptive and innate or pro-inflammatory and protective immune responses seen in progressive disease[155]. Conventional clinical trial designs might be underpowered to enable detection of disability progression independent of relapses and conventional secondary endpoints, such as white matter lesion activity, are poorly suited to progressive disease[155,156]. The latest clinical trials with new molecules and improved designs have produced more encouraging results[157], but we have not achieved what we need: a way of reliably slowing, stopping or preventing the chronic inflammation that leads to disability progression. New

approaches are needed. Advanced imaging methods that are sensitive to chronic inflammation are among the new tools could enable more informative clinical trials of potential therapies.

This Review highlights the importance of being able to assess and monitor inflammatory mechanisms through the disease course of MS. The challenge now is to validate advanced methods for imaging neuroinflammation sufficiently for their confident use in clinical trials and then to make some of the methods more practical for routine use in the clinic. We also still need to discover even better ways of visualizing the chronic inflammatory activity associated with progressive MS in vivo. With success, we should be better able to select patients who will benefit from newer treatments and accelerate development of therapeutics to address remaining, major unmet medical needs of people with progressive MS.

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Competing interests

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Boxes

Box 1 | Methods for in vivo imaging of chronic inflammation in MS

For each of the chronic inflammatory neuropathologies specified below, the imaging methods that can be used to visualize the chronic inflammation in vivo are listed.

New lesion activity

- Gadiolinium-enhanced T1 MRI[3,4,97]
- Longitudinal T2-weighted or fluid attenuated inversion recovery (FLAIR) MRI[3,4]

Mixed active–inactive lesions

- Enhancement with dynamic gadolinium contrast MRI[85-87]
- Quantitative longitudinal measures of T2 hyperintense lesion growth[90]
- T2* or susceptibility weighted MRI[92-95]
- Ultra-small paramagnetic iron particle contrast (USPIO) enhancement[96-99]

Cortical demyelinating lesions

- 3D double inversion recovery or phase-sensitive inversion recovery MRI[82,84,111]
- 3D magnetization-prepared rapid gradient echo MRI[83]
- Co-registration and multiplication of conventional 3D-T2 weighted and 3D-FLAIR[106]
- Ultra-high field 3D-FLAIR[107]

Leptomeningial inflammation

- Leptomeningial enhancement on T2-FLAIR MRI with gadolinium contrast[116,118,120]

Innate immune inactivation

- Translocator protein (TSPO) PET[124,125,127,128,130]
- ^{11}C-acetate PET[137]
- *Myo*-inositol proton magnetic resonance spectroscopy[142-144]

Box 2 | Translocator protein radioligands

A wide range of translocator protein (TSPO) PET radioligands are available; several have been validated for human use[158], and at least six (^{11}C-PK11195, ^{11}C-PBR28, ^{11}C-DPA713, ^{18}F-PBR111, ^{18}F-DPA714 and ^{18}F-GE180) have been used for studies in MS. The most important advantages of the newer, so-called second-generation TSPO radioligands are an increased affinity for TSPO relative to the first-generation ^{11}C-PK11195[159,160] and a higher target specificity[161]. A higher radioligand affinity increases the binding signal and, in some contexts, increases the sensitivity of PET to inflammatory neuropathology and clinically relevant differences between people with MS and healthy controls[127]. The choice between the second-generation radioligands depends on practical issues[158]. For example, some are fluorinated, meaning their half-lives are long enough for them to be distributed from a central manufacturing site to users working elsewhere. However, these ligands can have limitations; for example, ^{18}F-PBR111 is rapidly de-fluorinated in the body and the released fluoride binds to the skull, confounding accurate assessment of cortical uptake[129].

A limitation of many of the second generation radioligands is that they bind with lower affinity to TSPO in people who carry one carry a genetic polymorphism at the *TSPO* locus that substantially reduces binding of some other radioligands and that is common in Northern European populations[159]. Other TSPO radioligands that have been used to study MS (e.g., ^{18}F-GE180[162-

[164]) have an advantage in that they bind similarly to TSPO in all people. The largest difference in binding as a result of the polymorphism is seen with ^{11}C-PBR28, but the impact of this on a study can be managed by excluding people who are homozygous for the 'low-binding' allele and by analysing those who are heterozygous or homozygous for the 'high-binding' allele separately. Stratification of the population by genotype also can be used for ^{18}F-DPA714 PET, but the dynamic range of the signal with this radioligand in MS is lower than that for ^{11}C-PBR28, which limits its practical application to people who are homozygous for the 'high-binding' *TSPO* allele[165].

Figure Captions

Figure 1 | Leptomeningial inflammation in multiple sclerosis. A photomicrograph that shows binding of a B cell immunohistochemical marker (anti-CD20 antibody) to a cortical section over a sulcus. A dense meningeal cluster of B cells (arrows) overlies the cortical grey matter (arrow heads). This cluster is an example of the lymphoid-like meningeal B cell foci that appear to be chronic and that are associated with demyelination and neuronal injury and loss in the adjacent cortex (courtesy of Prof. R. Reynolds, Imperial College).

Figure 2 | Macrophages in a mixed active–inactive multiple sclerosis lesion. A photomicrograph that shows a mixed active–inactive lesion from a patient with secondary progressive multiple sclerosis. Luxol fast blue defines myelin-rich white matter with darker blue. A dense rim of highly activated MHC class II-positive microglia (yellow arrows) can be seen around a pale, central demyelinated core (white arrows) (courtesy of Prof. R. Reynolds, Imperial College).

Figure 3 | A slowly expanding multiple sclerosis lesion. Co-registered T1-weighted (upper panels) and T2-weighted (lower) axial brain MRI images were acquired from the same person with multiple sclerosis at baseline (a) and 12 months later (b). The lesion in the red box shows slow growth over the period of observation. The T1-weighted image intensity also appears to decrease over this period, consistent with progressive tissue rarefaction.

Figure 4 | PET imaging of microglial activation in secondary progressive multiple sclerosis. The axial 3D T1 MRI image on the left shows multiple T1-hypointense 'black holes'. The MRI image is overlaid with a co-registered parametric ^{11}C-PK11195 PET image from the same person on the right. The relative ^{11}C-PK11195 binding is illustrated as the distribution volume ratio in each voxel (colour scale bar). Red arrows indicate chronic lesions with increased microglial activation and higher ^{11}C-PK11195 binding at the lesion edge (mixed

active–inactive lesions), and white arrows indicate chronic lesions without notable microglial activation at the lesion edge (chronic inactive lesions). (courtesy of Prof. Laura Airas, University of Turku, Turku PET Centre, Finland).

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